Measurement of iron stores in cirrhosis using diethylenetriamine penta-acetic acid

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SUMMARY The chelating agent diethylenetriamine penta-acetic acid was used to measure iron stores in 83 patients with chronic liver disease. Iron chelation was normal in patients with chronic cholestasis. Chelation was increased above the control range in 14 out of 26 patients with alcoholic cirrhosis, in nine out of 28 patients with non-alcoholic cirrhosis, and in 11 out of 15 cirrhotics with a portacaval anastomosis. Iron stores in excess of 1.5 g were predicted from the results in 24 subjects; however, in only three were the values in the range found in propositi with untreated idiopathic haemochromatosis. Increased chelation did not correlate with hepatocellular impairment *per se* but was associated in 18 cases with surgical or large spontaneous portal systemic shunts. Exogenous factors for excess iron were present in three cases with alcoholic cirrhosis and portal systemic collaterals in one, but no special factor apart from alcoholism was apparent in the remainder.

The correlation between chelatable iron and stainable liver iron content was not close and was better in haemochromatosis than in other forms of cirrhosis; in some cases considerable siderosis was present with normal or only slightly increased chelation values.

A close association between iron excess and cirrhosis has long been recognized, and cirrhosis is regarded as potentially an iron-loading disorder (Kent and Popper, 1968). Alcoholism, particularly heavy wine consumption (Gilbert and Grenet, 1896; André, 1961; MacDonald, 1964; Perman, 1967), is accepted as an important factor, and portal systemic shunting may also have a pathogenetic role (Tuttle, Figueroa, and Grossman, 1959; Hoffbauer, 1960; Tisdale, 1961; Schaefer, Amick, Oikawa, and Schiff, 1962; Grace and Balint, 1966; Nixon, 1966). However, some degree of siderosis is common in liver disease generally (Zimmerman, Chomet, Kulesh, and McWhorter, 1967; Williams, Williams, Scheuer, Pitcher, Loiseau, and Sherlock, 1966) and its significance is often difficult to interpret. Quantitative estimates of storage iron in a large

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series of patients with cirrhosis have yet to be reported.

Chelating agents, such as diethylenetriamine penta-acetic acid (DTPA), provide an indirect means of measuring total body storage iron. A close and apparently specific relationship was found between DTPA-chelatable body iron and mobilizable storage iron in patients with iron excess, enabling the size of the stores to be reliably predicted (Barry, Cartei, and Sherlock, 1970). The present paper describes the findings with this technique in patients with chronic liver disease.

Methods

The methods used for determination of DTPA-chelatable iron, serum iron, and total iron-binding

capacity, and for assessing stainable liver iron, have already been described (Barry *et al*, 1970).

Patients Studied

Observations were made in 83 patients with chronic liver disease. The subjects (Table I) were divisible into groups with chronic cholestasis (14 cases), alcoholic cirrhosis (26 cases), non-alcoholic cirrhosis (28 cases), and cirrhosis with end-to-side portacaval anastomosis (15 cases). The control groups and patients with untreated and treated idiopathic haemochromatosis have been described previously (Barry *et al*, 1970). In addition there were seven patients with iron excess secondary respectively to sideroblastic anaemia (four cases), thalassaemia major, hereditary spherocytosis, and aplastic anaemia with multiple blood transfusions (one case each).

The diagnosis in the patients with chronic cholestasis was gallstones (two cases), primary biliary cirrhosis (three cases), traumatic and malignant biliary stricture (three cases respectively), drug-induced cholestasis (two cases), and Dubin-Johnson jaundice (one case). The mean duration of jaundice was 16 months (range two weeks-six years).

At least 12 of the patients with alcoholic cirrhosis had been drinking heavily up to the time of admission to hospital. Two were heavy wine drinkers, the others consuming mainly beer and spirits. One had received multiple transfusions elsewhere for a haemolytic anaemia. Five cases were referred following variceal haemorrhage having been transfused with 6-22 units of blood (mean 17.2). One had chronic encephalopathy due to large spontaneous collaterals.

