THE RELATIONSHIP BETWEEN FERRITIN AND HEMOSIDERIN IN RABBITS AND MAN*

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Iron is stored in tissues as ferritin and hemosiderin. These compounds are increased in a state of iron excess and become depleted with iron deficiency (2). Both may be considered to represent iron reserves which may be mobilized when needed.

The functional relationship between these two compounds has been a matter of speculation. It has been suggested that the ferritin fraction represents more "labile" iron reserves; it has been suggested also that ferritin and hemosiderin are different physical forms of the same iron-protein complex (3). The present studies are an attempt to clarify the relationship of these compounds in animals and man.

Methods

New Zealand white rabbits, weighing 2 to 4 kilos, were given various iron preparations. They were sacrificed by air embolism, and their livers were perfused through the portal vein with 0.9 per cent NaCl. Other tissues were not perfused. The analysis for ferritin and hemosiderin iron was performed immediately or occasionally after 24 hours storage in the frozen state.

Human tissues were obtained at autopsy and were kept frozen until fractionated.

The quantitative fractionation method employed to separate ferritin, hemosiderin, and hemoglobin in 10 gm. tissue aliquots and the preparation of crystalline ferritin and purified hemosiderin granules labeled with Fe$^{59}$ have been described in detail (4).

Radioactive iron in the tissue was processed by wet ashing, precipitation, and electroplating, and the two isotopes were differentially counted according to the method of Peacock et al. (5). The specific activity of the Fe$^{55}$ and Fe$^{59}$ was approximately 0.5 to 1 mc. per mg. of iron. In experiments

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in which both tracers were employed, the cross-counting was less than 5 per cent.

Saccharated oxide of radioiron was prepared by the method of Nissim and Robson (6).

Results

Quantitative Relationship between Ferritin and Hemosiderin Iron of Tissues

The content of ferritin and hemosiderin iron was determined in rabbits injected intravenously with 50 to 2000 mg. of iron as saccharated iron oxide. One group of animals was sacrificed within 1 month of the time of injection (Fig. 1, a). At lower levels, iron was present predominantly as ferritin, but with increasing iron the ratio was reversed and hemosiderin iron predominated. The second group of rabbits was sacrificed after 1 to 2 years (Fig. 1, b). While the total liver iron was higher in this group, a maximal level of about 200 mg. of ferritin iron per 100 gm. of tissue was found. This would indicate that, when adequate time is allowed for cellular adjustment, all storage iron beyond a certain amount is present as hemosiderin.

Comparable relationships between hemosiderin and ferritin were found in the splenic tissue of these two groups of rabbits (Fig. 2). The concen-

1 We are indebted to Smith, Kline, and French for the ferrojectin (saccharated iron oxide) used in these experiments.
tration of iron was greater in this tissue than in liver. This was anticipated, since the saccharated iron oxide administered is taken up by the reticulo-endothelial tissue and converted to storage iron (2). The amount of splenic iron diminished with time, and the liver analyses illustrated in Fig. 1, a and b indicate that this was attributable to a rerouting of iron to the liver.

Fig. 2. Fractionation of iron of rabbit spleen depicting early (a) and late (b) distribution of intravenously injected iron as saccharated iron oxide. The animals in group (a) were sacrificed 1 month after iron administration. Those in group (b) were sacrificed after 1 to 2 years. Each set of points (ferritin and hemosiderin) represents the fractionation of one rabbit liver.

Fig. 3. Fractionation of liver and splenic iron in man. Each set of points (ferritin and hemosiderin) represents the fractionation of one tissue. The squares refer to subjects in which storage fractions of both liver and spleen are graphed.

In human tissues at normal levels of iron content ferritin-hemosiderin ratios were variable, with a slight preponderance of ferritin iron. As the total iron increased, however, a progressively higher percentage of hemosiderin iron was found (Fig. 3).

