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Storage Iron Kinetics.
IV. Cellular Distribution of Ferritin Iron Stores in Rat Liver[1] (38017)

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There is evidence in both animals and man that the liver is the major site of body iron stores. In normal adult males for example, the liver accounts for approximately one-third of body iron stores or about 400 mg (1). Remaining sites of storage are widely dispersed throughout the body and thought to be about equally divided between skeletal muscle and the reticuloendothelial (RE) system (2). In states of iron overload, the proportion of storage iron within the liver is much greater.

Iron within the liver is contained in two discrete cellular compartments. The *Kupffer cells,* together with RE cells elsewhere in the body, contain ferritin and hemosiderin derived from catabolism of senescent red blood cells. The *hepatocytes* contain iron derived largely from uptake of plasma transferrin (3) and, to a lesser degree, from plasma hemoglobin and ferritin (4, 5). An important question in respect to hepatic ferritin metabolism is the relative distribution of liver iron between these two compartments. In the present study carried out in the rat, the proportion of ferritin iron in RE and parenchymal cells of the liver has been determined. This was accomplished by injecting radioiron-labeled heat-damaged red cells which are known to be taken up solely by RE cells. Determination was then made of the specific activity of whole liver ferritin and of ferritin from Kupffer cells isolated by selective digestion of parenchymal cells with a proteolytic enzyme. Because only RE cell ferritin was labeled, the specific activity of ferritin obtained from the whole liver was reduced in proportion to the relative size of the unlabeled parenchymal ferritin pool. Thus the contribution of Kupffer and parenchymal cells could be quantitated.

Methods. Studies were performed in female Wistar rats weighing 180–200 g. Animals were fed *ad lib.* on Purina rat pellets with an iron content of 380 μg/g of food. Injections and blood sampling were carried out through the tail veins. Animals were sacrificed under ether anesthesia by exsanguination through the abdominal aorta. Heat-damaged erythrocytes labeled with ^{59}Fe were prepared as described previously (5). The nonviable cells were injected intravenously into recipient animals in amounts not exceeding 5 mg of hemoglobin per rat. The labeled red cells were cleared from circulation with a half-time of less than 5 min. Maximum hepatic uptake of radioactivity during the first hour following injection averaged 80% of the injected dose.

Measurements of ferritin specific activity in hepatic tissue were performed in animals sacrificed 2 hr following injection of ^{59}Fe-labeled red cells, when over 30% of hepatic radioactivity was bound to ferritin. For each individual measurement, the livers of 4 animals were cut into 2–5-mm particles and combined. About one-quarter of this tissue was homogenized in 4 vol of distilled water for 5 min with a Vortex model 45 homogenizer and treated as described below to de-

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termine the specific activity of ferritin isolated from the whole liver. Hepatic RE cells were isolated from the remaining liver tissue by selective destruction of hepatic parenchymal cells with a proteolytic enzyme (6). The final RE cell isolate was obtained by centrifugation at 200*g* for 5 min, resuspension of the pellet in 5 ml water, and homogenization as described for whole liver.

Ferritin was isolated immunologically after first heating the whole liver homogenate or suspension of RE cells to 75° for 8 min to coagulate the bulk of nonferritin protein. Ferritin was precipitated from the supernatant by adding an excess of rabbit antihorse ferritin serum (7). Chemical iron was measured by first incubating the washed precipitate in 3 *N* HCl overnight at 37° to release iron from protein. After precipitation of the protein with 20% trichloroacetic acid, iron was measured colorimetrically with sulphonated bathophenanthrolene as described for serum iron determinations (8). The specific activity of ferritin ^{59}Fe was determined on duplicate samples of the trichloroacetic acid supernatant by obtaining a minimum of 20,000 counts with a NaI(Tl) well-type scintillation counter. In these single-isotope studies, the RE cell ferritin pool was calculated as the ratio of specific activities in whole liver and the Kupffer cell isolates.

In further studies, possible contamination of RE cell isolates by either parenchymal cells or parenchymal cell ferritin was assessed as follows. At the time of injection of heat-damaged RBC tagged with ^{59}Fe, animals were given a simultaneous intravenous injection of transferrin-bound ^{55}Fe. The latter was prepared by incubating 1 ml of freshly drawn rat plasma with 1 μCi of $^{55}FeCl_3$ (sp act 10–15 μCi/mg) which had been diluted in 0.005 *N* HCl and mixed with sufficient sterile 4% sodium citrate to ensure a molar ratio of citrate to iron in excess of 50:1. Previous studies have established that roughly 10% of injected transferrin-bound radioiron is localized in the liver 2 hr following injection and that virtually all of the radioactivity is contained in hepatocytes (5). In these double-isotope studies, ^{55}Fe and ^{59}Fe were measured simultaneously by a modification of the liquid scintillation method of Eakins and Brown (9). When transferrin ^{55}Fe was employed to label specifically parenchymal ferritin, contamination of Kupffer cell isolates by parenchymal ferritin could be calculated as the ratio of ^{55}Fe specific activity of RE and whole liver ferritin.

