Transferrin Iron, Chelatable Iron and Ferritin in
Idiopathic Haemochromatosis

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SUMMARY. In 12 patients with idiopathic haemochromatosis and varying degrees of
iron overload the serum ferritin concentration was related to the amount of iron
mobilized by venesection. There was no correlation between iron load and chelatable
iron. In 35 patients with normal iron stores following venesection therapy, both
transferrin iron and chelatable iron was pathologically increased.

The initial stages of iron accumulation in idiopathic haemochromatosis are charac-
terized by an imbalance between chelatable iron and ferritin, a phenomenon that
could explain the pathogenesis of the disease.

The patient with fully developed haemochromatosis has a high serum-iron concentration,
a high transferrin saturation and an increased total body-iron content. The latter is associated
with an increase in the amount of chelatable iron, measured after the administration of
desferrioxamine (DFO) or diethylene triamine penta-acetic acid (DTPA) (Barry, 1973;
Walker & Williams, 1974). While estimation of serum-iron concentration and transferrin
saturation are useful initial screening tests in the diagnosis of haemochromatosis they are of
little value in determining the size of the iron load. Chelation tests have been considered
useful in predicting the iron load existing at all stages of the disease and a correlation has been
found between chelatable iron and both liver-iron concentration (Barry et al., 1970a) and iron
mobilized by phlebotomy (Smith et al., 1969; Barry et al., 1970b). Others have found that
spurious high values for chelatable body iron may be obtained, especially in patients
reaccumulating iron after an initial course of venesection (Walker & Williams, 1974).

It has been shown that the concentration of ferritin in serum is closely related to total iron
stores in normal subjects and in patients with iron deficiency or iron overload secondary to
multiple transfusions (Jacobs et al., 1972). In normal subjects serum ferritin concentration is
directly related to the amount of mobilizable iron measured by repeated phlebotomy
(Walters et al., 1973). The present study was undertaken to determine the relationship of
initial serum ferritin concentration to the amount of iron mobilizable by venesection in
patients with idiopathic haemochromatosis and to compare this measurement with the
amount of chelatable iron in the same patients. In addition the opportunity was taken to
examine the interrelationship of transferrin iron, chelatable iron and ferritin in haemochromat-
totic patients in whom iron stores had been reduced to a low level by repeated venesection.

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Two groups of patients with idiopathic haemochromatosis were studied. In all cases the original diagnosis was supported by evidence of iron overload with no obvious primary cause and a liver biopsy showing increased parenchymal iron deposits and cirrhosis.

(1) Twelve patients with evidence of abnormal iron accumulation were treated by repeated venesection until normal iron status was regained. Five of these patients had been previously untreated and in seven cases there was evidence of iron reaccumulation after a period without venesection. Initial data is shown in Table I, together with the amount of blood subsequently removed.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Serum iron (µg/dl)</th>
<th>Transferrin saturation (%)</th>
<th>Serum ferritin (µg/l)</th>
<th>$F_v$ (µg/kg body weight)</th>
<th>Blood removed (l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 a</td>
<td>254</td>
<td>92</td>
<td>240</td>
<td>1493</td>
<td>8.0</td>
</tr>
<tr>
<td>2 a</td>
<td>180</td>
<td>70</td>
<td>55</td>
<td>538</td>
<td>5.0</td>
</tr>
<tr>
<td>3 a</td>
<td>262</td>
<td>86</td>
<td>570</td>
<td>1205</td>
<td>2.5</td>
</tr>
<tr>
<td>4 a</td>
<td>230</td>
<td>87</td>
<td>200</td>
<td>796</td>
<td>3.9</td>
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<tr>
<td>5 a</td>
<td>210</td>
<td>89</td>
<td>220</td>
<td>961</td>
<td>7.0</td>
</tr>
<tr>
<td>6 a</td>
<td>260</td>
<td>81</td>
<td>660</td>
<td>1633</td>
<td>10.8</td>
</tr>
<tr>
<td>7 b</td>
<td>330</td>
<td>97</td>
<td>5040</td>
<td>1299</td>
<td>38.0</td>
</tr>
<tr>
<td>8 b</td>
<td>380</td>
<td>95</td>
<td>1200</td>
<td>719</td>
<td>13.5</td>
</tr>
<tr>
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<td>210</td>
<td>79</td>
<td>304</td>
<td>1309</td>
<td>8.0</td>
</tr>
<tr>
<td>10 b</td>
<td>180</td>
<td>90</td>
<td>2200</td>
<td>1460</td>
<td>29.0</td>
</tr>
<tr>
<td>11 b</td>
<td>270</td>
<td>90</td>
<td>3840</td>
<td>1680</td>
<td>40.0</td>
</tr>
<tr>
<td>12 b</td>
<td>170</td>
<td>94</td>
<td>1867</td>
<td>862</td>
<td>25.0</td>
</tr>
<tr>
<td>Mean</td>
<td>244.7</td>
<td>87.5</td>
<td>1403.0</td>
<td>1156.9</td>
<td>15.9</td>
</tr>
<tr>
<td>SE</td>
<td>18.1</td>
<td>2.2</td>
<td>456.1</td>
<td>107.0</td>
<td>3.9</td>
</tr>
</tbody>
</table>

(a) Accumulating iron following cessation of phlebotomy therapy; (b) new patients previously untreated.