Iron Exchange between Plasma and Iron Storage Compounds

Radioiron was injected intravenously into rabbits as Fe59Cl3 in tracer amounts or as saccharated Fe59 oxide, and its distribution in the ferritin and hemosiderin fractions of the livers was determined. 10 to 25 per cent
of the inorganic radioiron, injected in tracer amounts (1 to 10 \( \gamma \) of iron) well within the binding capacity of the serum, was localized in the liver.

**Table I**

*Distribution of Liver Storage Iron Following Iron Administration*

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Form of iron administered*</th>
<th>Conditions of experiment†</th>
<th>Total iron per 100 gm. liver</th>
<th>Per cent iron as</th>
<th>Per cent radioactive iron as</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mg.</td>
<td>Ferritin</td>
<td>Hemosiderin</td>
</tr>
<tr>
<td>1</td>
<td>Fe*59Cl₂</td>
<td>Killed 24 hrs. after injection</td>
<td>8.5</td>
<td>77.5</td>
<td>22.5</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>&quot;</td>
<td>10.0</td>
<td>82.2</td>
<td>17.8</td>
</tr>
<tr>
<td>3</td>
<td>Fe*59Cl₂</td>
<td>Fe<em>59, 2 hrs. later 50 mg. Fe</em>55; killed after 24 hrs.</td>
<td>30.9</td>
<td>68.8</td>
<td>31.2</td>
</tr>
<tr>
<td>4</td>
<td>Fe*59Cl₂</td>
<td>Saccharated Fe*55</td>
<td>27.8</td>
<td>72.9</td>
<td>27.1</td>
</tr>
<tr>
<td>5</td>
<td>Fe*59Cl₂</td>
<td>Saccharated Fe*55</td>
<td>44.4</td>
<td>65.3</td>
<td>34.7</td>
</tr>
<tr>
<td>6</td>
<td>Saccharated Fe*55</td>
<td>200 mg. Fe<em>55 followed by Fe</em>59; killed after 10 days</td>
<td>90.0</td>
<td>66.5</td>
<td>33.5</td>
</tr>
<tr>
<td>7</td>
<td>Saccharated Fe*55</td>
<td>200 mg. Fe*55; killed after 11 days</td>
<td>72.9</td>
<td>62.0</td>
<td>38.0</td>
</tr>
<tr>
<td>8</td>
<td>Fe*59Cl₂</td>
<td>Killed after 24 hrs.</td>
<td>8.5</td>
<td>70.7</td>
<td>29.3</td>
</tr>
</tbody>
</table>

* Intravenously except for Rabbit 8, which received the iron intragastrically.
† When the amount of iron injected is not specified, it represents a tracer dose.

A slightly greater amount of the tagged iron was found in the ferritin fraction than would be anticipated from the distribution of non-radioactive ferritin and hemosiderin (Rabbits 1 and 2, Table I). When the tracer dose was followed by a large dose of saccharated iron, there was no shift of activity from ferritin to hemosiderin in 24 hours (Fe*59 data of Rabbit 3) and only a slight shift after 2 weeks (Fe*59 data of Rabbits 4 and 5). How-
ever, when Fe$^{55}$Cl$_3$ was injected after iron loading, a somewhat greater amount was found in the hemosiderin fraction (Rabbit 6). When saccharated Fe$^{55}$ oxide was injected in a large dosage of total iron, its distribution was more similar to that of the total iron of the tissue (Rabbits 3 to 7). In one animal iron administered orally was found to be deposited in a ratio similar to the ferritin-hemosiderin iron ratio in the tissue (Rabbit 8).

**Table II**  
**Distribution of Ferritin and Hemosiderin in Rabbit Liver after Intravenous Administration of These Compounds**  
Ferritin (4 mg. of iron per animal; 200,000 c.p.m. of Fe$^{59}$) and hemosiderin (10 mg. of iron per animal; 33,000 c.p.m. of Fe$^{69}$) were injected intravenously. The livers were fractionated for ferritin and hemosiderin iron. Each value represents an average of replicate determinations on duplicate 10 gm. samples of liver.