Results. The results of five separate studies using heat-damaged RBC tagged with ^{59}Fe to label the ferritin stores of RE cells are shown in Table I. The specific activity of ferritin isolated from whole liver was about one-fifth that of RE cells, indicating the extent to which RE cell ^{59}Fe ferritin had been diluted by unlabeled parenchymal cell ferritin. These studies suggested that a mean of 14.3% of hepatic ferritin (range 9.9–19.5) was contained in RE cells. While this figure may be taken as a maximal estimate of RE ferritin, it did not

TABLE I. Single-Isotope Measurement of the Intrahepatic Distribution of Ferritin Iron.

	Whole liver			Pronase (Kupffer cell) fraction			
Study	^{59}Ferritin (cpm/ml)	Ferritin iron (μg/ml)	Specific activity (cpm/μg)	^{59}Ferritin (cpm/ml)	Ferritin iron (μg/ml)	Specific activity (cpm/μg)	Ratio of specific activities
1	13,040	87.3	150	5,780	7.5	771	0.195
2	8,371	89.0	94	2,470	2.6	949	0.099
3	3,627	84.8	43	1,560	4.8	324	0.133
4	2,920	73.8	40	1,281	5.8	221	0.181
5	1,280	99.0	13	664	5.5	121	0.107
						Mean	0.143

TABLE II. Double-Isotope Measurement of the Intrahepatic Distribution of Ferritin Iron.

Study	A ^{59}Ferritin whole liver/pronase specific activity ratio	B ^{55}Ferritin pronase/whole liver specific activity ratio	Corrected cellular distribution of ferritin	
			RE cell[a] (% of total liver ferritin)	Parenchymal cell (% of total liver ferritin)
1	0.195	0.847	3.0	97.0
2	0.099	0.885	1.1	98.9
3	0.133	0.716	3.7	96.3
Mean	0.143	0.816	2.6	97.4

[a] % RE cell ferritin = A (1 − B) × 100.

exclude the possibility of significant contamination of the Kupffer cell fraction by parenchymal ferritin.

Dual-isotope studies in three sets of animals shown in Table II indicated that RE cell concentrates obtained with pronase were in fact significantly contaminated. Whereas parenchymal cell ferritin tagged with transferrin-bound ^{55}Fe should have been absent from the pronase digests, the specific activity of ^{55}Fe ferritin in the RE cell isolates was only slightly less (0.82) than the specific activity of whole-liver ^{55}Fe ferritin. When corrected for this cross-contamination by parenchymal cell ferritin, the RE cell ferritin was calculated to be only 2.6% of total-liver ferritin.

Discussion. Studies of the cellular distribution of nonheme iron between RE and parenchymal cells of rat liver have recently been reported by Van Wyk *et al.* (10). Their measurements, based on chemical determinations of whole liver and Kupffer cells isolated by pronase, indicated that virtually all (99%) of hepatic ferritin stores was found in parenchymal cells. Quantitation of ferritin iron in Kupffer cells in their studies depended on the accuracy of previous estimates of the number of Kupffer cells in the total liver and on the assumption that no cytoplasmic loss occurred during preparation of cellular suspensions. The data of Van Wyk *et al.* (10) showing an increase in Kupffer cell ferritin after hemoglobin injection was also of concern since hemoglobin iron has been shown to localize in the hepatocyte (4, 11). Because of these methodologic considerations, the present study was undertaken. The use of radioiron labels of parenchymal and RE ferritin obviated the need for any assumption as to the total mass or number of Kupffer cells in the liver. Despite the difference in techniques used, results of the present study were in good agreement with that of Van Wyk *et al.* and indicate that in the rat, hepatic ferritin stores can be equated with parenchymal ferritin stores and that the contribution of RE cells to total hepatic ferritin is negligible.

The present findings seem at variance with the usual histologic appearance of normal liver where stainable iron is located predominantly in Kupffer cells. Because iron stains detect hemosiderin rather than ferritin, the disparity may be explained by a different partition of storage iron between ferritin and hemosiderin in RE cells. In a recent study, splenic ferritin was found in growing female rats to represent only 25% of the 200 mg of nonheme iron in the spleen, whereas hepatic ferritin represented roughly 70% of the 1280 mg of nonheme iron in the liver (12). This suggests that as compared with the hepatocyte, RE cells have a higher proportion of storage iron in the form of hemosiderin. If one assumes that the ratio of ferritin to hemosiderin in the Kupffer cell is the same as in the RE cell of the spleen, the findings in the present study indicate that Kupffer cells contain 23 mg of ferritin iron and 69 mg of hemosiderin iron or a total of 92 (7%) of the 1280 mg of hepatic storage iron. Parenchymal cells of the liver would contain 850 and 340 mg of ferritin and hemosiderin iron, respectively. Such calculations emphasize the difference in behavior between

these two storage areas and the importance of the hepatocyte in total iron storage. The lack of visibility of hepatocyte iron by light microscopy undoubtedly relates to the large volume of hepatocyte tissue and the more dispersed form of its iron.

Summary. A study has been undertaken in rats to determine the distribution of ferritin iron stores between reticuloendothelial and parenchymal cells of the liver. After selective radioiron labeling of the reticuloendothelial cell ferritin compartment with heat-damaged erythrocytes, the ratio of specific activities of ferritin extracted from whole liver and from isolated Kupffer cells provided a measure of the size of ferritin iron stores within each cellular compartment. Kupffer cells were found to account for only 2.6% of total hepatic ferritin, indicating that in the rat, hepatic ferritin can be equated with parenchymal ferritin stores.

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