Heterogeneous Distribution of Transferrin Receptors on Parenchymal and Nonparenchymal Liver Cells: Biochemical and Morphological Evidence

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To investigate which cells of the liver express the receptor for transferrin, isolated rat liver cells produced by collagenase perfusion were fractionated by repeated differential centrifugation to produce hepatocytes (95% ± 1%, mean ± SD, n = 4) and nonparenchymal cells (97% ± 1%, n = 3). Saturable, high-affinity binding of 125I-transferrin was demonstrated on intact cells at 4°C, with average receptor numbers 20,900 ± 3,180 (mean ± SD, n = 4) for hepatocytes and 5,500 ± 1,520 (n = 3) for nonparenchymal cells. Total cellular receptors measured in detergent permeabilized hepatocytes were 42,000 ± 18,330 (mean ± SD, n = 3) per cell and 14,760 ± 7,120 (n = 3) per cell in the nonparenchymal fraction. Immunocytochemical demonstration of transferrin using antitransferrin, peroxidase antiperoxidase complex confirmed that both cell types bound transferrin. There was heterogeneity of the staining reaction since there was no detectable staining on 40% of hepatocytes and 60% of nonparenchymal cells. Microdensitometric analysis of the staining product corroborated the biochemical evidence that hepatocytes have, on average, more than three times more transferrin receptors than do nonparenchymal cells. These findings support the concept that the hepatocyte has a central role in the uptake and storage of transferrin iron.

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THE LIVER HAS a central role in mammalian iron metabolism, containing one-third of total body iron.¹ This is stored, mainly in the form of ferritin, in the hepatocytes and to a lesser extent in the nonparenchymal cells of the liver,² namely Kupffer and endothelial cells. On the basis of biochemical evidence, it has been suggested that the uptake of iron into this store is mediated through the presence of a specific saturable binding site on the hepatocyte, the receptor for transferrin.²⁻⁴ The histochemical demonstration of transferrin receptors, by use of a monoclonal antibody on both parenchymal and nonparenchymal cells in human liver tissue, supports this finding.⁵ In direct contrast to the above, however, Soda and Tavassoli and Kishimoto⁶ recently reported that they could demonstrate neither binding of transferrin-coated minibeads to parenchymal cells in rat liver by electron microscopy nor binding of radiolabeled transferrin at 4°C. They concluded that only hepatic endothelial cells express transferrin receptors.

This claim has led us to reexamine the question of which cells in the liver express receptors for transferrin. In the present study, we paid particular attention to purification of hepatocytes from nonparenchymal cells in preparations of rat liver cells obtained by collagenase perfusion.⁷ We present biochemical and morphological evidence that both rat hepatocytes and nonparenchymal liver cells express the receptor for transferrin.

MATERIALS AND METHODS

Cell preparations. Liver cells were obtained from male Wistar rats (200 to 250 g) using Berry and Friend's collagenase (type IV, Sigma, Poole, UK) perfusion method modified as described.⁸ After filtering through a nylon mesh, the total cell suspension was immediately transferred to 4°C and was kept at this temperature during further processing. Siliconized glassware and prechilled plastic tubes were used. The total cell yield was between 650 and 720 x 10⁶ cells per liver, and the viability was 98%, as judged by trypan blue exclusion. To prepare nonenzymatically separated hepatocytes, open fine-needle aspiration biopsies of livers⁹ in ether-anesthetized rats were performed. Approximately 10⁹ hepatocytes were obtained from a biopsy and washed three times in Hanks'¹⁰ buffered salt-solution (HBSS) containing 1% (wt/vol) bovine serum albumin (BSA) at 37°C. The cells were then spun onto slides and processed as described later for cells obtained by collagenase perfusion.

The total cell suspension from a collagenase-perfused liver was centrifuged at 50 g for 2 minutes in ice-cold HBSS containing 1% (wt/vol) BSA, and the supernatants were pooled.¹¹ The pellets were resuspended in wash buffer. These centrifugations were carried out to 10 times. The final pellet was kept at 4°C after resuspension. The collected supernatants were further centrifuged for 2 minutes at 50 g to remove the remaining hepatocytes, and the pellet obtained by centrifuging these at 700 g for 7 minutes was layered onto a discontinuous iso-osmotic Percoll (Pharmacia, Milton Keynes, Bucks, UK) gradient at a concentration of 10 x 10⁶ cells/15 mL of gradient. The gradients were prepared by adding the required volume of phosphate-buffered saline (PBS), (285 mOsm 1-¹ pH 7.4) to give Percoll solutions of densities 1.08, 1.075, 1.065, 1.055, and 1.045 g/mL. After centrifugation at 800 x 10⁴ rpm for 50 minutes at 4°C in a MSE Mistral Coolspin using a swing-out rotor, the fractions between 1.055 and 1.075 g/mL were collected and washed three times in wash buffer by spinning at 500 g for 7 minutes.