(2) Twenty-five previously treated patients in whom iron stores, as indicated by serum ferritin concentrations, were within the normal range. Most of these were in the process of reaccumulating iron.

In addition 47 healthy adult males from the hospital staff had blood samples taken for the estimation of serum ferritin, iron and total iron binding capacity.

**METHODS**

Serum-iron concentration and iron-binding capacity were measured by a modification of the method of Young & Hicks (1965) using magnesium carbonate as an adsorbent. Only about 10% of the iron contained in circulating ferritin is estimated by this technique and a circulating ferritin concentration of 5000 µg/l is not likely to lead to an increase in serum iron concentration of more than 10 µg/dl. Most of the ferritin concentrations in our samples were considerably less than this and no correction has been made. Serum-ferritin concentration was
measured by the immunoradiometric technique of Addison et al (1972). In vivo chelatable iron ($F_r$) was measured by the differential ferrioxamine test (Fielding, 1965) using a 6 hr collection of urine. The results are expressed as $\mu$g ferrioxamine per kg body weight.

![Graph 1](image1)

Fig 1. The total amount of blood removed from 12 patients with haemochromatosis in order to deplete iron stores together with the initial serum ferritin concentration.

![Graph 2](image2)

Fig 2. The total amount of blood removed from 12 patients with haemochromatosis in order to deplete iron stores together with the initial in vivo chelatable iron ($F_r$) in each case. The encircled points represent new patients (b), see Table I.

Venesection was carried out at frequent intervals until blood samples showed that the serum iron and transferrin saturation were in the normal or iron deficient range.

**RESULTS**

In the 12 untreated patients in group I there is a good correlation between the volume of
blood removed from each patient in order to deplete iron stores and the initial serum ferritin concentration before treatment; $r = 0.92$, $P < 0.001$ (Fig 1). There is, however, no correlation between the amount of blood removed by phlebotomy and the in vivo chelatable iron (Fig 2); $r = 0.4$, $P > 0.1$.

Table II. Serum ferritin and iron concentrations and transferrin saturation in 47 normal males and 25 patients with treated haemochromatosis

<table>
<thead>
<tr>
<th></th>
<th>Ferritin (µg/l)</th>
<th>Iron (µg/dl)</th>
<th>Transferrin saturation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal males</td>
<td>97.4 ± 10.1</td>
<td>110.5 ± 5.0</td>
<td>30.3 ± 1.4</td>
</tr>
<tr>
<td>Treated haemochromatosis</td>
<td>110.3 ± 16.6</td>
<td>157.8 ± 18.1</td>
<td>54.2 ± 6.5</td>
</tr>
</tbody>
</table>

Fig 3. Serum ferritin and iron concentration in 25 patients with normal iron stores. The horizontal line indicates the upper limit of normal values.

In the 25 patients in group 2, who were reaccumulating iron after iron stores had been reduced to normal or iron deficient levels by phlebotomy, both serum iron concentration and transferrin saturation were higher than normal (Table II). The chelatable iron was increased from a normal mean value of 226 (81–404) µg per kg body weight (Smith, 1968) to a mean value of 709 (118–1809) µg per kg body weight.

It can be seen from Figs 3, 4 and 5 that in those patients who had been made iron deficient by phlebotomy and had a serum ferritin concentration of 10 µg/l or less there was also a low
Fig 4. Serum ferritin concentration and transferrin saturation in 25 patients with normal iron stores. The horizontal line indicates the upper limit of normal values.

Fig 5. Serum ferritin concentration and in vivo chelatable iron in 25 patients with normal iron stores. The horizontal line indicates the upper limit of normal values.
serum iron concentration and transferrin saturation; chelatable iron was within the normal range. A slightly greater accumulation of iron in the body results in an early rise of serum iron concentration, transferrin saturation and chelatable iron to pathological levels, while serum ferritin concentration increases only slowly, remaining within the normal range. In two patients studied individually over a period of some months reaccumulation of iron was seen to result in a rise in serum iron and chelatable iron before any increase in ferritin concentration was observed. It is concluded that in patients with haemochromatosis who are reaccumulating iron after therapy normal levels of ferritin iron are associated with disproportionately high levels of transport and chelatable iron.