A diagnosis of active chronic hepatitis had been made at some time during the clinical course in 11 of the patients with non-alcoholic cirrhosis. Apart from three cases of Wilson's disease the pathogenesis of the cirrhosis in the remainder was unknown. Seven were referred following variceal haemorrhage, six having been transfused with 4-16 units of blood (mean 6.4); one case had received 91 units. Seven patients

required treatment for chronic portal systemic encephalopathy.

The portacaval anastomosis group comprised 13 cases of cryptogenic cirrhosis, one case of alcoholic cirrhosis, and one with probable primary biliary cirrhosis. The shunt had been present for a mean of 3.8 years (range 0.8-6.9 years). Nine patients required treatment for chronic encephalopathy.

Results

Details of the groups studied are summarized in Table I and the results are shown in Figure 1.

Fig. 1 DTPA-chelatable iron in liver diseases and idiopathic haemochromatosis. Δ = secondary haemochromatosis. The horizontal broken line indicates the upper limit of the control range.

Group (no.)	Hb (g/100 ml)	Serum Iron (μ g/100 ml)	Total Iron-binding Capacity (μ g/100 ml)	Urinary Iron (μ g)	DTPA-chelatable Iron (mg)	No. with Hepatic Siderosis/No. Biopsied
Control males (16)	14.9 $\pm$ 0.3	121 $\pm$ 8	345 $\pm$ 14	160 $\pm$ 20	0.87 $\pm$ 0.90	—
Control females (9)	14.3 $\pm$ 0.4	132 $\pm$ 19	395 $\pm$ 17	90 $\pm$ 10	0.54 $\pm$ 0.70	—
Untreated idiopathic haemochromatosis (7)	15.0 $\pm$ 0.6	300 $\pm$ 26	327 $\pm$ 22	9,959 $\pm$ 902	22.46 $\pm$ 1.93	7/7
Treated idiopathic haemochromatosis (9)	12.3 $\pm$ 0.6	66 $\pm$ 12	392 $\pm$ 15	157 $\pm$ 20	0.78 $\pm$ 0.80	0/6
Secondary haemochromatosis (7)	9.8 $\pm$ 1.1	271 $\pm$ 27	278 $\pm$ 25	9,066 $\pm$ 984	29.38 $\pm$ 3.12	4/4
Chronic cholestasis (14)	12.3 $\pm$ 0.4	114 $\pm$ 11	420 $\pm$ 17	166 $\pm$ 16	0.98 $\pm$ 0.10	0/13
Alcoholic cirrhosis (26)	13.4 $\pm$ 0.5	140 $\pm$ 14	320 $\pm$ 16	880 $\pm$ 292	4.26 $\pm$ 1.12	7/20
Non-alcoholic cirrhosis (28)	12.8 $\pm$ 0.3	114 $\pm$ 12	328 $\pm$ 16	334 $\pm$ 74	1.90 $\pm$ 0.29	7/27
Portacaval shunt (15)	12.8 $\pm$ 0.4	170 $\pm$ 11	260 $\pm$ 15	2,169 $\pm$ 780	6.53 $\pm$ 1.57	4/5

Table I Groups studied¹

¹Mean $\pm$ SEM value given

CONTROLS

The findings in controls have already been described (Barry *et al*, 1970). The values for DTPA-chelatable iron ranged from 0.29 to 1.59 mg. The upper limit of normal was taken as 1.60 mg.

IDIOPATHIC AND SECONDARY HAEMOCHROMATOSIS

The findings in the patients with secondary haemochromatosis did not differ significantly from those previously described in propositi with idiopathic haemochromatosis (Barry *et al*, 1970).

