IRON METABOLISM

THE PATHOPHYSIOLOGY OF IRON STORAGE

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IRON occupies a unique position in mammalian metabolism through its participation in intracellular respiratory processes. The literature over the past fifty years is well documented with observations on iron metabolism in animals and man. However, storage iron, because of difficulties in measurement and some question as to what it actually constitutes, has been the subject of but little discussion. The clinician becomes aware of the state of iron stores only when considering anemia due to iron deficiency or hemochromatosis which is an end stage of iron excess. Important to the proper regulation of iron metabolism between these two extremes is the maintenance of an effective but limited iron reserve. In the present article is presented a series of observations as well as a review of pertinent literature on the nature and behavior of this compartment of body iron.

Storage iron may be defined as the iron which can be mobilized from various body tissues for the formation of hemoglobin when needed. Such iron is stored intracellularly in a protein complex as ferritin and hemosiderin. The limited chemical data show a similarity between these two compounds. Hemosiderin may represent a condensation or clustering of ferritin molecules, although this is as yet an open question. The histologic appearance of hemosiderin is distinctive. In unstained preparations it is seen as golden granules and may be stained with appropriate compounds such as potassium ferrocyanide. Although ferritin is not visible as particulate material, it is possible that large amounts of ferritin may give a bluish cast to a cell when stained for iron. The interrelation of these very similar compounds is not clear, but at the present time there is no reason for their functional differentiation from the standpoint of iron storage. Each increases in the tissues in

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the presence of excess iron and each becomes depleted from the tissues when there is need for iron in erythropoiesis. It has been presumed that ferritin iron may be more readily available than hemosiderin iron, but evidence for this is incomplete.* A much smaller labile iron reserve has also been referred to on the basis of radioactive studies. Again, in the depletion of iron from tissues there would appear to be a rapid, continuous withdrawal of iron from these various sources. It seems justifiable to regard the iron reserves in toto as a single functioning physiologic unit rather than to attach any specific importance to their possible subdivisions.

Iron metabolism has been referred to as a closed system because of the minute amounts of iron excreted daily. In a normal man with about 5,000 milligrams of body iron, there is probably less than one milligram of iron assimilated from the diet per day and a smaller amount lost in urine and stool. Excretion appears to be a passive function, since excessive body iron will not appreciably increase it. According to a recent publication, several milligrams of iron may be lost daily through sweat. This observation is at variance with other data on iron balance in man, and has been refuted by studies employing radioactive iron where the possibility of external contamination was excluded. The turnover of iron between the individual and his environment, a fraction of a milligram per day, is slight compared with the internal turnover within the hemoglobin cycle of 25 milligrams of iron per day. There are other tissue iron complexes such as cytochrome and myoglobin. These appear to remain relatively constant despite large variations in body iron and represent but small fractions of body iron. It is felt that they are not of importance in a consideration of storage iron.

Since it appears difficult for even severely iron deficient individuals to absorb more than 5 milligrams of iron a day from a normal diet, the necessity for this body conservation of iron is apparent. Iron stores appear to accrue over a long period of time and, when depleted, are replaced slowly. The resiliency of the organism in meeting emergencies involving iron metabolism will depend to a large extent on an ability to maintain suitable iron stores.

There are various ways of determining iron reserves. They may be measured by a forced depletion of body iron through phlebotomy, by chemical analysis of tissues, by histologic examination of tissues, by studying the tissue localization of radioactive iron, and finally, by radioautographs which are of particular assistance in determining individual cell localization of iron. Observations on iron depletion by phlebotomy will be reported elsewhere. In the present study, these other methods have been employed with the hope of more clearly integrating our knowledge of the pathophysiology of iron storage, particularly in conditions of iron excess.

Iron is widely distributed in the mammalian body and the ability to store iron is shared by many tissues. Two important localities of iron storage for the purpose of the following discussion should be recognized: reticulo-endothelial and parenchymal storage. The former includes the fixed phagocytic tissue of the reticulo-endothelial

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* As discussed in section VII, very large aggregates of hemosiderin may be more slowly mobilized, especially when they are found extracellularly or present in such excessive amounts within the cell as to interfere with cell function.
system and wandering tissue macrophages. All of these cells appear to be equally competent to store iron, although the particular location of one group of cells in an area of blood breakdown may allow greater iron uptake. The term parenchymal is used to indicate the polygonal cells of the liver, glandular tissue of the pancreas and adrenals, and other secretory cells throughout the body. As will be evident subsequently, the liver far exceeds other parenchymal tissue in its capacity for iron storage.

Since much important work has been done on dogs in the past, and experimental data are to be presented on them, it is helpful that there is considerable similarity between the internal iron metabolism of dog and man. In both dog and man, normal iron reserves have been estimated at about 20 per cent of body iron.\(^7\)\(^8\) Erythrocyte iron turnover in both species proceeds at a rate of approximately 1 per cent a day.\(^1\)\(^1\) Radioactive turnover studies have shown similar rates of utilization of radioiron for hemoglobin production.\(^1\)\(^5\) On the basis of such similarities, we have felt justified in using data obtained in these animals to formulate a clinical concept of iron metabolism.

In the course of our studies many different tissues have been analyzed for iron by chemical, radioactive and histologic methods. Three organs have been selected for particular discussion since they characterize different types of iron storage. The liver was chosen because of the particular ability of its parenchyma to store iron and because of the presence of an extensive reticulo-endothelial network, the spleen because it represents reticulo-endothelial tissue with special implications related to blood cell breakdown, and the kidney because of its ability to take up hemoglobin iron. In sections I to V, factors determining initial distribution of iron will be discussed. In section VI and VII, the condition of iron excess is dealt with, particularly as it is related to hemochromatosis.

**Materials and Methods**

A variety of animal species and experimental technics have been employed to evaluate the functional state of storage iron under various conditions. Dogs were employed to determine the localization of storage iron and its availability for hemoglobin production. Increases in total carcass iron were measured in mice. Rats were most suitable for studies of tissue localization of radioactive hemoglobin and tagged erythrocytes. Radioautographs to determine tissue localization of injected iron also utilized rat tissues. The organs of patients with transfusion or idiopathic hemochromatosis were analyzed for their iron content.