DISCUSSION

In normal subjects the concentration of ferritin in serum is closely correlated with the amount of mobilizable iron in the body measured by the phlebotomy technique (Walters et al., 1973). In patients with aplastic anaemia the serum ferritin concentration is related to the amount of iron administered in the form of blood transfusions (Jacobs et al., 1972) and in thalassaemic subjects a good correlation has been shown between serum ferritin concentration and the iron content of liver biopsy samples (Letsky et al., 1973). The present study indicates a similar good correlation between serum ferritin concentration and the total iron load as estimated by phlebotomy in patients with haemochromatosis. These data, together with the findings in iron-deficient patients (Jacobs et al., 1972), confirm the overall relationship between circulating ferritin and storage iron in a wide variety of conditions.

In the last few years many workers have attempted to assess body iron stores indirectly by measuring urinary iron excretion following the injection of an iron chelating agent. Wöhler (1964) showed that the intramuscular injection of 500 mg desferrioxamine is followed by an increase in urinary iron excretion during the subsequent 6 hr. The amount excreted is increased in subjects with iron overload. Fielding (1965) suggested that the amount of iron excreted in the urine did not always bear a constant relationship to the amount chelated in vivo and recommended a differential ferrioxamine test which would enable the amount of iron chelated in vivo to be calculated. A modification of this test has been used in the present study.

Balcerzak et al. (1968) and Olsson (1972) found a close linear correlation between desferrioxamine induced iron excretion and iron stores measured by the phlebotomy technique in normal subjects. Hallberg et al. (1966) found a correlation between post-desferrioxamine iron excretion and the iron content of the liver. In gross iron overload correlations have been reported between chelatable iron and both liver-iron concentration (Barry et al., 1970a) and iron mobilized by phlebotomy (Smith et al., 1969; Barry et al., 1970b). While this evidence suggests that chelatable iron is related to iron stores observations on the site of action of desferrioxamine indicate that iron in the storage compound ferritin is not itself directly chelatable. Lipschitz et al. (1971a) have shown that liver and spleen ferritin in rats can be reduced by repeated injections of desferrioxamine but this does not occur when erythropoiesis is reduced by hypertransfusion with a consequent reduction in reticuloendothelial (RE) iron release to the plasma. They concluded that desferrioxamine obtains iron predominantly from a compound or compounds on the pathway between ferritin and plasma transferrin.
The nature of this chelatable iron is not known but it corresponds to the 'pre-release' iron pool in reticuloendothelial cells also postulated by Lipschitz et al (1971b) to explain their experiments on RE iron release. The iron chelated by desferrioxamine usually appears to be related to the amount of ferritin present in the body but there are a number of instances where this is not so. Karabus & Fielding (1967) showed that in megaloblastic and haemolytic anaemias the amount of chelatable iron may be disproportionately high and, in some cases, even higher than in cases of untreated haemochromatosis. In the megaloblastic anaemias the chelatable iron is rapidly reduced by specific therapy as normal erythropoiesis is established (Karabus & Fielding, 1967; Balcerzak et al, 1968). A disproportionately high chelatable iron also occurs in iron deficiency anaemia, as a result of intramedullary haemolysis, and paradoxically this falls after the administration of iron (Brunström et al, 1968). Cumming et al (1967) have shown that in iron overload states the urinary-iron excretion after desferrioxamine is increased if haemolysis is induced by phenylhydrazine.

Karabus & Fielding (1967) considered their results to indicate the presence of a chelatable iron pool in reticuloendothelial cells the size of which is determined on the one hand by the inflow of iron from haem catabolism and on the other hand by the outflow to plasma or to stable storage compounds such as ferritin. This pool is in equilibrium with ferritin in the cell and is the one sampled by the chelator. This concept has been extended by Lipschitz et al (1971b) and Lynch et al (1974) who suggest that the mechanism for diverting intracellular iron into storage forms is by enlargement of the chelatable 'pre-release' pool which results in the induction of ferritin synthesis. Conversely iron mobilized from ferritin passes through the 'pre-release' pool en route to transferrin.