Assessment of cellular composition. Aliquots (5 x 10⁶ cells) of the well-mixed suspensions of purified cell preparations were spun onto slides after the separation process, using a Shandon Cytospin 2 at 350 rpm for 15 minutes, air-dried, fixed in cold acetone, and stained with hematoxilin. A total of 2,000 cells from high-power fields representing central and peripheral areas of two specimens of each fraction were examined on a Leitz 20 EB microscope fitted with eyepiece graticules. Hepatocytes and nonparenchymal cells could easily be distinguished by their typical size and morphology.¹² Kupffer cells were identified by staining for their endogenous marker enzyme peroxidase¹³ according to Graham and Karnovsky.¹⁴

Proteins. Transferrin was purified from pooled human and pooled rat plasma to which ferrous ammonium sulphate was added to iron-load the transferrin. A modification of the method of Okada and colleagues¹⁵ was used, in which the ammonium sulphate fractions precipitated between 35% and 75% saturation were desalted by extensive dialysis against distilled water and chromatographed at 4°C on a column (gel bed, 2 x 60 cm) of diethylaminoethyl
(DEAE)-Sepharose (Pharmacia), using a linear 50- to 350-mmol/L gradient of Tris/HCl, pH 8. The pink fractions were pooled, concentrated by ultrafiltration, and reapplied to the column using a 50- to 160-mmol/L Tris gradient as before. Fractions with A470/A410 ratios of >1.0 were pooled and rechromatographed to yield fractions of ratios of >1.3. This procedure gave a single protein peak in the case of human transferrin and two distinct peaks with rat transferrin. These rat isoform transferrins, previously characterized as T₀ and T₁, were present in a ratio of ~2:1, respectively. The major isoform transferrin T₀ from three separate preparations of rat transferrin was used in the present study. These isoforms have been previously shown to donate iron equally to the liver and other tissues in the rat.17

The diferric transferrin preparations were radiolabeled with 125I (Amersham International, Bucks, UK) using solid-phase lactoperoxidase as previously described,18 and the iron saturation of the proteins was confirmed after iodination by polyacrylamide gel electrophoresis (PAGE) in 6 mol/L of urea.19 Specific activities of the labeled proteins ranged from 500 to 900 cpm/ng. To prepare 125I-labeled apotransferrin, the labeled diferric species were dialyzed at 4 °C against three changes of 0.1 mol/L of sodium citrate/acetate acid, pH 4.5, followed by exhaustive dialysis against deionized double-distilled water.18

Binding assays. Binding assays on intact cells were performed by adding cells to HBSS 1% BSA containing 125I-labeled transferrin in a final 0.5-mL vol in flat-bottomed polystyrene tubes. The tubes were gassed with 95% O₂/5% CO₂ and incubated with shaking for 90 minutes at 4 °C. Parallel incubations were performed with at least 10-fold excess of unlabeled human transferrin to estimate nonspecific binding. The cells were washed three times in ice-cold PBS by centrifuging at 500 g at 4 °C and were counted in a γ counter.

To determine the binding affinities of 125I-labeled diferric human and rat transferrins to the rat transferrin receptor, rat reticulocytes were produced by phenylhydrazine injection19 and 2 × 10⁶ washed reticulocytes were added to each tube; 0.1 to 20 μg/mL of 125I-labeled transferrin was added, as was unlabeled transferrin when indicated. In some experiments, 125I-labeled rat and human apotransferrins were used. The binding buffers contained 100 mmol/L of desferrioxamine (Ciba Pharmaceuticals, Horsham, Sussex, UK) to maintain the proteins in the iron-free form.18 Because apotransferrin binds with lower affinity to the transferrin receptor than does the diferric species, a higher concentration of the labeled proteins (1 to 200 μg/mL) was used in the binding studies to ensure saturation of the receptor.18