The dogs used in these experiments were adult mongrels in good condition, dewormed and immunized against distemper. At the beginning of the experimental period their erythrocyte values were normal and the experimental procedures described below did not in any instance produce appreciable changes. Four groups of dogs are listed in table 1. Dogs in Group 1 received no treatment and were considered normal. Group 1A represented a similar group of animals in which large amounts of iron-ascorbate gelatin\(^*\) were injected intravenously.\(^1\)\(^7\) These injections in amounts of 30 to 100 milligrams of iron per day were well tolerated with some flushing and nausea. The dog in Group 1B was given injections of red cells. The blood was kept in citrate for ten days and then transfused. This blood was rapidly destroyed, occasionally producing hemoglobinuria. The dogs in Groups 1 and 2 were kept on kennel rations of 100 to 400 grams of Kibbles per day and about one half pound of meat per week. They maintained their weight throughout the experimental period. In Group 3 there were 3 dogs given a diet of Purina Dog Chow plus 1 per cent ferric citrate. The dogs in Group 4 were placed on a diet of corn grits and 2 per

\(^*\) The iron-ascorbate gelatin was kindly supplied by The Knox Gelatin Corporation.
### Table 1. Chemical and Histologic Studies of Iron Distribution

<table>
<thead>
<tr>
<th>DOG</th>
<th>Conc. Fe mg./100 Gm.</th>
<th>Mg. Fe/organ</th>
<th>Histologic Fe</th>
<th>Expil Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Spleen</td>
<td>Kidney</td>
<td>Liver</td>
</tr>
<tr>
<td>1</td>
<td>26</td>
<td>16</td>
<td>2.5</td>
<td>86</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>13</td>
<td>3.0</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>74</td>
<td>3.3</td>
<td>130</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>26</td>
<td>4.2</td>
<td>136</td>
</tr>
<tr>
<td>5</td>
<td>22</td>
<td>50</td>
<td>5.6</td>
<td>98</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>30</td>
<td>1.9</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>19</td>
<td>35</td>
<td>3.4</td>
</tr>
</tbody>
</table>

### Group 1A—Parenteral Fe Administration

<table>
<thead>
<tr>
<th></th>
<th>Conc. Fe mg./100 Gm.</th>
<th>Mg. Fe/organ</th>
<th>Histologic Fe</th>
<th>Expil Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>160</td>
<td>180</td>
<td>19</td>
<td>810</td>
</tr>
<tr>
<td>8</td>
<td>363</td>
<td>25</td>
<td>19</td>
<td>2500</td>
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<td>9</td>
<td>700</td>
<td>412</td>
<td>19</td>
<td>168</td>
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<td>650</td>
<td>236</td>
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<td>4000</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>468</td>
<td>269</td>
<td>33</td>
</tr>
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</table>

### Group 1B—Parenteral Red Cell Administration

<table>
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<tr>
<th></th>
<th>Conc. Fe mg./100 Gm.</th>
<th>Mg. Fe/organ</th>
<th>Histologic Fe</th>
<th>Expil Conditions</th>
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</thead>
<tbody>
<tr>
<td>11</td>
<td>122</td>
<td>383</td>
<td>19.6</td>
<td>650</td>
</tr>
</tbody>
</table>

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### Group 3—Purina plus 2% Fe citrate Diet

<table>
<thead>
<tr>
<th></th>
<th>115</th>
<th>210</th>
<th>6.6</th>
<th>101</th>
<th>7.8</th>
<th>5.9</th>
<th>0</th>
<th>+++</th>
<th>++++</th>
<th>Purina + 2% Fe cit., 69 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>21</td>
<td>20</td>
<td>6.0</td>
<td>118</td>
<td>9.3</td>
<td>4.0</td>
<td>++</td>
<td>+++</td>
<td>++++</td>
<td>Purina + 2% Fe cit., 81 days</td>
</tr>
<tr>
<td>13</td>
<td>27</td>
<td>36</td>
<td>6.0</td>
<td>215</td>
<td>18.0</td>
<td></td>
<td>++</td>
<td>+++</td>
<td>++++</td>
<td>Purina + 2% Fe cit., 139 days</td>
</tr>
<tr>
<td>14</td>
<td>66</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

Average: 38, 33, 6.3, 144, 11.7, 4.9

### Group 4—Corn grit plus 2% Fe citrate Diet

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<tr>
<th></th>
<th>115</th>
<th>250</th>
<th>5.6</th>
<th>1040</th>
<th>72</th>
<th>4.1</th>
<th>+++</th>
<th>++++</th>
<th>+++++</th>
<th>Diet of corn grit + 2% Fe citrate for 60 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>310</td>
<td>300</td>
<td>20.0</td>
<td>1260</td>
<td>120</td>
<td>5.4</td>
<td>++++</td>
<td>+++++</td>
<td>+++++</td>
<td>Diet of corn grit + 2% Fe citrate for 87 days</td>
</tr>
<tr>
<td>16</td>
<td>680</td>
<td>365</td>
<td>47.0</td>
<td>1560</td>
<td>62</td>
<td>10.0</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>Diet of corn grit + 2% Fe citrate for 137 days</td>
</tr>
<tr>
<td>17</td>
<td>560</td>
<td>475</td>
<td>25.0</td>
<td>1670</td>
<td>66</td>
<td>13.0</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>Diet of corn grit + 2% Fe citrate for 139 days</td>
</tr>
<tr>
<td>18</td>
<td>710</td>
<td>320</td>
<td>37.0</td>
<td>1730</td>
<td>61</td>
<td>11.0</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>Diet of corn grit + 2% Fe citrate for 175 days</td>
</tr>
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Average: 479, 340, 23.1, 1652, 76, 12.7
Table 1.—Radioactive Studies of Iron Distribution

<table>
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<tr>
<th>Dog</th>
<th>Total Activity counts/min.</th>
<th>Counts/100 Gm.</th>
<th>Counts/organ</th>
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<tr>
<td></td>
<td></td>
<td>Liver</td>
<td>Spleen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood</td>
<td>Liver</td>
</tr>
<tr>
<td>5</td>
<td>3,200,000 (Fe55)</td>
<td>607,000</td>
<td>14,300</td>
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<tr>
<td></td>
<td></td>
<td>4,700,000</td>
<td>500,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>526,000</td>
<td>1,540</td>
</tr>
<tr>
<td>6</td>
<td>1,000,000 (Fe59)</td>
<td>38,000</td>
<td>70,000</td>
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<tr>
<td></td>
<td></td>
<td>9,730</td>
<td>9,300</td>
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<tr>
<td></td>
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<td>85,000</td>
<td>2,700</td>
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<tr>
<td>12</td>
<td>850,000</td>
<td>36,000</td>
<td>51,000</td>
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<tr>
<td></td>
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<td>130,000</td>
<td>12,100</td>
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<tr>
<td>16</td>
<td>850,000</td>
<td>5,700</td>
<td>3,000</td>
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<td></td>
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<td>18,800</td>
<td>1,240</td>
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<tr>
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<td>4,500,000</td>
<td>4,750</td>
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<tr>
<td>9</td>
<td>270,000</td>
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<td>17,000</td>
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<tr>
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<td>175,000</td>
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<td></td>
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<td>17,200</td>
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% Activity

