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Studies on the Rat Liver Following Iron Overload: An Analysis of Iron and Lysosomal Enzymes in Isolated Parenchymal and Non-Parenchymal Cells

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Summary. Iron overload is known to affect the liver. In order to study the effect of iron on various liver cellular and subcellular compartments and the alterations due to mobilization of iron, an experimental model has been developed previously. In this study iron stores in parenchymal and non-parenchymal cells have been investigated during iron loading and unloading. Following completion of the experimental procedures, liver cells were isolated by means of collagenase perfusion (parenchymal cells) and pronase treatment (nonparenchymal cells). It was found that iron overload did not result in significantly increased levels of three lysosomal enzymes, and that the enzyme activities were not altered as iron was mobilized. Iron stores were localized largely in parenchymal cells, and these stores decreased after cessation of iron loading. The iron content was further lowered if the animals were bled. The non-parenchymal cells of the liver initially stored a relatively small part of the administered iron but this increased in the two months following iron loading. On the other hand if the animals were bled there was a pronounced decrease in iron content of these cells as well as in parenchymal cells.

It is concluded that iron overload does not affect lysosomal enzymes and that iron stores in both parenchymal and non-parenchymal cells can be mobilized in response to increased demand.

Key words: Liver – Iron overload – Lysosomal enzymes – Iron mobilization – Kupffer cells

Introduction

The rat liver parenchyma is composed of different kinds of cells – hepatocytes (parenchymal cells) and sinusoidal lining cells (non-parenchymal cells).

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The sinusoidal lining cells are endothelial cells, Küpffer cells and fat-storing cells (Wisse 1970). These cells differ in morphology and function. Most reports dealing with the liver focus on the parenchymal cells. During iron overload following repeated iron injections, iron is stored not only in the parenchymal but also in Kupffer cells (Shoden and Sturgeon 1968; Knook et al. 1977; Hultcrantz et al. 1979; Lake-Bakaar 1980). We have previously studied the mobilization of iron from increased liver iron stores by means of venisection. Ultrastructural and biochemical investigations (Hultcrantz et al. 1980; Hultcrantz and Glaumann 1982) indicated that Küpffer cells, when compared with hepatocytes, mobilize their iron compartment more rapidly in order to meet increased demand.

In this study we have isolated parenchymal and sinusoidal cells from rat liver in order to quantitate possible differences in iron content between different liver cells during iron overloading and unloading.

Materials and Methods

Male Sprague Dawley rats (b.w. 200–220 g) were given 15 intramuscular injections of iron (Jectofer®, Astra, Södertälje, Sweden) in a total dose of 50 mg Fe^{3+}/100 g during a three week period. The animals were divided into three equal groups. One group was sacrificed immediately following Jectofer treatment. The second group were bled at weekly intervals as described earlier (Hultcrantz et al. 1980). The third group received no further treatment before sacrifice. Untreated animals were used as controls.

The liver was perfused *in situ* through the portal vein without recirculation for 5 min with calcium-free Hank's solution in 0.6 mM EGTA. The perfusion fluid was gased with 95% O_2 and 5% CO_2 throughout and sodium bicarbonate was added as a buffer. During this period the liver was removed and placed on a net.

In order to isolate the different liver cells, the liver was then perfused in vitro with a recirculating 0.075% collagenase solution (Boehringer-Mannheim, FRG) in Hank's solution with 4 mM Ca^{2+} for 10–15 min (Arborgh et al. 1973). The capsule was removed and a liver cell suspension was prepared by shaking the liver in cold Hank's solution buffered with HEPES (pH 7.45). The suspension was filtered through a nylon cloth. About 90% of the cells in the resulting cell suspension, designated as the *initial cell suspension* (ICS), were viable as indicated by resistance to trypan blue. Cell counts were carried out in a Bürker chamber.