Data obtained in the present group of 12 haemochromatotic patients show no correlation between chelatable iron and the amount of storage iron measured by phlebotomy or the initial serum ferritin concentration. Previous data suggested that iron stores can be predicted from F, values throughout the range of iron overload (Smith et al, 1969) but this was derived somewhat differently from the present study. In the present investigation seven out of the 12 patients had only moderately increased iron stores which had reaccumulated after an initial course of therapy followed by no treatment. In each case the relationship between initial F, value and the total iron removed during the entire course of venesections was examined. The earlier study of Smith et al (1969) was concerned with nine patients whose initial mean F, was similar to that of the present group but who were all previously untreated. Repeated F, values were obtained during the course of venesection and the difference between values was related to the amount of iron removed during the corresponding time. Quite apart from the different methodology, the difference between the two sets of data may be related to the inclusion of some patients with only slightly increased stores in the present group and to the fact that the iron stores in our patients were, in most cases, the result of reaccumulation. The findings in haemochromatotic patients with low iron stores who are reaccumulating after having been fully treated show that it is in just this group that the dissociation between iron stores and chelatable iron is most marked.

Table II shows the mean serum ferritin concentration to be 97.4 μg/l in a group of healthy men aged 18-44 years. The highest normal value was 220 μg/l. Untreated patients with haemochromatosis when first seen have much higher levels (Jacobs et al, 1972), the lowest value seen up to the present time being in excess of 900 μg/l. In the 25 patients in whom iron
stores were normal as judged by serum ferritin concentration (Table II) both the mean serum iron concentration and transferrin saturation were abnormally elevated and the mean chelatable iron (F) of 709 μg/kg body weight is above the upper limit of normal values. These abnormalities are not apparent in those cases where venesection has resulted in gross iron depletion but they appear as soon as iron reaccumulation commences. As the process of iron loading begins the first abnormal signs to appear are gross increases both in transport iron and chelatable iron and these occur long before there is any increase in the amount of ferritin produced (Figs 3, 4 and 5). There is no reason to believe that the original process of iron accumulation in this disease is any different. The rapid rise in serum-iron concentration after the cessation of treatment in haemochromatosis has long been recognized and the present data suggest that although this transport iron compartment is in equilibrium with the chelatable iron pool it is not related to ferritin iron in the expected manner. The stimulation of ferritin synthesis by iron is a well-recognized phenomenon (Crichton, 1973) and Lipschitz et al (1971b) have suggested that the immediate stimulus is enlargement of the chelatable pool. This implies a constant relationship between the size of the chelatable and the ferritin pool and explains why chelatable iron is usually proportional to ferritin iron stores. In patients with haemochromatosis who are beginning to accumulate iron there appears to be a disproportionately large increase in the chelatable pool and transferrin iron pool without any increase in ferritin levels. This suggests that the normal intracellular relationship between iron and ferritin may be disturbed in this condition, with a decreased sensitivity of the ferritin synthetic mechanism to iron in the chelatable pool and a relative decrease in the amount of ferritin formed.

The possibility that haemochromatosis, a disease characterized by increased iron loading, may be associated with a decreased capacity to form ferritin seems at first unlikely, but further examination shows that this hypothesis is not only tenable but may go some way towards explaining the pathogenesis of the disorder.

Karabus & Fielding (1967) drew an analogy between the chelatable iron pool in RE cells and a similar pool in the epithelial cells of the small intestine. It has been demonstrated that iron is in a chelatable form during its transit across the small intestinal mucosa (Jacobs et al, 1969). Recent work in the field of iron absorption has provided ample evidence for the presence of a non-ferritin iron pool in intestinal cells associated with the transport of iron from the luminal to the serosal surface (Jacobs, 1973). In normal circumstances iron taken up from the intestinal lumen is found initially in a non-ferritin fraction of the cell from which it is transferred to the plasma. Iron remaining in the non-ferritin fraction stimulates ferritin synthesis and is then sequestered in the cell as ferritin, being unavailable for serosal transfer. If the present observations reflect an imbalance between the chelatable pool and ferritin in intestinal cells as well as in RE cells then the absorption of iron would be affected. Enlargement of the chelatable pool and impairment of ferritin synthesis would result in an increase in the amount of iron available for transferrin binding and consequently an increase in absorption. Crosby (1963) has demonstrated a failure of the intestinal epithelium to form F (ferritin) bodies in haemochromatosis, an observation in keeping with the present hypothesis.

It is concluded that in patients with haemochromatosis the circulating ferritin is related to body stores of ferritin. In those patients who are beginning to accumulate iron there appears to be an imbalance between intracellular chelatable iron and ferritin, which may be caused by
an impaired synthesis of the proteins, resulting in a high transferrin iron concentration. If such an imbalance occurred in the small intestinal epithelium this would account for the inappropriately high iron absorption found in this condition and the consequent increase in iron load. Iron excretion is limited and the increasing amount of iron accumulating within the body will eventually reach the level necessary to stimulate ferritin synthesis. It seems more likely that the tissue damage that occurs later in the evolution of the disease is a consequence of enlargement of the chelatable iron pool than to the accumulation of ferritin.

REFERENCES