<table>
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<tr>
<th>Dog</th>
<th>Blood</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidney</th>
</tr>
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<tbody>
<tr>
<td>5</td>
<td>15</td>
<td>86</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>55</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
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<td>1.5</td>
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<td>16</td>
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<tr>
<td>1</td>
<td>2.5</td>
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<td>0.4</td>
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<tr>
<td>9</td>
<td>10</td>
<td>3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>17</td>
<td>10</td>
<td>3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>18</td>
<td>10</td>
<td>3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Data on Experiment

- 1 mg. Fe+++ I.V.—perfusion 6 hours later
- 1 mg. Fe+++ I.V.—perfusion 5 hours later
- 1 mg. Fe+++ plus iron binding protein I.V.—perfusion 6 hours later
- 1 mg. Fe+++—perfusion 30 days later
- 2 mg. Fe+++ I.V.—perfusion 30 days later
- 1 mg. Fe+++ I.V.—perfused 21 days later. On purina and Fe diet
- 1 mg. Fe+++ I.V.—perfused 2 days later. On corn grit and Fe diet
- 10 mg. Fe+++ by stomach tube—perfused in 4 hours
- 10 mg. Fe+++ by stomach tube—perfused in 4 hours
- Radioactivity given as tagged red cells. Dog had enlarged Fe stores through Fe injection.
- Radioactivity given as tagged cells. Animal on corn grits and Fe diet
- Radioactivity given as tagged cells. Animal on corn grits and Fe diet
- 1 mg. Fe+++ I.V. followed by injection of nonradioactive Fe. Killed 15 mos. later.
- 1 mg. Fe+++ I.V. in the same animal 20 days before perfusion.
- 1 mg. Fe+++ I.V. followed by injection of nonradioactive Fe and perfused 15 mos. later.
- 1 mg. Fe+++ I.V. in same animal 20 days before perfusion.
Materials were supplied by the Ansco Corporation.

1 cent ferric citrate. This diet was known to result in the excessive absorption of iron.\(^8\) Radioactive iron as ferric ammonium citrate was given to selected animals (table 2) by mouth or intravenously to obtain information regarding the distribution and movement of one fraction of iron in a variety of different states of iron metabolism.

At the termination of the experimental period, the animals were viviperfused as described by Whipple\(^9\) to render the tissues free of hemoglobin. Ten-gram samples of various body tissues were wet ashed and, in the case of the more important organs of iron storage, several samples were taken. Iron analyses were done on the aliquots of the digest using a buffer solution with thioglycolate as a reducing agent and orthophenanthroline as an indicator. The intensity of the developed color was read on an Evelyn colorimeter. Carrier iron was then added to the radioactive samples to a total of 10 milligrams of iron per sample. The electroplating and counting techniques employed have been previously reported.\(^{18}\) Since it was not possible to determine the total carcass iron and since entire organs in the case of dogs were not digested, we regard the figures reported as only roughly quantitative. Such data, however, would appear sufficient to establish the direction of iron movement and to detect the larger areas of storage.

For measurements of total carcass iron, mice were used (table 3). The diet of corn grits and 2 per cent ferric citrate employed was the same as for dogs of group 4. Weight changes and histologic appearance of tissues were similar to those reported previously in rats.\(^8\) The mice were dry ashed according to the method of Vosburgh et al.,\(^{21}\) slightly modified. Ashed samples were shaken in thiocyanate and the iron thiocyanate complex extracted into isopropyl ether. The iron was determined colorimetrically using orthophenanthroline after reduction of the iron.

### Table 3.

<table>
<thead>
<tr>
<th>Days on Diet</th>
<th>0</th>
<th>4</th>
<th>9</th>
<th>12</th>
<th>17</th>
<th>20</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver iron (mg.)</td>
<td>0.15</td>
<td>0.37</td>
<td>1.01</td>
<td>1.36</td>
<td>2.16</td>
<td>2.86</td>
<td>3.26</td>
</tr>
<tr>
<td>Carcass iron (mg.)</td>
<td>0.75</td>
<td>1.03</td>
<td>1.02</td>
<td>0.94</td>
<td>1.32</td>
<td>1.15</td>
<td>1.18</td>
</tr>
<tr>
<td>Total body iron (mg.)</td>
<td>0.90</td>
<td>1.60</td>
<td>1.03</td>
<td>1.30</td>
<td>3.48</td>
<td>4.01</td>
<td>4.44</td>
</tr>
<tr>
<td>% iron in liver</td>
<td>17</td>
<td>36</td>
<td>50</td>
<td>59</td>
<td>62</td>
<td>71</td>
<td>74</td>
</tr>
</tbody>
</table>

To determine cell localization of iron, radioautographs were prepared using the technic of Leblond\(^{23}\). Two hundred gram normal rats were used as experimental animals. A total of about 1 milligram of Fe\(^{59}\) was given intravenously by repeated injections in the form of ferric ammonium citrate. The rats were sacrificed ten days later and perfused to remove the radioactivity contained in the circulating red cells. Medium lantern slides and Ansco photographic emulsions* were used. Tissue sections contained 2.5 to 10 counts per minute and were developed over a period of 36 to 140 days. Images were formed at an earlier time on the slides coated with the larger grained Ansco emulsion than on those coated with the smaller grained medium lantern slides.

In experiments on the tissue localization of nonviable erythrocytes and hemoglobin, tagged cells were first prepared by the injection of radioiron into animals to be used as donors. After sufficient time had elapsed for most of this iron to be incorporated into hemoglobin, blood was withdrawn. The red cells were kept in citrate for a period of five to ten days. Erythrocytes so preserved are, for the most part, nonviable and are removed almost immediately from the circulation.\(^{23}\) Hemoglobin was prepared by the repeated freezing and thawing of blood and the stroma was centrifuged off as described in more detail elsewhere.\(^{24}\) The recipient animals were perfused two to four hours after the injection of red cells or hemoglobin solution. The localization of radioactivity was determined by processing and counting the perfused organs.