The binding of diferric transferrin to liver cells was performed by incubating 1.8 ± 1.0 × 10⁶ nonparenchymal cells, or 2 ± 0.25 × 10⁶ parenchymal cells to each tube with 0.1 to 20 μg/mL of 125I-human transferrin. Alternatively, in experiments to evaluate receptor number in a competitive setting, a fixed amount (~2,500 cpm) of 125I-diferric human transferrin, labeled to high specific activity (~5,000 cpm/ng) was added to each tube. An increasing amount of unlabeled human or rat transferrin (up to 0.5 mg/mL) was added, and the incubations were carried out at 4 °C for 90 minutes with shaking. Cells were washed in PBS and counted as before.

Total cellular receptor number (the sum of membrane and intracellular receptors), was determined on detergent-solubilized cell extracts.20 Six to 12 × 10⁸ cells were dissolved in 1.2 mL of PBS containing 0.1% (vol/vol) Triton X-100 (Sigma) and 1 mmol/L of phenylmethylsulfonyl fluoride (Sigma) and centrifuged at 12,000 g for 15 minutes at 4 °C. The supernatant was divided into 0.2-mL aliquots in 1.5-mL microcentrifuge tubes, and 20 μg of unlabeled human transferrin was added to two of these. After 15 minutes at 22 °C, 0.3 mL of 0.1 mol/L of Tris/citrate pH 5, containing 0.1% (wt/vol) BSA, 0.1% (vol/vol) Triton X-100, and 200 ng of 125I-human transferrin (diferric) was added to all tubes. These were incubated for 30 minutes at 37 °C, and the transferrin–receptor complex was precipitated by the addition of 0.8 mL of polyethylene glycol 6,000 (12% wt/vol) in 0.1 mol/L of Tris/citrate pH 5 containing human γ-globulin (0.1% wt/vol) for 30 minutes at 4 °C. The tubes were centrifuged at 12,000 g for 15 minutes at 4 °C, and the supernatant and precipitate were counted in a γ counter.

Immunoperoxidase technique. Cytofilm monolayer cell preparations of the mixed postperfusion population and purified preparations of parenchymal and nonparenchymal cells were air-dried, fixed in cold acetone for 20 seconds,21 and further processed as described by Faulk and co-workers,22 modified for the indirect immunoperoxidase method at 4 °C. Endogenous peroxidase was blocked by incubation with 1.5% H₂O₂–PBS (vol/vol) for 30 minutes; incubation with transferrin (0.53 mg/mL) was carried out for 30 minutes, followed by two washes in PBS for 15 minutes. To prevent nonspecific background staining, the specimens were then incubated with 20% normal swine serum (Dakopatts, High Wycombe, Bucks, UK) for 10 minutes. Because human transferrin binds to the rat transferrin receptor with higher affinity than does the native protein (described in Results section), we used human transferrin and a specific heat-inactivated (30 minutes at 56 °C) antisera against human transferrin raised in rabbits20 at the highest possible dilution. Radial immunodiffusion and immunoelectrophoresis24 indicated that this antisera was specific for human transferrin and failed to react with rat transferrin. Control incubations were performed with: (a) purified rat transferrin as ligand, (b) with human apotransferrin in PBS containing 100 μmol/L of desferrioxamine (Ciba) as ligand, (c) without transferrin, and (d) without antibody. Commercially available bridging antibody and peroxidase–antiperoxidase (PAP)-complex were used in recommended dilutions (Dakopatts). The peroxidase reaction was performed with 0.5% (wt/vol) 3'-3'-DAB (Sigma) Tris-HCl pH 7.8 solution for 2 minutes at room temperature. H₂O₂ was added to a final concentration of 0.3% (vol/vol) just before use.

Microdensitometric quantitation. The colored product of the peroxidase reaction on a specimen containing the mixed parenchymal–nonparenchymal cell populations was quantitated with a Vickers M85 scanning and integrating microscope. Absorbance at 430 nm was measured with a 40× objective, spot size 1 (0.5 mm in diameter in the optical plane of the specimen), field size of 25 mm for parenchymal cells or 10 mm for nonparenchymal cells. Under these conditions, single cells of randomly chosen clusters throughout the specimen were evaluated after the microscope was calibrated as described.25,26 The absorption expressed in arbitrary machine units was corrected for the slide-background, which was assessed by scanning a cell-free area of the specimen.