CHRONIC CHOLESTASIS

DTPA-chelatable iron showed no significant deviation from the control range in the 14 patients studied. The mean value was higher than in controls but the difference was not significant. Many of the patients had a mild hypochromic normocytic anaemia, not obviously due to iron deficiency, and it is possible that redistribution of iron from the haemoglobin compartment to the stores may account for the slightly higher chelation values than in the controls. This was supported by the presence of a weak inverse correlation between DTPA-chelatable iron and haemoglobin concentration in the 10 male patients ($r = -0.70$, $P < 0.05$).

ALCOHOLIC CIRRHOSIS

DTPA-chelatable iron was raised in 14 of the 26 subjects, the values in nine indicating an iron load > 1.5 g; two of these were heavy wine

drinkers, another had been transfused on many occasions for a haemolytic anaemia, and one had chronic encephalopathy due to large spontaneous collaterals.

NON-ALCOHOLIC CIRRHOSIS

DTPA-chelatable iron was raised in nine of the 28 patients, but in only four were the values indicative of an iron load > 1.5 g. Six of the patients with raised values had chronic encephalopathy due to large spontaneous collaterals.

FACTORS ASSOCIATED WITH INCREASED CHELATION

These were examined using the combined results of the alcoholic and non-alcoholic cirrhosis groups.

Siderosis

Stainable liver iron was demonstrable in 14 out of the 47 cirrhotics biopsied. It was present in only three (9.4%) of 28 biopsied cases with normal DTPA-chelatable iron, as compared with 11 (58%) of 19 biopsied cases with increased chelation. The correlation between stainable liver iron grade and DTPA-chelatable iron was not close, being better in haemochromatosis (Fig. 2) than in other forms of cirrhosis (Fig. 3).

Poor liver cell function

The 34 patients without demonstrable hepatic siderosis were divided into two groups, those with normal (25 cases) and those with increased chelatable iron (8 cases) respectively. As shown in Table II these groups did not differ significantly

Fig. 2 Relation between DTPA-chelatable iron and stainable liver cell iron in patients with haemochromatosis.

Fig. 3 Relation between DTPA-chelatable iron and stainable liver iron in patients with cirrhosis. Δ = portacaval anastomosis.

	Normal Chelation	Increased Chelation	t Test
No. in group	26	8	—
DTPA-chelatable iron (mg)	1.14 ± 0.06	2.71 ± 0.35	—
Hb (g/100 ml)	13.0 ± 0.4	12.2 ± 0.7	P>0.3
Serum iron (μg/100 ml)	110 ± 12	114 ± 22	P>0.8
Total iron-binding capacity (μg/100 ml)	345 ± 16	337 ± 22	P>0.7
Serum bilirubin (mg/100 ml)	2.0 ± 0.6	3.0 ± 1.7	P>0.4
Asparate transaminase (IU/l)	42 ± 12	40 ± 8	P>0.4
Serum albumin (g/100 ml)	3.8 ± 0.2	3.6 ± 0.4	P>0.4

Table II *Cirrhosis with siderosis: comparison of groups with normal and increased DTPA-chelatable iron*¹

¹Mean ± SEM values are given.

with respect to liver function, suggesting that increased iron chelation by DTPA in cirrhosis is not a function of hepatocellular impairment *per se*, as has been found with desferrioxamine.

Spontaneous portal systemic shunts

Seven (30.4%) of the 23 cirrhotics with increased DTPA-chelatable iron had spontaneous portal systemic collaterals of sufficient size to cause symptomatic encephalopathy. Only one (3.2%) of the 31 patients with normal chelatable iron had encephalopathy. Details of the patients with encephalopathy are summarized in Table III.

Serum iron concentration

The relationship between serum iron and DTPA-chelatable iron in haemochromatosis differed from that in other forms of cirrhosis and in neither case could be satisfactorily expressed as a single mathematical function. This will be the subject of a separate paper. Increased chelation was associated with a serum iron level > 205 μg/100 ml (the 95% upper confidence limit of our normal range) in 34 out of 42 observations in haemochromatosis, and in six out of 25 observations in alcoholic and cryptogenic cirrhosis. A raised serum iron level was associated with normal chelatable iron only once in 42 observations.