Iron analyses of tissues were correlated with the amount of iron seen histologically. All histologic studies were made on animal tissues which had been collected in neutral formalin and stained for iron as previously described.\(^{18}\) The sternal marrow iron was determined by examining aspirated marrow.\(^{25}\)

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* Materials were supplied by the Ansco Corporation.
An attempt was made to differentiate and measure tissue hemosiderin and ferritin on the basis of solubility. Ferritin is soluble in saline, while hemosiderin can be dissolved only in acid solution. A tissue homogenate was prepared in the Waring blender and repeatedly washed with saline and separated by centrifugation. In the supernatant, hemoglobin was identified by the pyridine-hemochromogen reaction. Mono- or di-molecular iron was reduced by hydroquinone. Ferritin iron was not reduced by hydroquinone, but was reduced by Na$_2$S$_2$O$_3$. Hemoglobin iron was not affected by either of these agents. After reduction the iron was determined quantitatively with orthophenanthroline as an indicator. The hemosiderin iron was extracted from the precipitate with 10 per cent HCl and determined in the usual way. Using this method, duplicate analyses were accurate to approximately plus or minus 5 per cent, while recovery experiments gave yields of 70-80 per cent. Details of this method will be published later.

EXPERIMENTAL OBSERVATIONS AND DISCUSSION

I. Normal Iron Storage

In dogs, a value for normal iron storage may be derived by the comparison of the perfused carcass of a normal animal with an animal rendered iron deficient, since in a phlebotomized animal all available iron will have been extracted from the tissues in an attempt to overcome the induced anemia. Such studies would indicate that storage constitutes approximately 20 per cent of total body iron. The iron content of the liver, spleen and kidney in 6 supposedly normal dogs is recorded in table I, Group I for comparison with subsequent data. Depletion studies by others would indicate that all but about 5 milligrams of the average of 87 milligrams of liver iron would be available for hemoglobin production. Small and variable amounts of hemosiderin were seen histologically in the reticuloendothelial cells of the spleen and to a lesser degree in the bone marrow and Kupffer cells. In some animals, none was visible despite the presence of appreciable iron as determined by chemical analysis. No iron was demonstrated histologically in parenchymal cells although it is likely that in these animals much of the iron was distributed diffusely in these cells as ferritin. Histologic and chemical data indicated somewhat higher concentration of iron in the spleen than liver in these animals. Due to the respective size of these organs, however, total iron storage was about eight times greater in the liver.

To study further the distribution of iron through various periods of time, tracer doses of iron were injected intravenously. The amount of iron given did not exceed the iron binding capacity of the dogs' serum and thus represented a true labelling of the serum iron compartment. Six hours after injection, the concentrations of radioiron in the liver and spleen were approximately equal (table I, Dogs 4 and 5). At the end of twenty-one days the ratio of radioactivity in the liver and spleen still remained about equal per gram of tissue despite the fact that most of the iron had been withdrawn from the tissue and incorporated into circulating red cells (table 2, Dogs 2 and 3). This would indicate a similar availability of iron for hemoglobin production in liver and spleen. It is worthwhile noting from the standpoint of tissue irradiation that between 10 and 35 per cent of the injected radioactivity remained in the liver. This constitutes most of the localized tissue radiation since 60 to 80 per cent of the injected radioactivity is incorporated in the circulating red cell mass.

* The authors appreciate the advice of Dr. S. Granick in relation to these studies.
II. Storage of Iron Absorbed from the Gastro-intestinal Tract

Iron in the lumen of the gastro-intestinal tract may be absorbed and temporarily stored as ferritin\textsuperscript{2} and perhaps hemosiderin\textsuperscript{3} in the epithelial cells of the mucosa. This iron then enters the metabolic pool of body iron by way of the serum iron-binding protein. Balance studies of iron absorption have been difficult in animals and man because of the very small amount of iron absorbed daily in proportion to total dietary iron. The trickle of iron gaining access to the body may best be followed by feeding radioiron. In such experiments (table 2, Animals 1 and 13), equal amounts of radioactivity per gram of tissue appear to be assimilated by spleen and liver. The distribution is similar to that observed after the intravenous injection of radioiron. This is not surprising, since in both instances the iron is distributed by way of the iron binding protein of the serum to the tissues.

The regulation of iron absorption is in some way dependent on the body needs for iron. Absorption is greatly increased when iron deficiency anemia is produced in experimental animals or in man.\textsuperscript{31,32} The absorbed iron in iron deficiency anemia, however, is “earmarked” for erythropoiesis. Once the demand of the bone marrow is met, available evidence would indicate that the accelerated absorption of iron stops and that reserves remain in a depleted state.\textsuperscript{11,12} Fontes and Thivolle\textsuperscript{11} found that supplementary iron given to such depleted dogs for six months to a year was not sufficient to replete the iron stores. Likewise, in animals with normal iron reserves it is difficult to appreciably increase the total body iron by feeding iron. In dogs of Group 3 (table 1), large amounts of dietary iron were given over prolonged periods with only slight increases in tissue iron. Such experiments indicate a rather remarkable ability of the organism under most circumstances to defend itself against the accumulation of iron excess.

It is possible to overcome this so-called mucosal block by altering the composition of the diet. The excessive absorption of iron under such circumstances allows one to study the sequence of events in iron storage.\textsuperscript{18,33} In such animals there is an increase in serum iron with complete saturation of the iron binding protein in several weeks. Liver iron increases to 20 milligrams per hundred grams wet tissue before an increase in hemosiderin is observed microscopically. Thereafter, progressive hemosiderosis is visible in both parenchymal and reticulo-endothelial systems. The extent of the excessive iron storage in dogs is recorded in table 1. The histologic appearance of the liver of Dog 9 is shown in figure 1A. There is a greater localization of iron in the periportal parenchymal portion than in the central position of the lobule. This distribution is probably not due to the source of entry of iron from the gastro-intestinal tract, since a similar distribution is seen after the intravenous injection of iron (fig. 1B). The greater capacity of portal cells to store iron is consistent with their ability to deal with other substances as well, perhaps related to the blood supply of the liver lobule.\textsuperscript{34} Along with iron deposits in the liver parenchyma, the reticulo-endothelial tissue of liver, spleen and macrophages throughout the body show deposits of iron. Eventually other parenchymal tissues show iron deposits, including duodenal mucosa, Brunner’s glands, the outer zone of the adrenal, proximal tubules of the kidney and acinar tissues of the pancreas. It has been pointed out\textsuperscript{18} that in our opinion this distribu-
tion of iron is quite similar to that seen in idiopathic hemochromatosis and in what might be referred to as the dietary hemochromatosis (cytosiderosis) reported by Gillman et al.\textsuperscript{55}

The extent to which the liver acts as the chief storage depot for absorbed iron is shown by total carcass analyses on mice (table 3). These animals were maintained on a diet of corn grits and two per cent ferric citrate for prolonged periods. A comparison of liver iron with total carcass iron demonstrates the superior ability of the liver in dealing with the absorbed iron. In the mouse, 17 per cent of total body iron was initially present in the liver. This doubled after four days on the diet and after twenty-four days the liver had increased to 74 per cent of the total body iron.

