Brief Report

Clearance of Iron from Hemochromatotic and Normal Transferrin in Vivo

By Munsey S. Wheby, Stanley P. Balcerzak, Pearl Anderson and William H. Crosby

The basic defect underlying the phenomenon of iron loading in patients with idiopathic hemochromatosis is unknown. It has been suggested that the iron binding protein, transferrin, may have an abnormal avidity for iron. The experiments to be described were designed to test this possibility in vivo, by comparing simultaneously, the clearance of iron from normal and hemochromatotic plasma in normal and hemochromatotic subjects.

Methods

Each patient with idiopathic hemochromatosis was paired with a normal volunteer whose plasma had approximately the same total iron binding capacity. Seventy ml. of sterile plasma were obtained from each subject. The hemochromatotic plasma was incubated at 37 C. for 45 minutes with Fe59* and the normal plasma with Fe55.* Radioactive standards were prepared from aliquots of the labelled plasmas. Plasma iron content and total iron-binding capacity before and after radio iron addition was measured in other aliquots. Both subjects simultaneously were injected intravenously with an accurately weighed quantity of each of the two radioactive plasma samples. Blood samples were then drawn from each subject, at timed intervals over the next 120 minutes. Two ml. of these plasma samples and the standards were digested, electroplated and counted for Fe59 and Fe55 using the simultaneous liquid scintillation counting procedure, described by Dern and Hart. All samples were counted in duplicate with a counting error of 1 per cent or less. The validity of using these two isotopes for simultaneous iron clearance studies has been shown previously. As a further check on the method, simultaneous plasma iron clearance studies were done on 2 healthy males using their own plasma tagged with both Fe55 and Fe59.

In all subjects blood was obtained 2 weeks after the clearance study to determine red cell incorporation of the isotopes. Plasma volume and blood volume were calculated from the initial dilution of both Fe59 and Fe55, using the value of 0.92 as the ratio of body hematocrit to venous hematocrit.

Subjects

Idiopathic Hemochromatosis

F. G., a 56-year-old retired officer was reported previously. The diagnosis of idiopathic hemochromatosis was made in 1958 on the basis of liver cirrhosis with esophageal varices, diabetes mellitus, elevated serum iron, saturated iron binding capacity and removal, by phlebotomy, of 22 Gm. of iron in 11 months. He had undergone a series of phlebotomies preceding the present study. At the time of the study he seemed to be well, but in July 1962, 2 months after the present study was completed, he died with a malignant hepatoma.

B. W., a 54-year-old officer also was reported previously. The diagnosis of idiopathic
hemochromatosis was based on liver cirrhosis with heavy iron deposition in the liver, diabetes mellitus, elevated serum iron, saturated iron binding capacity, and removal, by phlebotomy, of 22 Gm. of iron in 11 months.

Neither of these patients had a history of excessive alcohol intake, unusual diet containing excessive iron, iron therapy or blood transfusions. Prior to study both patients were phlebotomized to insure sufficient unsaturated iron binding capacity to bind the isotope.

Controls

F. M., a 23-year-old healthy male, had never donated blood.
G. C., a 33-year-old healthy male, had donated 500 ml. of blood 7 months prior to the study.
M, and D, were both healthy males who had been blood donors on numerous occasions in the past. Neither had been bled during the year before this study.

RESULTS

The significant data for M, and D, the two subjects given their own doubly tagged plasma, are shown in table 1. Clearance of each isotope was approximately the same as is shown graphically in figure 1. The rapid clearances presumably reflect decreased body iron stores, a consequence of previous donations of blood.
HEMOCHROMATOTIC AND NORMAL TRANSFERRIN

