Relationship of hepatic iron concentration to histochemical grading and to total chelatable body iron in conditions associated with iron overload

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SUMMARY A simple method based on atomic absorption spectrophotometry and using protein concentration as the reference standard was used for measuring the total tissue iron concentration in needle liver biopsy specimens from 47 patients with varying levels of body iron stores. The values obtained showed a close correlation over a wide range with measurements of total chelatable body iron stores. A comparison with the histochemical assessment of iron deposition showed that only those with grades 3 and 4 had significant increases in iron concentration which is in accord with other clinical observations in such patients. A liver biopsy can thus be of value in the quantitative determination of total body iron overload as well as in the diagnosis of underlying liver disease.

In haemochromatosis measurement of the total body iron stores is particularly useful at the time of diagnosis and during the follow-up period after completion of venesection therapy when iron may accumulate again. Currently favoured methods are those based on the use of iron-chelating agents such as desferrioxamine and diethylenetriamine penta-acetic acid. However, in practice, since the liver is the major site of iron storage in this disease, the most frequently used method for assessing iron overload is by liver biopsy with subjective grading of the iron present in the sections when stained histochemically. Relatively few reports have appeared on the quantitative measurement of hepatic iron concentration in needle biopsy specimens mainly due to lack of suitable and accurate methods. Recently, however, Barry and Sherlock (1971) described a method which in a series of patients with varying degrees of iron overload gave values which correlated closely with estimates of total body iron stores by a chelation method. The technique we have been using is based on the measurement of iron by atomic absorption spectrophotometry which has many practical advantages and also has a different standard of reference. This paper describes the results obtained in 47 patients which have also been compared with histochemical subjective assessment of hepatic iron deposition and the quantitative measurement of total

chelatable body iron stores by the differential ferrioxamine test.

Patients and Methods

Of the 47 patients examined, 26 had primary idiopathic haemochromatosis and were at various stages of treatment by multiple venesection. Five close relatives of patients with haemochromatosis were also investigated and there were three patients with secondary iron overload due to prolonged oral iron ingestion in one and to congenital spherocytosis in two. Seven patients had cryptogenic cirrhosis and four alcoholic cirrhosis, two of the latter having presented with porphyria cutanea tarda. The remaining two patients on final analysis were considered to have no evidence of liver disease. Total chelatable body iron stores were estimated using the differential ferrioxamine test of Fielding (Fielding, 1965; Smith, Miller, Pitcher, Lestas, Dymock, and Williams, 1969).

Percutaneous liver biopsy was obtained in the majority of cases using the Tru-cut needle. After removal from the needle one half of the biopsy was taken for standard histological processing, which included staining by Perls's technique for iron, the amount present being graded from 1-4 according to Scheuer, Williams, and Muir (1962). The other half, with a wet weight of 10 to 20 mg, was gently blotted

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and then kept at -20°C until the time of determination, the most practical number of specimens to measure at one time being 20. After homogenization of the biopsy a small portion was taken for protein estimation using Lowry's technique. The remaining volume of homogenate was wet ashed using a 1:1 mixture of concentrated sulphuric acid and nitric acids as a digestant and hydrogen peroxide as oxidant. The resultant solution was quantitatively transferred to a 5 ml volumetric flask and made up to the mark with deionized water. The iron concentration was measured using a Southern Analytical A 3000 atomic absorption spectrophotometer and related to the protein concentration. The latter was adopted because of the poor reproducibility found when results were related to the wet weight.

Results

When attempts were made to study the reproducibility of the methods using specimens of liver obtained at necropsy the results were poor, probably due to the autolysis and degradation of protein which had occurred. However, excellent reproducibility was obtained in a study of a cirrhotic liver examined immediately after removal from a patient being treated by orthotopic liver transplantation. In multiple specimens with weights similar to those obtained by percutaneous liver biopsy and taken from different areas of the liver, the mean iron concentration was $1,126 \mu\text{g Fe/g protein}$ with a standard deviation of 98 and a coefficient of variation of 8.7%.

As expected from the composition of the series of patients examined, the value for iron concentration had a wide range (184 to $82,545 \mu\text{g Fe/g protein}$), but an analysis of the relation to the measurements of total chelatable body iron stores (Fig. 1) which was possible in 35 of the 47 patients, showed a close and statistically highly significant linear relationship ($r = 0.87$, $P < 0.001$). The intercept of the regression line does not differ significantly from zero.