It would then appear from tracer studies that the distribution of iron is the same whether absorbed from the gastrointestinal tract or injected in amounts carried by the iron binding protein of the serum. Small amounts of iron gaining access to the body are stored principally in the liver and the same pattern is found when greater amounts of iron are absorbed.

III. Parenteral Iron Administration

Since iron is essentially a "one way substance" with little excretion, its introduction parenterally results in an absolute increase in storage iron proportionate to the amount of iron given minus the amount which is used for blood production.

There are many excellent histological studies of the parenteral administration of iron by different routes.\textsuperscript{3,4,12} The results are influenced by the form in which the

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**Fig. 1A (top left).**—The distribution of excessive iron gaining access to the body through absorption. This dog (\*\textsubscript{2}) had been on a corn grit and iron diet for 139 days. The iron is stained blue and is most prominent in the parenchymal cells in the periportal areas of the liver.

**Fig. 1B (top right).**—The distribution of excessive iron given intravenously. The dog (\*\textsubscript{9}) had received 3920 mg. of ferrous ascorbate-gelatin twenty-one months previously. The bulk of the iron is deposited in loci of reticulo-endothelial cells, although small amounts are visible in the hepatic parenchyma.

**Fig. 1C (center left).**—Hemosiderosis of the sternal marrow following administration of iron-saccharide. This man, previously iron deficient with no sternal marrow hemosiderin, received 1000 mg. of iron saccharide intravenously. At the time of the puncture he had used 600 mg. for new blood formation and 1400 mg. were estimated to be in tissue stores. The iron shows up as fine blue granules against the otherwise unstained sternal marrow.

**Fig. 1D (center right).**—The reticulo-endothelial distribution of iron-saccharide in the liver. The photomicrograph is a liver biopsy from the same patient (\*\textsubscript{C}) stained with prussian blue and basic fuchsin. The reticulo-endothelial cells are outlined in blue due to their iron content. Fine red granules with staining characteristics of hemosiderin are visible in the hepatic parenchymal cells.

**Fig. 1E (bottom left).**—Large hemosiderin aggregates in sternal marrow. The preparation is unstained. This dog had been given 2455 mg. of iron intravenously and after months was phlebotomized 1697 mg. of iron. The iron remaining was in the form of very large masses either extracellular or obscuring normal cell architecture.

**Fig. 1F (bottom right).**—Transfusion hemochromatosis. This specimen is from the liver of an 8 year old child who died following approximately 100 transfusions given over a period of eight years. The diagnosis through this period was aplastic anemia. At necropsy a nodular pigment cirrhosis was found. In the illustration the periportal fibrosis is evident. With appropriate staining iron deposits are seen in the hepatic parenchyma and particularly in the portal areas. (The authors are indebted to Drs. Diamond and Farber, Children's Hospital, Boston.)
iron is administered, the total amount given and the time allowed to lapse before examination.

The injection of iron subcutaneously or intramuscularly is attended by such severe local reactions that it has been impossible to give any appreciable amount by this route. On the other hand, considerable amounts may be given intravenously. Preparations for intravenous injections may be divided into easily dissociable and poorly dissociable forms or ionizable and unionizable forms. Experience has shown that only small amounts of dissociable iron such as ferrous chloride or ferric ammonium citrate may be given at one time. It appears likely that whenever the iron binding capacity of the serum in man is exceeded by such an iron preparation (when more than 5 to 10 milligrams is given intravenously), a toxic reaction results. This includes flushing, nausea and vomiting, shock, central nervous depression and coma. To obtain some idea of the tissue distribution of this type of iron, rats were repeatedly injected with small amounts of Fe as ferric ammonium citrate. The amount given was sufficient to exceed the binding capacity of the serum and cutaneous flushing was observed. Two weeks thereafter, the animals were vivipерfused and radioautographs prepared according to the technic of Leblond. These are shown in figure 2, A to D. The iron was not visible in histologic preparations stained for iron. It will be observed that in the spleen the deposition of tagged iron was particularly heavy in the reticulo-endothelial cells and around certain blood vessels and in the periphery of the lymph follicles. In the liver the deposition was heaviest in the parenchymal tissue and periportal in distribution. In the kidney the convoluted tubules were particularly heavily impregnated in contrast to the glomeruli and collecting tubules. The acinar tissue of the pancreas was much more heavily involved than the islets and stroma. The heavy uptake of the parenchymal tissue in these animals was somewhat surprising. One might speculate that the toxicity of easily ionizable iron preparations when given in amounts exceeding the iron-binding capacity of the serum would be related to this excessive uptake by parenchymal cells.

The injection of poorly dissociable iron compounds such as iron ascorbate, iron ascorbate gelatin and iron saccharide is far better tolerated. These preparations are selectively taken up by the reticulo-endothelial cells of the body. This was well illustrated in a patient suffering from iron deficiency who was given 2,000 milligrams of saccharated iron intravenously over a period of two weeks. Liver biopsy was obtained during a gastric resection and stained for iron (fig. 1D). At the time of the biopsy it was estimated that 600 milligrams had been utilized for hemoglobin production leaving 1,400 milligrams of iron stored in the tissues. Each Kupffer cell is conspicuously stained blue due to its iron content, while no iron was visible in the parenchymal tissue. The sternal marrow obtained by aspiration showed abundant hemosiderin granules (fig. 1C).

Animals in Group 2 (table 1) were given large amounts of iron ascorbate gelatin and observed over a period of as long as two years. Histologically, massive deposits of iron were seen in the reticulo-endothelial tissue of the body. The macrophages of all tissues, particularly in the lymph nodes at the base of the liver were heavily laden with iron. In the biliary epithelium and gall bladder epithelium, hemosiderin...
FIG. 2.A. A radioautograph of the distribution of radioiron in the liver lobule. The blackened areas of heaviest concentration of isotope are periportal in distribution. Localization of the injected iron is greatest in the cytoplasm of parenchymal cells. Nuclei contain little or no radioactivity, Kupffer cells have a lower concentration than parenchymal tissue.