Table 1

<table>
<thead>
<tr>
<th>Subject</th>
<th>Hematocrit (%)</th>
<th>Plasma Iron before Iron Added (ug./100 ml)</th>
<th>Total Iron-Binding Capacity before Iron Added (ug./100 ml)</th>
<th>Plasma Iron after Iron Added (ug./100 ml)</th>
<th>Total Iron-Binding Capacity after Iron Added (ug./100 ml)</th>
<th>Substrate iron reticulocyte</th>
<th>RBC Incorporation at 14 Days %</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>46</td>
<td>56</td>
<td>219</td>
<td>71</td>
<td>219</td>
<td>56</td>
<td>53</td>
</tr>
<tr>
<td>M</td>
<td>45</td>
<td>51</td>
<td>211</td>
<td>110</td>
<td>203</td>
<td>52</td>
<td>51</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Subject</th>
<th>Diagnosis</th>
<th>Hematocrit (%)</th>
<th>Plasma Iron before Iron Added (ug./100 ml)</th>
<th>Total Iron-Binding Capacity before Iron Added (ug./100 ml)</th>
<th>Plasma Iron after Iron Added (ug./100 ml)</th>
<th>Total Iron-Binding Capacity after Iron Added (ug./100 ml)</th>
<th>Hemochromatotic Plasma</th>
<th>Normal Plasma</th>
<th>RBC Incorporation at 14 Days %</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. M.</td>
<td>Normal</td>
<td>41.5</td>
<td>84</td>
<td>280</td>
<td>93</td>
<td>289</td>
<td>120</td>
<td>120</td>
<td>83 75</td>
</tr>
<tr>
<td>F. G.</td>
<td>Hemochromatosis</td>
<td>36</td>
<td>37</td>
<td>261</td>
<td>32</td>
<td>244</td>
<td>26</td>
<td>24</td>
<td>82 89</td>
</tr>
<tr>
<td>G. C.</td>
<td>Normal</td>
<td>43</td>
<td>99</td>
<td>234</td>
<td>116</td>
<td>226</td>
<td>92</td>
<td>88</td>
<td>57 61</td>
</tr>
<tr>
<td>B. W.</td>
<td>Hemochromatosis</td>
<td>46.5</td>
<td>81</td>
<td>237</td>
<td>73</td>
<td>221</td>
<td>57</td>
<td>53</td>
<td>89 100</td>
</tr>
</tbody>
</table>

Table 2 contains the pertinent data for the studies pairing the normal and hemochromatotic subjects. The disappearance of iron from hemochromatotic plasma was almost identical to the disappearance from normal plasma in both the normal and the hemochromatotic subject. Figure 2 shows the pair of experiments done in G. C. and B. W.

Subsequent red cell incorporation of each isotope did not differ significantly in any of the studies as shown in tables 1 and 2. The explanation for the decreased incorporation of both isotopes found in G. C. is unknown.

DISCUSSION

These results indicate that with respect to in vivo iron transport, hemochromatotic transferrin does not differ from normal transferrin. If the iron binding protein of the patient with hemochromatosis handles iron in an abnormal manner, such a defect is too subtle to be demonstrated by this technic. Bothwell and co-workers recently reported identical findings in a patient with idiopathic hemochromatosis. In addition they were unable to show any abnormality of hemochromatotic transferrin using several chemical methods.

Since the increased amount of body iron in idiopathic hemochromatosis enters via the gastrointestinal tract, the suggestion that hemochromatotic transferrin might be abnormal implies that transferrin is important in the regulation...
Fig. 2.—Plasma radioactivity in G. C., (upper graph) a normal subject, after simultaneous intravenous injection of Fe$^{55}$ bound to his own plasma and Fe$^{59}$ bound to the plasma of B. W., a patient with hemochromatosis.

Plasma radioactivity in B. W., (lower graph) a patient with hemochromatosis, after simultaneous intravenous injection of Fe$^{59}$ bound to his own plasma and Fe$^{55}$ bound to the plasma of the normal subject, G. C.

of iron absorption. Recent studies$^{11,12}$ have demonstrated that iron is absorbed quite well despite complete saturation of circulating transferrin. The patient with congenital absence of transferrin, reported by Heilmeyer and co-workers,$^{13}$ was found to have greater than normal iron absorption. These reports indicate that transferrin does not have a direct or immediate influence on the control of iron absorption.

The findings in the present study make it unlikely that the basic defect in idiopathic hemochromatosis involves the iron binding protein transferrin.

**Summary**

The iron transport function of transferrin from normal subjects and patients with idiopathic hemachromatosis has been studied using the radioisotopes Fe$^{59}$ and Fe$^{55}$. It was concluded that transferrin from hemochromatotic patients functions in a normal manner in vivo.

**Summario in Interlingua**

Le function, in le transporto de ferro, de transferrina ab subjectos normal e ab subjectos con hemochromatosis esseva studiate con le uso del radio-
isotopos $\text{Fe}^{59}$ e $\text{Fe}^{55}$. Esseva concluïte que transferrina ab patientes hemo-
cromatotic functiona normalmente in vivo.

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