RESULTS

Comparison of binding of human and rat transferrins to the rat transferrin receptor. To distinguish between receptor-bound ligand and endogenous transferrin in the immunocytochemical staining of liver cells, we occupied the cellular receptors with human diferric transferrin and recognized this with a polyclonal anti-human transferrin antiserum that did not react detectably with rat transferrin. For this reason, experiments were performed first to characterize the binding of 125I-human transferrin to the rat transferrin receptor and then to compare this with the binding of iodinated rat transferrin. Equilibrium binding experiments were performed at 4 °C using rat reticulocytes and 125I-human diferric transferrin and 125I-rat diferric transferrin as ligands. As shown in Fig 1, in which the data from a representative experiment have been plotted according to the method of Scatchard,27 human transferrin (Kₜ, 4.7 × 10⁸ 1 mol⁻¹) bound
after three washings varied between 30% and decreased slightly by the separation procedure but remained ±
tial centrifugation to 30%

obtained by collagenase perfusion was reduced by differen-

2.5 ± (Ka 2.9
4 'C in air,
5% CO,

trations of 6.9 ±
or rat transfernin was used to compete for binding.

human
experiments in which a fixed amount of '251-human diferric
binding
data for human transferrin (0) K, 4.6 x 10'
mol') apotransfernins demonstrated very

Similar
numbers of receptors were recognized by the

higher affinity (Ka 6.5 ±

consistently
higher

5)

ferrin (K1 0.9 ±

times

5)

2

mL (mean

3)

Fifty percent inhibition of binding was achieved by concen-

transferrin was added to rat reticulocytes, and unlabeled

difference in the affinity of binding was obtained from

higher

5

to the rat transferrin receptor with an affinity nearly 5 times
higher than that of the homologous transferrin (K, 1 x 108 1 mol'). Similar numbers of receptors were recognized by the
two transferrin species. Three preparations of human transferrin purified from different batches of serum bound with
consistently higher affinity (K, 6.5 ± 2 x 108 1 mol',

mean + SD) than that of three preparations of rat transferrin (K, 0.9 ± 0.4 x 108 1 mol'). Confirmation of this
difference in the affinity of binding was obtained from experiments in which a fixed amount of 125I-human diferric transferrin was added to rat reticulocytes, and unlabeled human or rat transferrin was used to compete for binding. Fifty percent inhibition of binding was achieved by concen-

trations of 6.9 ± 2.6 x 10^-8 mol/L and 4.3 ± 2.8 x 10^-7 mol/L (mean ± SD, n = 3) with human and rat transferrin, respectively.

The differential in binding affinity was not present, how-
ever, in the case of the iron-free proteins, since both human
(K, 2.9 ± 3.3 x 103 1 mol'; mean ± SD, n = 3) and rat (K, 2.5 ± 2.4 x 10 -1 mol') apotransferrins demonstrated very

low affinity for the receptor.

Cell purification. The total yield of hepatocytes obtained by collagenase perfusion was reduced by differen-
tial centrifugation to 30% ± 6% (mean + SD, n = 3).

Recovery of nonparenchymal cells from the Percoll gradients after three washings varied between 30% and 50% of the cell number applied. The initial cell viability of >98% was
decreased slightly by the separation procedure but remained at 95%. The purity of the cell suspension used for the receptor assays was 95% ± 1% (mean + SD, n = 4) for hepatocytes and 97% ± 1% (mean ± SD, n = 3) for nonparenchymal cells. This latter fraction contained 30% ± 3% (mean + SD, n = 3) mononuclear cells positive for endogenous peroxidase.

Immunocytochemical demonstration of transferrin binding. In all preparations of liver cells, human transferrin had bound to hepatocytes and nonparenchymal cells, as shown by the antibody-mediated PAP staining of both types of cell (Fig 2). The staining product on the hepatocytes (H in Fig 2) and nonparenchymal cells (NP in Fig 2) was granular and distributed over the cells. The staining reaction was regarded as specific since (a) preincubation of the specimen with purified rat transferrin, (b) omission of the human transferrin, or (c) omission of antibody to human transferrin abolished the staining. Furthermore, when human apotransferrin was used as ligand, under conditions to ensure that it remained iron-free,14 staining was also abolished. Figure 2 shows that there was individual variation in the staining of the hepatocytes since the cells in the lower part of the Fig 2 showed granular staining whereas those in the upper part demonstrated minimal staining. This heterogeneity was further investigated by staining preparations of purified hepato-
cytes and nonparenchymal cells. As shown in detail in the following section, 60% of hepatocytes (Fig 3a) showed granular staining of variable intensity, with the remaining cells showing diffuse, very weak staining. In nonparenchymal cells, staining was seen on 30% to 40% of the cells, with the remaining cells showing very weak staining (Fig 3b). This heterogeneity of staining of hepatocytes was also seen on cells that had not been exposed to collagenase and that had been produced by fine-needle liver biopsy. Figure 4 shows two views of such a preparation.