FIG. 2.B. Distribution of radioactive iron in the spleen. The iron appears to be localized in the reticulo-endothelial elements of the spleen. The deposition is particularly heavy at the periphery of lymph follicles and occasionally around central arterioles.
FIG. 3C.—Distribution of radioactive iron in the kidney. Heaviest deposits are in the proximal convoluted tubules. Less activity was found in glomerular and collecting tubular areas.

granules were prominent. There were small amounts of hemosiderin in parenchymal tissues, especially of the liver in several animals. In Dog 10, hemosiderin was found
more widely distributed, including Brunner’s glands, acinar tissue of the pancreas, zona glomerulosa of the adrenal and convoluted tubules of the kidney. However, the amount of iron visible in the reticulo-endothelial tissue was much greater than that in the parenchymal tissue in contrast to absorption experiments (fig. 1B).

It was of interest to determine the localization of radioactive iron in these iron heavy animals. In two of the dogs, Dogs 7 and 10 (table 2), radioactive iron (Fe55) had been given at the very onset of the experiment. At the time of death more than a year later, the concentration of radioactivity in the splenic tissue in both animals exceeded that found in the liver. The greater concentration in the spleen is consistent with the pattern of blood destruction (Section IV). The iron for the most part was originally incorporated in the red cell mass. When these red cells were broken down, little of the iron was reutilized because of the massive amount of non-radioactive iron which had been injected in the meantime. The radioactive iron from red cells, therefore, would tend to remain deposited in the area of breakdown and the increased concentration in the spleen is a reflection of the activity of this organ in blood destruction. Since whole carcass analyses of these animals were not performed, it is impossible to determine whether the decrease in total activity in blood, liver, kidney, and spleen of the original radioiron was due to a redistribution within the body of the animal or to blood loss not recognized through the experimental period. Radioactive iron (Fe59) administered shortly before sacrificing the animals at a time when the iron storage was large, showed a deposition of 85 per cent in the liver of one dog and 95 per cent in the second dog. The concentration in hepatic and splenic tissue was identical.

Recently, and for the first time, parenteral iron has assumed clinical importance because of the availability of a relatively nontoxic preparation of saccharated iron. This may be given in amounts of as much as several hundred milligrams in a single intravenous injection without serious toxic reactions. Its clinical efficacy for hemoglobin production is demonstrated by a number of reports.41–49 Its distribution, as shown in figure 1, C and D, is first reticulo-endothelial. The reports of Cappel41 and Polson48 suggest that the iron later moves into parenchymal tissue as well. Iron-deficient individuals tolerate larger doses than do normal subjects, but in either case a tolerance is obtained with repeated injections. This may be related to the appearance of tissue receptors which allow the body more efficiently to handle iron.50 It was of interest that a liver biopsy after the injection of saccharated iron (fig. 1D) showed hemofuscin.

IV. Red Cell and Hemoglobin Iron

Each day iron is liberated from the stores of erythrocytes and rapidly rerouted to the bone marrow where it is reincorporated into hemoglobin as demonstrated by isotope technics.51,52 The rate of blood destruction and production is so well controlled in this cycle that under normal conditions the relative size of the red cell compartment and storage iron compartment remains unchanged. Under pathologic conditions, a piling up of iron in storage depots from excessive red cell breakdown may occur. This is observed in hemolytic anemia and following transfusions
Fig. 3A.—Red cell localization (top). The data were obtained from rats killed at the specified time intervals and injected with identical amounts of poorly preserved blood. The donor rat had been injected with radioactive iron so that the transfused blood contained radioactive iron and its tissue localization could be determined. In this experiment the spleen had 4 to 5 times the concentration of the tagged erythrocytes shown by other tissues measured. In other experiments splenic localization has been less conspicuous, which was interpreted to mean more intravascular and less extravascular hemolysis related to the state of preservation.

Fig. 3B.—Hemoglobin localization (bottom). Hemoglobin solution prepared from radioactive donor blood was injected in amounts to produce an initial hemoglobinemia of 60 mg. per cent. Subsequent localization in the kidney was about 4 times that in spleen and liver/Gm. tissue.

for bone marrow dysfunction. The exact mechanism of red cell and hemoglobin catabolism is unknown. However, phagocytosis of erythrocytes by the reticulo-
endothelial system has been observed on the one hand, and in other pathologic states, intravascular breakdown of erythrocytes occurs. In short term experiments employing tagged nonviable red cells and hemoglobin solutions, it has been possible to determine the immediate tissue localization of these products. In figures 3 and 4 are shown the comparative abilities of the spleen, kidney and liver in two groups of rats to take up hemoglobin and erythrocytes. The greater effectiveness of the spleen in dealing with whole erythrocytes and the localization of free hemoglobin in the kidney is apparent. In Dog 11 of series 2B (table 1), large amounts of nonviable red cells were transfused over a period of weeks. These red cells were poorly preserved and at times appreciable hemoglobinuria followed their administration. The distribution of iron within the tissues of the animal was quite comparable to those animals receiving large doses of iron ascorbate gelatin and to the results obtained by the use of radioactive tagged erythrocytes. There were,

in addition, a number of pigments and scars involving wedges of tubular cells in the kidney. Other observations in animals and man would indicate that the hemosiderosis following breakdown of red cells is localized predominantly within the reticulo-endothelial system.

Hemoglobin requires particular consideration because of the role of the kidney in its processing. Evidence in the literature would indicate that hemoglobin in the plasma at levels below "renal threshold" is filtered through the glomeruli and reabsorbed by the tubules. With serum hemoglobin levels between 30 and 150 milligrams per cent, much greater concentrations of hemoglobin iron are localized in kidney than in splenic or hepatic tissue of the rat despite the absence of hemoglobinuria. Histologic studies employing larger amounts of hemoglobin show extensive hemoglobin and hemosiderin deposits in the convoluted tubules compatible with this reabsorption phenomenon.

Increased ferritin content has likewise been demonstrated in renal tubular tissue during periods of hemoglobinuria. It may then be anticipated that the pattern of iron localization following
breakdown of blood will vary somewhat depending on whether the breakdown is chiefly intravascular or extravascular.