Parenchymal cells (PC) were isolated from an aliquot of this cell suspension by low grade centrifugation (3 times at 50 × g). About 50% of the parenchymal cells from the initial cell suspension was recovered in the final pellet, and more than 90% of these resisted trypan blue. Another aliquot of the initial cell suspension was used for preparation of *non-parenchymal cells* (NPC). This portion was incubated with 0.25% pronase (Calbiochem) in Hank's solution buffered with HEPES (pH 7.45) (Mills and Zucker-Franklin 1969; Berg and Boman 1973). Separation of non-parenchymal cells was achieved by centrifugation of the digested material at 500 × g for 3.5 min with four washings of the ensuing pellet. About 40% of the pelleted cells were Küpffer cells as identified by light and electron microscopy. 90–95% of the cells resisted trypan blue. 40–50% of the non-parenchymal cells present in the initial cell suspension were recovered in the final pellet.

Acid phosphatase, aryl sulphatase and cathepsin D activities were determined (Bowers et al. 1967). Iron was quantified using an atomic absorption spectrophotometer as described previously (Hultcrantz and Glaumann 1982). Protein was determined according to Lowry et al. (1951).

Some of the pellets prepared after isolation of the parenchymal and non-parenchymal cells were used for electron microscopy. The pellets were fixed in glutaraldehyde and embedded in Epon. Thin sections were stained with lead citrate and examined in a Jeol 100 C electron microscope.

Table 1. Activity of aryl sulphatase in parenchymal and non-parenchymal cell fractions from iron loaded rat liver. Activity expressed as nmoles 4-nitrocatechol released/min/mg protein. Each value represents the mean of 5–7 experiments and standard error of the mean. ICS, initial cell suspension; PC, parenchymal cells; NPC, non-parenchymal cells

Animals	ICS	PC	NPC
Controls	7.81 ± 0.30	6.63 ± 0.32	48.1 ± 3.1
Immediately following Jectofer treatment	7.57 ± 0.36	6.13 ± 0.42	65.7 ± 8.1
Two months, non-bled	6.12 ± 0.91	6.86 ± 0.73	55.1 ± 8.2
Two months, bled	7.83 ± 0.81	5.73 ± 1.2	62.1 ± 3.8

Table 2. Concentration of iron in parenchymal and non-parenchymal cell fractions from iron loaded rat liver. Concentration of iron expressed as μg iron/mg protein. Each value represents the mean of 5–7 experiments and standard error of the mean

Animals	Iron content in different cell fractions μg iron/mg protein		
	ICS	PC	NPC
Control	0.496 ± 0.043	0.546 ± 0.044	0.105 ± 0.035
Immediately following Jectofer treatment	6.52 ± 0.41	7.23 ± 0.37	1.75 ± 0.23
Two months, non-bled	4.32 ± 0.40	4.55 ± 0.30	4.57 ± 0.45
Two months, bled	2.44 ± 0.15	3.60 ± 0.24	0.531 ± 0.085

Results

The protein contents and the activities of three different lysosomal enzymes were measured in the various cell fractions. Only the results for aryl sulphatase are given in Table 1. The values for protein and lysosomal enzymes were not altered in any of the cell fractions from the iron-loaded groups compared with controls. The protein content/10^6 cells was about 1.6 mg for parenchymal cells and about 0.20 mg for non-parenchymal cells. The values for acid phosphatase and cathepsin D are not given in table, but they were not altered in the different groups, and were similar to those reported previously (Arborgh et al. 1973, 1974; Munthe-Kaas et al. 1976).