Microdensitometry. To provide an objective comparison of the intensity of the color products on nonparenchymal cells

![Fig 1. Comparison of the binding of human and rat transferrin to rat reticulocytes. Rat reticulocytes were produced by phenylhydrazine injection and incubated (2 x 10^6 cells/0.5 mL of Hanks' Balanced Salt Solution, 1% bovine serum albumin) with up to 10 μg/mL of 125I-labeled human or rat transferrin for 90 minutes at 4 °C in air, 5% CO2. Non-specific binding was estimated in the presence of 100-fold excess of unlabeled human or rat transferrin, respectively. Cells were washed three times in phosphate-buffered saline (PBS) at 4 °C and counted in a γ counter. The binding data for human transferrin (○) K, 4.6 x 10^-6 1/mol^-1; and rat transferrin (★) K, 1 x 10^-8 1/mol^-1 were plotted according to the method of Scatchard.27](https://www.bloodjournal.org/content/266/5/266/F1)

![Fig 2. Anti-human transferrin antibody peroxidase-anti-peroxidase (PAP)-stained preparation of mixed rat liver cells (postperfusion). Hepatocytes (H) and nonparenchymal cells (NP) can easily be identified by their different size and shape. Staining reaction varies between neighboring hepatocytes, suggesting differences in transferrin binding. Leitz photomicroscope, ×40 objective, Ilford Pan F. original magnification; current magnification ×19.](https://www.bloodjournal.org/content/266/5/266/F2)
Fig 3. Anti-human transferrin antibody peroxidase–anti-peroxidase (PAP)-stained preparations for purified (A) hepatocytes and (B) nonparenchymal cells. Hepatocytes and nonparenchymal cells show individual variation in staining intensity, with a subgroup of both cell types showing minimal staining.

(n = 380) and hepatocytes (n = 350), a preparation of mixed cells was scanned using a microdensitometer. An estimate of the absorbance due to nonspecific staining and light scattering was obtained by evaluating 20 cells of each type on a control slide from which the antitransferrin antibody had been omitted during the processing. As shown in the histogram (Fig 5) 150 nonparenchymal cells and 205 hepatocytes gave absorbance values greater than those obtained from cells on the control slide. In the nonparenchymal cells, values ranged from 5 to 35 machine units, whereas values in the hepatocytes ranged from 15 to 130 machine units. Comparison of the range of absorbance for the two groups using the Mann-Whitney test showed that they were significantly different (P < .001).

Binding of 125I-human transferrin to hepatocytes and nonparenchymal cells. Because of the evidence that dferic human transferrin binds with high affinity to the rat transferrin receptor, this ligand was used in a series of experiments to investigate the binding of transferrin to purified preparations of hepatocytes and nonparenchymal cells. There was saturable binding of 125I-transferrin at 4 °C to cells of both fractions (Fig 6) with saturation occurring at >12.5 nmol/L of transferrin. Scatchard analysis of the data (Fig 7) showed that 125I-transferrin bound to 20,900 ± 3,160 (mean ± SD, n = 4) sites per cell on parenchymal cells with a K_a of 2.6 ± 0.8 x 10⁸ 1 mol⁻¹ (mean ± SD, n = 4). In the nonparenchymal cell fraction transferrin bound to 5,500 ± 1,520 (mean ± SD, n = 3) sites per cell with a K_a of...
To estimate the number of transferrin binding sites present on nonparenchymal cells. To investigate transferrin binding sites the sites were detected using this procedure as compared with of receptors, assays were performed on ben parenchymal cell fraction and 20% of that in the nonparenchymal cell fraction. For more binding. As shown in unlabeled human or rat transferrin was used to compete for purified parenchymal abscissa indicates that 1.62 x 10^5 molecules of transferrin was added, and unlabeled transferrin (0 to 0.5 mg/mL) was added to compete for binding.