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Form of iron administered</th>
<th>Time of sacrifice after administration</th>
<th>Total iron per 100 gm. liver</th>
<th>Per cent iron as</th>
<th>Per cent Fe$^{59}$ as</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>min.</td>
<td>mg.</td>
<td>Ferritin</td>
<td>Hemosiderin</td>
</tr>
<tr>
<td>F1</td>
<td>Ferritin</td>
<td>30</td>
<td>9.5</td>
<td>79</td>
<td>21</td>
</tr>
<tr>
<td>F2</td>
<td>&quot;</td>
<td>1</td>
<td>7.5</td>
<td>56</td>
<td>44</td>
</tr>
<tr>
<td>F3</td>
<td>&quot;</td>
<td>2</td>
<td>14.2</td>
<td>83</td>
<td>17</td>
</tr>
<tr>
<td>F4</td>
<td>&quot;</td>
<td>6</td>
<td>26.3</td>
<td>57</td>
<td>43</td>
</tr>
<tr>
<td>F5</td>
<td>&quot;</td>
<td>24</td>
<td>18.2</td>
<td>58</td>
<td>42</td>
</tr>
<tr>
<td>H1</td>
<td>Hemosiderin</td>
<td>6</td>
<td>17.6</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>H2</td>
<td>&quot;</td>
<td>24</td>
<td>30.8</td>
<td>66</td>
<td>34</td>
</tr>
</tbody>
</table>

**Interrelationship of Ferritin and Hemosiderin Administered Intravenously**  
Fe$^{55}$-labeled ferritin and hemosiderin were injected intravenously into rabbits which were sacrificed from 30 minutes to 24 hours after injection. The tagged compounds rapidly disappeared from the blood stream. The data reported in Table II show that, whether administered as ferritin or hemosiderin, the radioactivity was found distributed in the liver between the two fractions proportional to the over-all distribution of these compounds.

**Mobilization of Iron from Ferritin and Hemosiderin Storage Fractions**  
In order to determine whether either one of these iron storage compounds was mobilized preferentially, two groups of rabbits were injected with 250 mg. of Fe$^{55}$ as the saccharated iron oxide and a tracer amount of iron as Fe$^{58}$Cl$_3$. The rabbits in Group I served as controls and were not bled. The rabbits of Group II were bled to 500 ml. over a period of 3 weeks,
at which time both groups were sacrificed. These bleedings represented the removal of as much as 180 mg. of iron and resulted in an appreciable reduction in total liver iron compared with that of the control animals. This did not disturb the ferritin-hemosiderin iron ratio or the distribution of radioiron given as either the saccharated oxide or the chloride (Table III).

**Table III**

_Mobilization of Iron from Ferritin and Hemosiderin of Rabbit Liver_

All rabbits (Groups I and II) were injected intravenously with saccharated oxide of Fe\(^{55}\) (250 mg. of iron) followed by a tracer amount of iron as Fe\(^{54}\)Cl\(_2\). The animals in Group II were bled to 500 ml. over 3 weeks, at which time both groups were sacrificed. The livers were fractionated for ferritin and hemosiderin iron. Each value represents an average of replicate determinations on duplicate 10 gm. samples of liver.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Rabbit No.</th>
<th>Total iron per 100 gm. liver</th>
<th>Per cent iron as</th>
<th>Per cent Fe(^{55}) as</th>
<th>Per cent Fe(^{54}) as</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg.</td>
<td>Ferritin</td>
<td>Hemosiderin</td>
<td>Ferritin</td>
</tr>
<tr>
<td>I. Controls</td>
<td>C1</td>
<td>97</td>
<td>59</td>
<td>41</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>130</td>
<td>54</td>
<td>46</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>C3</td>
<td>136</td>
<td>43</td>
<td>57</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>C4</td>
<td>132</td>
<td>48</td>
<td>52</td>
<td>73</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>124</td>
<td>51</td>
<td>49</td>
<td>78</td>
</tr>
<tr>
<td>II. Bled</td>
<td>B1</td>
<td>48</td>
<td>52</td>
<td>48</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>48</td>
<td>55</td>
<td>35</td>
<td>86</td>
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<td></td>
<td>B3</td>
<td>96</td>
<td>48</td>
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<td>80</td>
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<tr>
<td></td>
<td>B4</td>
<td>63</td>
<td>40</td>
<td>60</td>
<td>73</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>64</td>
<td>51</td>
<td>49</td>
<td>79</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The relationships between ferritin and hemosiderin in iron deposition and iron mobilization have been described. From the studies reported in Tables I to III, it is obvious that hemosiderin and ferritin are intimately related, and a number of pathways in storage and mobilization of iron within the cell appear possible (Fig. 4). Inorganic iron in tracer amounts, whether given alone or followed by a loading dose of saccharated iron, is found in somewhat greater amount in the ferritin fraction than would be expected from the distribution of non-labeled compounds in the tissue (Pathway 1, Fig. 4, A). This is also true of ingested iron.