As has been demonstrated earlier, Jectofer administration increases the iron content of rat liver (Hultcrantz and Glaumann 1982). The concentration of iron in the initial cell suspension from the iron-loaded group (Table 2) was somewhat lower than we have previously reported for liver homogenates from iron-treated animals (Hultcrantz and Glaumann 1982). The decrease in iron in the initial cell suspension following mobilization by bleeding was of the same order of magnitude as shown previously for the corresponding homogenate (Table 2). The loss of iron in the parenchymal cell suspension followed the changes in the initial cell suspension in the bled and non-bled groups. In contrast with parenchymal cells, the iron content

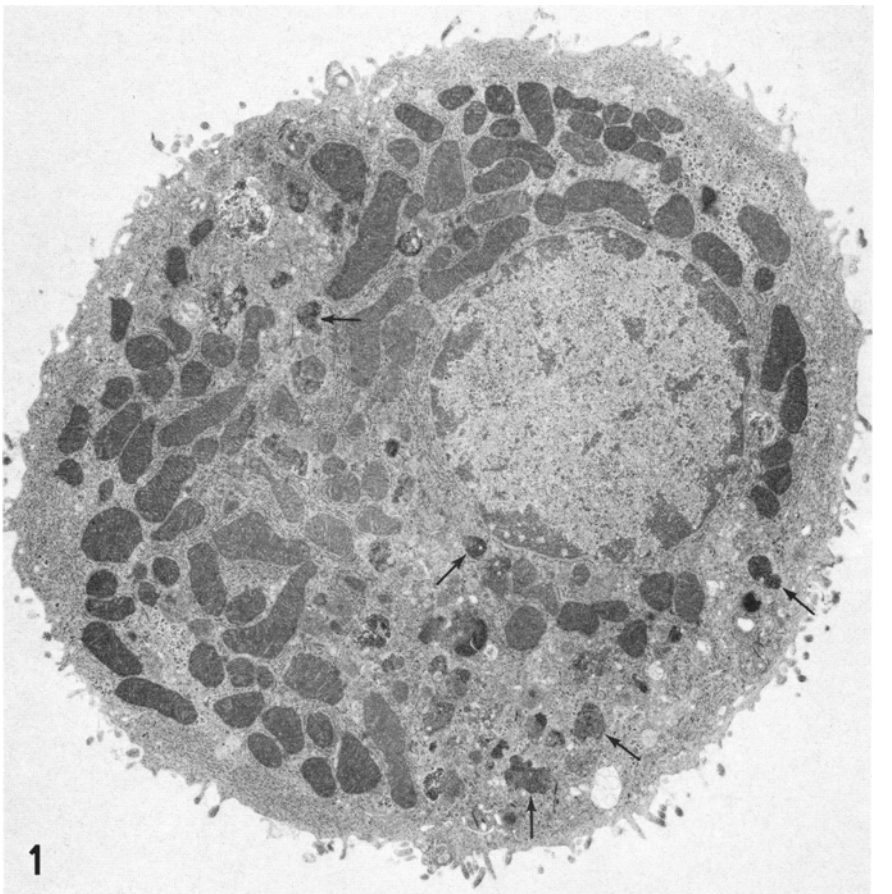
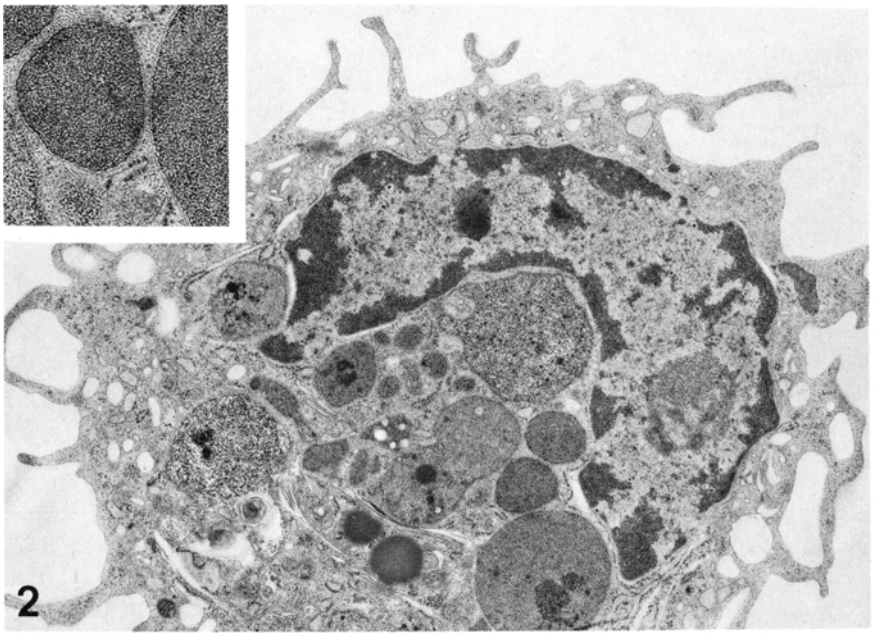