V. **Focal Iron Distribution**

Whenever blood or blood products are liberated locally in the body, a hemosiderosis of that area may result. With the chronic pulmonary congestion accompanying mitral stenosis, the depositions of iron may be so extensive as to be visualized roentgenologically and are evident at postmortem examination by the rusty color of the pulmonary tissues. This is undoubtedly due to the continuous extravasation and breakdown of erythrocytes in that area. Extensive pulmonary iron is also found in workers exposed to certain dust-laden occupations. Pulmonary siderosis is particularly heavy in a clinical syndrome associated with repeated intrapulmonary bleedings. Pigmentation of the brain and central nervous system has been described frequently in syphilis and following hemorrhage. Extensive skin pigmentation may be seen following purpura. This is particularly evident in the legs in the presence of circulatory difficulties. In these various areas where blood is broken down, wandering macrophages take up the metal. Lymph nodes draining these areas frequently show hemosiderosis. This is prominent in lymph nodes in hemochromatosis where drainage from the heavily laden parenchymal tissue of the liver results in massive siderosis of the lymph nodes at the base of the liver. Focal iron deposits may be considered, therefore, to have no greater significance than to indicate previous breakdown of blood in the areas involved, or deposition of iron through inhalation or injection. The rate of mobilizing iron from these areas is undoubtedly related to the general need for iron.

VI. **The Recognition of Excess Iron Stores**

In man there are several methods for the recognition of the state of iron stores. The serum iron compartment is in many ways an excellent indicator. The serum iron is high in hemochromatosis and the iron carrying protein is almost completely saturated; in iron deficiency, the serum iron is low and the iron binding protein unsaturated. However, there may be considerable enlargement or depletion of iron stores in the presence of normal serum iron values. Infection may produce unsaturation of the iron binding protein in a patient with greatly enlarged iron stores (hemochromatosis).

Sternal marrow obtained by puncture allows one to examine a small piece of the reticulo-endothelial system for iron. No hemosiderin is visible in sternal marrow in iron deficiency. In conditions of iron excess, whatever their origin (infection, hemolytic anemia, multiple transfusions, idiopathic hemochromatosis), sternal marrow hemosiderin is increased. In those conditions in which reticuloendothelial localization of iron appears predominantly, such as following injection of saccharated iron, smaller amounts of iron are the more conspicuous. The results of the storage of 1600 milligrams of saccharated iron in a previously iron deficient patient is shown in figure 1C.

The distribution of radioiron given intravenously is influenced by the size of iron stores. With enlarged iron depots, less radioiron appears in circulating
erythrocytes. However, the uptake of radioactivity is influenced by bone marrow function as well so that interpretation is difficult in the presence of anemia.

Other tests have been employed to recognize iron excess. Hemosiderinuria is found particularly in conditions associated with intravascular hemolysis and also in hemochromatosis. Diffuse hemosiderosis of the skin is found in hemochromatosis at a late stage of this disease. It is of diagnostic importance in hemochromatosis only when the iron is located in the secretory cells of sweat glands. A skin test has been employed using potassium ferrocyanide to demonstrate hemosiderin but this is frequently unreliable. A liver punch or biopsy is the most accurate way of evaluating iron stores since the liver is the most important storehouse for iron. This procedure carries too great a hazard to be employed as a routine diagnostic procedure for iron excess.

VII. Interpretation of Excessive iron Stores

All intracellular ferritin and hemosiderin iron would appear to be available for hemoglobin production should the need arise. This is indicated histologically by the disappearance of hemosiderin and chemically by the removal of all but a very small fraction of liver iron in dogs following bleeding. The hemosiderosis of the tissues in infection would appear to represent a bone marrow dysfunction rather than unavailability of iron. Indeed, when iron deficiency is severe, injected iron may be utilized for hemoglobin production in normal fashion despite the presence of inflammation. In our opinion, it is only when deposits of iron within the cell become so large as to interfere with cell function or destroy the cell that the mobilization of iron is impaired. This is illustrated in figure 1E. In a dog, two years following the injection of 2,400 milligrams of iron, some 1,700 milligrams were removed by repeated phlebotomies. At this point, hematopoiesis decreased and the serum iron fell to low levels. In effect, the dog was no longer able to mobilize enough iron to support blood production. At autopsy, hemosiderin was present in tissues but was present as large lumps. This and other in vitro evidence emphasize the importance of the cell in the mobilization of storage iron.

It has been postulated that the large iron stores in hemochromatosis are the result of abnormal cellular metabolism—that iron accumulates in the cell in some abnormal form. Accordingly, an attempt was made to characterize the liver iron in idiopathic hemochromatosis and compare it with a liver of an animal previously injected with large amounts of iron. The distribution of iron between saline soluble and insoluble fractions of the liver homogenates is shown in table 4. The two fractions which are thought to represent ferritin and hemosiderin maintained a constant relationship in both instances. This would support the thesis that both are a result of excessive storage of iron. The removal of iron from the livers of patients with idiopathic hemochromatosis by phlebotomy also indicates the availability of this tissue iron for hemoglobin production. It would thus appear that the storage mechanism is acting quite similarly in these different instances.

Storage iron may be increased at the expense of the circulating red cell mass. Thus, with development of any anemia other than iron deficiency anemia (i.e. hemolytic anemia, infection, uremia, pernicious anemia, myelophthisic
anemia, anemia of the newborn, etc.), there will be a transfer of iron from the circulating blood to the tissues. This occurs without change in the total body iron since iron excretion is negligible. The observations of Fanger et al. illustrate this. Among 24 patients who died of severe untreated pernicious anemia, an excessive deposition of iron was seen in both parenchymal tissue and reticulo-endothelial tissue of the liver in 21 instances. In contrast to this, in only 2 of 11 patients with pernicious anemia under adequate treatment at the time of death were significant amounts of hemosiderin seen in the liver. This type of relative increase in iron stores is portrayed diagrammatically in figure 4. The excess in storage iron under such circumstances is limited to one or two grams, the amount which can be transferred from the red cell compartment.

An absolute increase in body iron occurs as a result of an increased iron intake through one of the routes described in previous sections. In dietary or idiopathic hemochromatosis, there is a progressive absorption of iron beyond the needs of the body. Experimentally, extremely large doses of iron, a very low phosphate content of the diet, and ligation of the pancreatic ducts would all seem capable of producing progressive increase in body iron stores. The observations of Gillman and Gillman are of particular interest. They have described deposition of large amounts of iron and fibrosis of the liver in South African natives who were on a poor diet consisting chiefly of corn meal. The distribution of iron in all of these conditions in which the iron gains entrance through the gastro-intestinal tract is strikingly similar.

Massive deposits of iron may occur following the administration of many transfusions and following the injection of iron. The initial distribution with these different routes of administration may be quite different as demonstrated diagrammatically in figure 5. However, internal redistribution of iron is constantly taking place. It has recently become evident that the morphologic end results of iron from transfusions and those in dietary and idiopathic hemochromatosis may be indistinguishable.