In contrast, no correlation could be demonstrated between the values for iron concentration and the histochemical grading of hepatic iron (Fig. 2). Twenty patients had no discernible iron deposition but the iron concentrations in this group were very similar and the mean not statistically different from those found in the patients with grades 1 and 2 on histochemical assessment. Iron concentrations were higher in those patients with histochemical grades 3 and 4 and although the range was wide there was little overlap with the concentrations found in grades 1 and 2. The numbers of patients in each group are relatively small and if the findings in both grades 3 and 4 are combined together then the mean value for iron concentration of $28,888 \mu\text{g Fe/g protein}$ is significantly higher ($P < 0.001$) than the mean of

Fig. 1 Relationship between hepatic iron concentration and the total chelatable body iron stores measured by the differential ferrioxamine test. Uncrossed circles represent patients with primary idiopathic haemochromatosis or their relatives.

Fig. 2 Relationship between the iron concentration and the histochemical grading of hepatic iron deposition.

4,115 $\mu\text{g Fe/g}$ protein found when patients with grades 0, 1, and 2 are grouped together.

All the patients with histochemical grades 3 or 4 had primary haemochromatosis with one exception. The latter was a patient with cryptogenic cirrhosis and a portacaval shunt who had developed secondary iron overload. The histochemical grading was 3 and the value for iron concentration of 39,000 $\mu\text{g Fe/g}$ protein was the second highest in the series.

Discussion

The technique described here for measuring iron concentration in liver biopsy specimens has, in practice, proved simple to use. One theoretical objection is that it measures both haem and non-haem iron but the former represents a decreasing percentage of the total as the amount of iron deposited in the liver increases. According to Scott and McCoy (1944) liver tissue contained about 8% blood which could represent a significant amount if concentration of iron was low, and indeed this might explain some of the scatter of results found in the histochemical grades 0-2.

It was of interest to find such a close correlation between the measurements of hepatic iron concentration and total chelatable body iron as measured by the differential ferrioxamine test. That the latter gives a true measure of total body iron has been shown by the correlation found in previous studies between serial measurements by the differential ferrioxamine test and direct quantitation of the amount of iron removed during venesection therapy (Smith *et al*, 1969; Barry, Cartei, and Sherlock, 1970). The close correlation found between iron concentration and body iron stores would suggest that the theoretical objection raised to the former that there might be marked variation in the amounts of iron deposited in different areas of the liver does not hold.

The present findings, as well as those of Barry and Sherlock (1971), also show that the determination of hepatic iron concentration can be of practical use in obtaining a reliable estimate of total body iron. In many ways it is easier and quicker in laboratory time to measure liver iron concentration than to perform one of the chelation tests, particularly since most of the patients will need a liver biopsy for diagnostic reasons it being as necessary to assess the severity of underlying liver damage as it is to know the amount of iron deposition. However, accurate determinations of iron concentration are dependent on obtaining an adequate sized liver biopsy and the use of a Tru-cut needle makes this possible even in cirrhosis (Rake, Murray-Lyon, Ansell, and Williams, 1969). The fragmented biopsy often obtained in cirrhosis

when the Menghini needle is used rarely provides sufficient material for both histological examination and measurement of iron concentration.

The present findings also show that although the histochemical assessment of hepatic iron deposition into grades 1-4 was thought when initially described to represent a reliable quantitative grading of the iron deposition, this is not so. Hepatic iron concentration in those with grades 1 and 2 was not significantly higher than in those without discernible iron on histological examination. In support of this is the clinical observation that patients with cirrhosis and secondary iron overload in whom the iron grades of 1 or 2 are more usual than 3 or 4 often become anaemic quite rapidly when treated by venesections, 2-3 g of iron only being removed. This also applies to the affected relatives of patients with haemochromatosis in the majority of whom the hepatic siderosis falls within grades 1-2 (Williams, Scheuer, and Sherlock, 1962). Furthermore, measurements of the total chelatable body iron stores in such relatives have shown only slight increases or values within the normal range (Dymock, Miller, Godfrey, and Williams, 1970). Studies indicating a poor correlation between the total chelatable body iron and the histochemical iron grading (Smith, Studley, and Williams, 1967) may also be explained by our present findings.

In most of the previous studies dry weight has been adopted as the standard to which iron concentrations have been related. It has been suggested recently that if there is a significant amount of fatty change in the liver then this will give a falsely low value for hepatic iron concentration if dry weight is used as the standard because of the low water content of fat (Lundin, Lundvall, and Weinfeld, 1971). The protein standard such as we used is probably more satisfactory although the same authors have presented evidence suggesting that the protein concentration in the liver may be lower than normal in cirrhosis.

The finding of grades 1 and 2 hepatic siderosis in the presence of normal total body iron stores and with normal hepatic iron concentrations needs further comment. It has been suggested by Weinfeld, Lundin, and Lundvall (1968) that such stainable liver iron should be considered a normal finding but this does not explain why some liver biopsies with similar measurements of iron concentration have histochemically demonstrable iron deposition and others do not. Another aspect which requires further study is whether in the apparently affected relatives of patients with haemochromatosis the minor hepatic siderosis develops as a result of redistribution within the body without any overall increase in total body iron.

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