Fig. 1. Electron micrograph showing part of an isolated iron-loaded parenchymal cell. Several iron-laden lysosomes are seen (*arrows*). × 7,500

of non-parenchymal cells increased after cessation of iron administration. However, bleeding also effectively mobilized the iron from non-parenchymal cells.

Electron micrographs of the isolated cells are shown in Figs. 1 to 3. The parenchymal cells of all experimental groups had electron-dense iron-containing particles (IP) in their lysosomes and scattered in their cytoplasm. No clear-cut difference in the IP content could be seen in parenchymal cells from the three experimental groups.

The isolated non-parenchymal cells showed the same features reported previously (Emeis and Planque 1976; Knook et al. 1977). The lysosomal system of the Küpffer cells was filled with IP in the animal sacrificed immediately after completed Jectofer treatment. The non-bled group showed similar appearances (Fig. 2). In contrast, no IP could be identified in Küpffer cells isolated from bled animals or from controls (Fig. 3). Some of the Küpffer cells in all groups contained minor amounts of phagocytosed material. The other cell types in this fraction lacked features of iron overload.



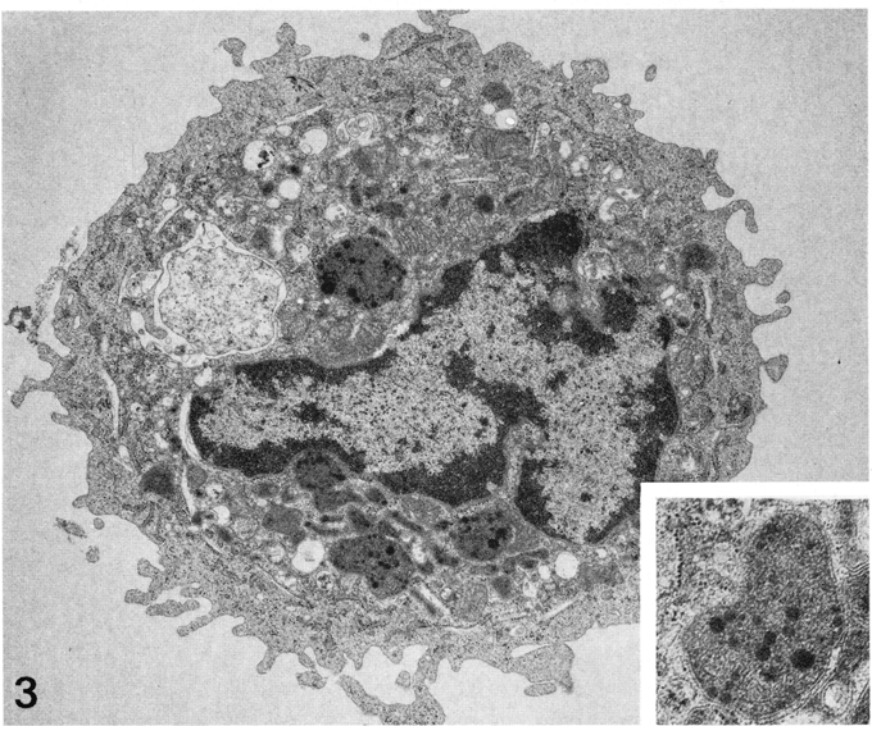


Fig. 2. Electron micrograph showing an isolated Küpffer cell from iron-loaded non-bled rat liver. Many lysosomes filled with iron-containing particles are seen, ×10,000. *Inset:* Iron-loaded lysosome. ×25,000