The extent to which iron per se may be responsible for cirrhosis of the liver remains an open question since no great success has been achieved in the production of fibrosis in experimental animals by iron injection or blood administration. However, the repeated occurrence of liver fibrosis in transfusion hemosiderosis gives support to the hypothesis that iron may be an important, if not the only etiologic factor in producing these tissue changes. It would appear that over a hundred transfusions are usually necessary to produce a picture of hemochromatosis.

### Table 4.—Liver Iron Partition

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<tr>
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<th>Hemoglobin Iron</th>
<th>Soluble Iron Fraction</th>
<th>Insoluble Iron Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemosiderotic dog (perfused)</td>
<td>-</td>
<td>31.9</td>
<td>68.1</td>
</tr>
<tr>
<td>Patient with hemochromatosis</td>
<td>32.2</td>
<td>67.8</td>
<td></td>
</tr>
<tr>
<td>Patient with hemochromatosis</td>
<td>31.6</td>
<td>68.4</td>
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</tr>
</tbody>
</table>
Fig. 5.—Diagram of iron metabolism. GI = gastro-intestinal tract, SI = serum iron, BM = bone marrow, K = kidney, RE = reticulo-endothelial tissue, L = liver. Normal relationships of storage areas to erythrocyte iron are shown at the top of the figure. Absorbed iron is stored in the liver and also reticulo-endothelial system. With injected red cells there is an increased breakdown of erythrocytes in the reticulo-endothelial system and to a lesser extent intravenously. The iron which accumulates in the reticulo-endothelial system is transferred over a period of time to the liver. Injected hemoglobin is taken up by the kidney, liver parenchyma, and reticulo-endothelial system. While at low levels of hemoglobinemia other tissues may play a more important role, at levels approaching or exceeding renal threshold, the kidney assimilates large amounts of pigment. Secondary redistribution of iron again occurs. Iron injected as iron saccharide is taken up almost exclusively by the reticulo-endothelial tissues but later moves on to the liver. Thus, in each instance the pathologic picture of hemochromatosis may be the end result.
with liver fibrosis and impaired glucose tolerance in man. In transfusion hemochromatosis, in contrast to the idiopathic type, fibrosis of the spleen appears to precede and often is more pronounced than liver fibrosis.\cite{53,81} This is consistent with the observation that the localization of the broken down red cell iron is initially greater in the spleen than in other tissues. More acute liver damage may also be seen in transfusion hemochromatosis. This may be attributable to the more rapid deposition of iron or the additional burden of anemia. It is indeed a conspicuous finding that the liver is the organ containing by far the greatest amount of iron in hemochromatosis and is also the organ which is most frequently damaged.

One of the functions of the liver would appear to be the regulation of the level of serum iron. The elevated serum iron observed initially during excessive iron absorption in experimental animals may well be due to the fact that the rate of absorption of iron exceeds the rate at which the liver may store it. When the animal is placed on a low iron diet, the serum iron level will fall to normal limits. After prolonged and progressive iron deposition, a situation resembling idiopathic hemochromatosis is reached when the serum iron remains persistently elevated. The serum iron-binding protein is saturated with iron and the serum iron level ranges between 200 and 350 gamma per 100 ml. of serum. It is our experience that the iron pigmentation of the skin following transfusions is found only in those patients with markedly elevated serum iron levels. This is interpreted to mean that the liver, the “shock organ” for iron absorption, is no longer able to regulate the serum iron level and iron is overflowing into secondary tissue receptors. The serum iron may be elevated by conditions affecting the bone marrow as well. It seems likely from clinical observations that lesions of the bone marrow producing elevation of serum iron allow considerably more generalized parenchymal iron deposition than would be found with a similar amount of iron without bone marrow block.

**Summary**

On the basis of experimental and clinical observations and a review of the literature, a concept of the behavior of storage iron in relation to body iron metabolism has been formulated.

Storage iron is defined as tissue iron which is available for hemoglobin synthesis when the need arises. This iron is stored intracellularly in protein complex as ferritin and hemosiderin. It would appear that wherever the cell is functionally intact, such iron is available for general body needs.

Iron is transported by a globulin of the serum to and from the various tissues of the body to satisfy their metabolism. Surplus iron carried by this iron-binding protein is deposited chiefly in the liver.

Storage iron may be increased in two ways. The first mechanism results from the inability of the body to excrete significant amounts of iron. Because of this, any decrease in circulating red cell iron (any anemia other than blood loss or iron deficiency anemia) is accompanied by a shift of iron to the tissue compartment. The total amount of body iron remains constant and is merely redistributed.
This is to be contrasted with the absolute increase in body iron and enlarged iron stores which follow excessive iron absorption or parenteral iron administration. Enlarged iron stores in either instance may be evaluated by examination of sternal marrow or determination of the serum iron and saturation of the iron binding protein.

In states of iron excess, differences in initial distribution are observed, depending on the route of administration and type of iron compound employed. Iron absorbed from the gastro-intestinal tract and soluble iron salts injected in small amounts are transported by the iron-binding protein of the serum and stored predominantly in the liver. Colloidal iron given intravenously is taken up by the reticulo-endothelial tissue. Erythrocytes appear to localize in greatest concentration in the spleen, while greater amounts of hemoglobin iron are found in the renal parenchyma. These latter differences in distribution reflect the capacity of various body tissues to assimilate different iron compounds, which while present in the plasma are not carried by the iron-binding protein.

Over a period of time an internal redistribution of iron from these various sites occurs through the serum iron compartment. The liver becomes progressively loaded with iron. When the capacity of the liver to store iron is exceeded, the serum iron increases and secondary tissue receptors begin to fill with iron. That iron in large amounts is toxic to tissues is suggested by the occurrence of fibrosis in the organs most heavily laden with iron. This sequence of events, whether following excessive iron absorption or parenteral iron administration is believed to be responsible for the clinical and pathologic picture of hemochromatosis.

REFERENCES

10. Moore, C. V.: Personal communication.
1006    IRON METABOLISM


74 FINCH, C. A.: Unpublished date.


IRON METABOLISM


65 Finch, C. A., and Finch, S.: Haemochromatosis. (To be published.)


71 Kinney, T., Hagedorn, M., and Finch, C. A.: Iron absorption in depancreatized dogs. (To be published.)


74 Yuile, C. L., and Young, L. E.: Personal communication.

75 Finch, C. A.: Unpublished data.