Loading doses of saccharated iron, in the early stages of iron deposition
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(24 hours), show relatively larger amounts in the hemosiderin fraction, while the original tracer dose of iron has not altered its initial distribution. This would imply that the loading iron either is directly adsorbed on to hemosiderin (Pathway 2, Fig. 4, A) or is incorporated into ferritin which undergoes aggregate formation without admixture with ferritin already present (Pathway 3). Long term studies in iron loading (Figs. 1 and 2) indicate that a maximal level of ferritin is attained beyond which further iron storage is reflected quantitatively as hemosiderin. This may result from a conversion of ferritin to hemosiderin (Pathway 4, Fig. 4, A) and possibly from a direct iron deposition as hemosiderin (Pathway 2). Intracellular exchange between ferritin and hemosiderin iron is suggested also by short term loading experiments (14 and 18 days) in which colloidal iron as the saccharated oxide was administered. A redistribution, either through a disaggregation of tagged iron from hemosiderin to ferritin or a transfer of adsorbed iron from hemosiderin to ferritin (Pathway 5), is implied. In addition, tagged iron, injected as ferritin and hemosiderin, is localized in the tissues in proportion to their ferritin-hemosiderin content. The strong binding of iron by these compounds would seem to preclude any appreciable exchange of radioiron in the serum. Thus, the injected hemosiderin or ferritin, after its uptake by the cell, must be altered to assume the physical characteristics of both (Pathways 4 and 5) or the iron of these compounds must be split off and redistributed within the cell.

Whereas a number of exchanges between ferritin and hemosiderin iron have been demonstrated, the observations are consistent with the concept that hemosiderin represents an aggregate of ferritin molecules and that the tendency for aggregate formation is increased with increased cell iron.

![Diagram](http://www.jbc.org/) Fig. 4. Possible relationships between ferritin and hemosiderin in iron deposition and iron mobilization. The physiological significance of any specific pathway of exchange of iron is not implied.
Studies of the mobilization of iron, although they do not permit speculation concerning the intracellular exchange between the iron storage compounds, indicate that iron is mobilized from both ferritin and hemosiderin (Fig. 4, B). Only when hemosiderin is present in excessive amounts, forming aggregates so large as to interfere with cell function, is the mobilization of iron impaired (2).

The authors wish to acknowledge the valuable technical assistance of Mr. Wilbur Linde and Mr. Robert McKay.

SUMMARY

Human and rabbit tissues of widely varying iron content were fractionated quantitatively to determine the pattern of distribution of ferritin and hemosiderin iron.

At physiological levels of tissue iron a slight preponderance of ferritin over hemosiderin iron was found. With increasing concentrations of iron, hemosiderin stores predominated. At high levels, additional storage iron was reflected by a quantitative increase in hemosiderin.

Radioiron, administered orally or parenterally in tracer amounts, was stored as hemosiderin as well as ferritin, and, when mobilization of iron occurs, it has been shown to be derived from both ferritin and hemosiderin fractions of the tissue. Pathways of iron exchange between cell and plasma and the intracellular arrangement have been discussed.

The results indicate that these two types of storage iron are intimately associated and are functionally indistinguishable. It appears likely that these compounds differ only in physical form.

BIBLIOGRAPHY

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