![Fig 6](image)

Fig 6. Concentration-dependent binding of human 125I-transferrin to purified (A) hepatocytes and (B) nonparenchymal cells at 4 °C. Up to 3 x 10^6 cells were incubated in 0.5 mL of Hanks' balanced salt solution, 1% bovine serum albumin with 0.1 to 20 μg/mL of transferrin for 90 minutes under air, 5% CO₂. Cells were washed three times in phosphate-buffered saline at 4 °C and counted in a γ counter. Specific binding (Δ) was calculated by correcting total binding for nonspecifically bound counts (O) determined in the presence of 100-fold excess of unlabeled transferrin.

3.3 ± 1.9 x 10^5 mol⁻¹ (mean ± SD, n - 3). At saturation, nonspecific binding was 15% of the total binding in the parenchymal cell fraction and 20% of that in the nonparenchymal cell fraction. To investigate transferrin binding sites in a competitive setting, 125I-human transferrin was added to purified parenchymal and nonparenchymal cells and either unlabeled human or rat transferrin was used to compete for binding. As shown in Table 1, similar numbers of binding sites were detected using this procedure as compared with the method used in Fig 7, with approximately four times more binding sites demonstrated on parenchymal cells than on nonparenchymal cells.

The above studies using intact cells at 4 °C provide an estimate of the number of transferrin binding sites present on the external plasma membrane. To estimate the total number of receptors, assays were performed on extracts of cells that had been solubilized in a detergent buffer. This treatment permeabilizes the cells and renders intracellular receptors available for binding as well as those present on the cell membrane. With this technique, transferrin bound to 42,000 ± 18,330 (mean ± SD, n = 3) sites per cell in the parenchymal fraction, and 14,760 ± 7,120 (n = 3) sites per cell in the nonparenchymal fraction.

**DISCUSSION**

The use of differential centrifugation and colloidal silica (Percoll) gradient centrifugation enabled us to prepare highly purified preparations of parenchymal and nonparenchymal cells from rat liver perfusates. This treatment is unlikely to have altered the characteristics of the cells significantly since they retained high viability (> 90%) in both fractions and previous work has shown that Percoll does not change the surface properties of isolated liver cells.

The hepatocyte is well established as the major site of transferrin synthesis, to avoid cross-reaction between endogenous transferrin and the antitransferrin antibody used during the immunocytochemical localization of receptor-bound ligand, we used human transferrin to occupy the receptors and its antibody, which did not react detectably with rat transferrin. Evidence for the specificity of this system came from the finding that staining could be abolished by occupying the receptors with rat transferrin and also by using human apotransferrin as ligand since at physiologic pH the iron-free form of transferrin has low affinity for its receptor.

Because of this dependence on human transferrin, experiments were performed to characterize and compare the binding of human and of rat transferrin to the receptor on rat reticulocytes. Equilibrium binding experiments indicated that dimeric human transferrin bound with high affinity to the rat receptor; furthermore, it bound with higher affinity than did rat transferrin. This difference in affinity was confirmed by the finding of at least a tenfold difference in the concentration of human (6.9 x 10⁻⁴ mol/L) and rat (4.3 x 10⁻⁷ mol/L) dimeric transferrin required to displace 50% of 125I-labeled human transferrin from rat reticulocytes. In contrast, both human and rat apotransferrins bound with an affinity ~1 order of magnitude less than the dimeric proteins. This finding is in agreement with previous data showing very low binding affinity between the homologous apotransferrin and rabbit reticulocytes and rat liver cells.

The present study provides morphological and biochemical evidence that human transferrin bound to receptors on
parenchymal as well as on nonparenchymal cells of rat liver. Intensity of staining showed individual variation, however, due to the specific binding of transferrin, as assessed visually and by microdensitometry, suggesting that some hepatocytes could express several times more receptors than the average number found using the radio-binding assays. The average of 20,000 surface receptors is in agreement with a previous report from this laboratory\(^2\) in which hepatocytes that had not undergone extensive purification to rid the parenchymal cells of contaminating nonparenchymal cells were used and rat transferrin was the ligand. The amount of transferrin bound in the present study, in which the titrations were done at 4 °C, was ~50% that reported previously for rat hepatocytes\(^3\-5\) in which the analyses were performed at 37 °C. At the lower temperature, only surface receptors are occupied by ligand, whereas at 37 °C, endocytosis occurs and the full cellular complement of receptors is occupied.\(^6\) Confirmation of the presence of a large intracellular pool of transferrin receptors in hepatocytes, as has been described for other types of receptor such as that for asialoglycoproteins,\(^4\) was obtained from binding assays performed on permeabilized cells. This method showed that the total receptor number was >40,000 per cell.