Fig. 3. Electron micrograph showing an isolated Küpffer cell from a bled animal. No iron-containing particles are noted in the lysosomes. ×9,000. *Inset:* Lysosome without visible ferritin or hemosiderin particles. ×25,000

Discussion

Apparently iron treatment does not induce lysosomal enzyme activities as might be expected, since an increase in the lysosomal volume density has been described for both parenchymal and Küpffer cells (Hultcrantz 1982). This fits with our results from homogenates in which enzyme activities also human unaltered (Hultcrantz and Glaumann 1982). The changes in iron concentrations in the non-parenchymal cells isolated from the different experimental groups resembled the gross pattern we noted in the spleen using the same experimental model (Hultcrantz and Glaumann 1982). Apparently both the iron-storing macrophages in the spleen and their hepatic counterparts the Küpffer cells, respond in the similar way during different stages of iron storage and mobilization.

The volume density and protein content are much higher in parenchymal than non-parenchymal cells (Blouin et al. 1977). The data in Table 2 also show that the non-parenchymal cells and more specifically the Küpffer cells store only a minor part of the total liver iron as compared with parenchymal cells. However, the Küpffer cells only constitute 40% of the cells in our non-parenchymal cell fractions. Since the other cell types probably have a much lower concentration of iron, the actual amount of iron present in Küpffer cells is likely to be higher than the values given. Consequently, the amount of iron mobilized from parenchymal cells in the bled group is greater than the part mobilized from non-parenchymal cells. Following cessation of iron administration the parenchymal cells in the non-bled group loose iron, whereas the iron content of non-parenchymal cells increases. A possible explanation for this is that following increased storage of iron the Küpffer cells may only release a small part, or may do so at reduced rates as shown by Lipschitz et al. 1971. Since at the same time they continue to phagocytose and degrade iron-containing red blood corpuscles and other cell debris, the net result would be an increase in their iron content. Alternatively, ferritin, possibly released from parenchymal cells may be endocytosed by Küpffer cells as happens when ferritin is administered intravenously (Henell, submitted for publication). On the other hand, during induced unloading caused by bleeding the Küpffer cells show a net decrease in iron in response to the increased demand. Both parenchymal cells (Baker et al. 1977) and reticuloendothelial cells (Shoden and Sturgeon 1968; Lipschitz et al. 1971; Beamish et al. 1977; Hultcrantz et al. 1980) have been shown to increase the release of iron when there is an enhanced demand for iron outside the cells, but the mechanisms involved are unknown. It is also unclear whether the two systems are functionally interrelated or whether they work as two separate iron storing compartments. Since the reticuloendothelial cells are known to be the main source of iron for maturing reticulocytes in the bone marrow a redistribution of iron from liver parenchymal cells to reticuloendothelial cells could occur to facilitate the further transport of iron to the bone marrow.

The ultrastructural findings in iron-loaded Küpffer cells are similar to

those of Knook et al. (1977). These authors examined isolated non-parenchymal cells from iron-loaded rat liver, and like us found signs of iron loading only in the Kupffer cells. These findings also favour the assumption outlined above that alterations in iron content in the non-parenchymal cell fraction is due mainly to alterations in the iron content of Küpffer cells.

Conclusions

Jectofer treatment does not alter the lysosomal enzyme activities in any liver cell type. Following iron overload iron is localized mainly in the parenchymal cells. Following cessation of iron injections iron is mobilized from the parenchymal cells and accumulates in continuously non-parenchymal cells. During iron demand induced by bleeding, iron is depleted from both parenchymal cells and Küpffer cells.

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