PORTACAVAL ANASTOMOSIS

DTPA-chelatable iron was normal in four and elevated in 11 patients with an end-to-side portacaval anastomosis. The mean value was higher than that in other cirrhotics (Table I) and was associated with a higher mean serum iron level and a greater incidence of siderosis. However, in only one case (the only alcoholic in the group) was iron chelation in the range observed in propositi with idiopathic haemochromatosis.

Chelatable iron in the portacaval patients was significantly greater than in cirrhotics with recent haematemesis awaiting shunt surgery, though not in cirrhotics with chronic encephalopathy due to large spontaneous collaterals (Fig. 4). Details of these groups are summarized in Table III. The portacaval patients differed significantly from the cirrhotics studied before operation with regard to serum iron and total iron-binding capacity and in the incidence of hepatic siderosis, though not in the amount of exogenous iron administered throughout the clinical course. Comparison of the portacaval patients with the cirrhotics with large spontaneous shunts revealed no significant difference in iron status or liver function. However, a similar comparison with all other cryptogenic cirrhotics showed that chelatable iron and serum iron values were significantly higher in the portacaval patients although the two groups were comparable with regard to liver function (Table III). These findings suggest that in cirrhosis portal systemic shunting *per se* is associated with increased iron chelation, the effect occurring independently of hepatocellular impairment.

In the portacaval patients chelatable iron showed no correlation with time since operation ($r = -0.22$) or with the total quantity of iron or blood administered throughout the clinical course ($r = -0.07$).

Discussion

Iron chelation by desferrioxamine has been found to vary significantly with hepatocellular

	Surgical Shunts ¹	Cirrhosis and Recent Haematemesis	Cirrhosis with Encephalopathy	All Other Cryptogenic Cirrhosis
No. in group	15	10	8	16
DTPA-chelatable iron (mg)	6.53 ± 1.57	1.18 ± 0.15 ^a	3.64 ± 0.66	1.62 ± 0.34 ^a
Serum iron (μg/100 ml)	170 ± 11	98 ± 17 ^a	148 ± 28	116 ± 17 ^a
Total iron-binding capacity (μg/100 ml)	260 ± 15	393 ± 21 ^a	265 ± 25	321 ± 22
Hb (g/100 ml)	12.8 ± 0.4	12.9 ± 0.4	12.7 ± 0.7	13.3 ± 0.4
Iron administered (g) ⁴	2.42 ± 0.41	4.38 ± 1.87	—	—
No. with siderosis/no. biopsied	4/5	0/10	4/6	3/15
Serum bilirubin (mg/100 ml)	2.7 ± 0.4	1.3 ± 0.3 ^a	2.1 ± 0.6	3.1 ± 1.1
Asparate transaminase (IU/l)	28 ± 4	19 ± 3 ^a	27 ± 5	56 ± 18
Serum albumin (g/100 ml)	3.3 ± 0.1	4.2 ± 0.1 ^a	3.0 ± 0.3	3.7 ± 0.3

Table III *Comparison of surgical shunt patients with other cirrhosis groups*⁵

¹Test of difference (Wilcoxon) from surgical shunt group not significant unless indicated.

^aP<0.01.

^bP<0.02.

⁴By IM injection or as transfused haemoglobin-iron.

⁵Mean ± SEM values are given.

Fig. 4 Comparison of DTPA-chelatable iron in patients with a portacaval anastomosis, patients with recent haematemesis awaiting shunt surgery, and patients with chronic encephalopathy (PSE) due to spontaneous collaterals.