Approximately 35% of the cells in the nonparenchymal fraction were peroxidase positive (Kupffer cells), a proportion similar to that observed in the intact liver,\(^6\) and it is likely that the separation techniques used here produced a representative population of nonparenchymal cells. Although we cannot comment at this stage on the distribution of transferrin receptors on different subpopulations of nonparenchymal cells, it is of interest that ~60% of these cells showed negligible binding of transferrin, suggesting (as with the hepatocytes) that an individual cell could have more receptors than the average found on this mixed population (surface ~5,000; total ~15,000) using the biochemical assays.

Despite the differences in receptor numbers expressed by the parenchymal and nonparenchymal cells, both bound transferrin with high affinity and similar association constants, which suggests that the cells express the same type of receptor. The values for the association constants obtained fall within the range reported for a variety of cultured cell lines\(^2,3,3\) as well as normal cells.\(^3\)

The finding that parenchymal cells have approximately three times the number of surface and total receptors as nonparenchymal cells is corroborated by the immunocytochemical demonstration of the binding of transferrin to the two cell types. Although the technique of microdensitometry cannot be used for the absolute quantitation of ligand binding, a comparison of the median absorbance range of the color reaction suggests that approximately three times more transferrin was bound by hepatocytes than by nonparenchymal cells. We interpret the data showing heterogeneity of the color reaction between individual hepatocytes as indicating differences in receptor densities and the absence of transferrin binding to a proportion of hepatocytes and nonparenchymal cells as indicating that some cells express so few receptors that they are not detected by the techniques used here. Although transferrin receptors are maximally expressed by proliferating cells,\(^3,6,33\) this is unlikely to be the cause of receptor heterogeneity among hepatocytes, because the proportion of cells in the liver undergoing cell division is very small.\(^2\) The heterogeneity may reflect different roles in iron metabolism of hepatocytes from different parts of the lobule, as has been shown for other hepatic functions.\(^3\)

Despite a recent claim to the contrary,\(^40\) selective proteolytic damage to the transferrin receptors is unlikely since the perfusion buffers included a trypsin inhibitor\(^4\) and heterogeneity of staining was also seen on mechanically separated hepatocytes that had not been exposed to proteases. A study of the distribution of ferritin among individual hepatocytes would be of interest since it has been shown recently that there is an inverse relationship between the concentration of ferritin protein and expression of transferrin receptors by cultured human cells.\(^39\)

Our results do not substantiate the claim of Kishimoto and Tavassoli that transferrin receptors are expressed only by the nonparenchymal and specifically the endothelial cells of rat liver. This conclusion was based on their finding that 125I-rat transferrin-coated minibeads failed to bind to isolated rat hepatocytes but were apparently specifically bound by endothelial cells. The uptake of minibeads could be a reflection of the accessibility of transferrin receptors on the different cell types since the membrane of endothelial cells is smooth, permitting access of immobilized ligand to its receptor. This contrasts with the more irregular membrane of the hepatocytes and Kupffer cells, which could compromise accessibility of ligand immobilized on relatively large beads. As discussed above, more recent claims by those workers that surface transferrin receptors are damaged by collagenase\(^40\) are also unlikely, as we found that receptors were detectable on hepatocytes isolated by collagenase perfusion as well as by mechanical means. The concentration of 125I-transferrin required to achieve saturable binding to liver cells in the studies of Kishimoto and Tavassoli was in excess of 1 μmol/L, which is in contrast to the results of the present study and to the experience of many workers using crude suspensions of hepatocytes\(^2,3,5\) and cultured cells\(^2,3,3,3\) in which saturation was achieved in concentrations ~1 order of magnitude less.

In conclusion, our finding that hepatocytes express, on average, three to four times more receptors than do nonparenchymal cells supports the concept that the hepatocyte has a central role in the uptake and storage of transferrin iron.

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