function in chronic alcoholism and in other forms of cirrhosis (Schnack and Wewalka, 1964; Barry, Cartei, and Sherlock, 1969; Lundvall and Weinfeld, 1969), and in our experience does not provide a reliable index of body storage iron in patients with chronic liver disease. However, the same limitation need not necessarily apply to DTPA, which differs from desferrioxamine on both chemical and pharmacological grounds. Studies in patients with iron excess indicated that DTPA-chelatable iron specifically reflects the mobilizable iron stores (Barry *et al*, 1970); similar studies in the same patients with desferrioxamine showed that this compound derived significant amounts of iron from non-storage sources. Analysis of the present results in subjects with chronic liver disease revealed no tendency for the effect of DTPA to vary with hepatocellular function *per se*, the normal findings in patients with chronic cholestasis, in particular, contrasting sharply with the greatly increased effect of desferrioxamine in this group (Barry *et al*, 1969). Furthermore, in as yet unpublished studies we have found a very close correlation between DTPA-chelatable iron and total liver iron concentration in patients with various forms of cirrhosis as well as in those with idiopathic and secondary haemochromatosis. We therefore feel justified in treating the present chelation results as if they solely reflected body storage iron,

predicting the latter by means of the previously defined relationship (Barry *et al*, 1970): iron stores (g) = 0.46 × DTPA-chelatable iron (mg).

Thirty-four out of a total of 69 cirrhotics had chelation values within the control range, corresponding to a total body storage iron content of less than 750 mg. The values in another 11 were elevated but indicative of an iron store of less than 1.5 g. Twenty-four cirrhotics had a predicted iron load exceeding 1.5 g; 10 of these had a portacaval anastomosis, nine had alcoholic cirrhosis, and four (including one of the alcoholics) had chronic encephalopathy due to large portal systemic collaterals; one patient with cryptogenic cirrhosis, who did not fall into any of the above categories, had an iron load of 3 g for which no cause was apparent.

Although it is widely believed that iron excess and chronic alcoholism are frequently associated, recent quantitative studies of liver storage iron in alcoholics without cirrhosis have given conflicting results, high values being found in Queensland (Powell, 1966) and normal values in Sweden (Lundvall, Weinfeld, and Lundin, 1969). In the present study iron chelation was increased in 14 out of 26 alcoholics with cirrhosis. Obvious exogenous factors for iron excess, in the form of heavy wine consumption or multiple blood transfusions for a haemolytic anaemia, were present in the three patients with the highest values. The remaining cases were all beer and spirit drinkers and in only one of these, whose iron load was confirmed by subsequent venesection, were the predicted stores greater than 2.8 g. These findings therefore support the view that alcoholic cirrhosis is potentially an iron-loading state but suggest that in the absence of excessive iron intake, as in wine drinkers, the degree of accumulation falls far short of that found in propositi with idiopathic haemochromatosis. In view of the low iron content of popular British beers and spirits (Table IV) it is necessary to postulate increased absorption from an essentially normal dietary iron intake to account for the moderate iron excess in our patients. This might result from the known ability of alcohol to increase iron absorption (Charlton, Jacobs, Seftel, and Bothwell, 1964) or perhaps be secondarily related to hepatic or pancreatic injury.

Beverage (No. of brands tested)	Iron Concentration (mg/l) ^a	
	Mean ± SEM	Range
Red wine (5)	12.25 ± 1.21	8.35-16.83
White wine (4)	7.50 ± 1.41	3.17-11.08
Sherry and port (4)	4.65 ± 0.88	2.82- 7.32
Cider (1)	2.64	
Stout (1)	0.81	
Beer (4)	0.27 ± 0.04	0.22 - 0.37
Whisky (1)	0.15	
Gin (1)	0.06	

Table IV Iron content of some alcoholic beverages available in London

^aDetermined by wet ashing.

The association between increased iron chelation and the presence of large portal systemic collaterals applied both to patients with surgical shunts and to cirrhotics with spontaneous collaterals of sufficient size to cause chronic encephalopathy. Comparison of these with other cirrhotic groups suggested that the effect was related to the presence of the shunt rather than to any concomitant deterioration in liver function, and the higher serum iron and lower values of total iron-binding capacity as well as the greater incidence of hepatic siderosis were further indications of iron excess in these patients. Experimental studies in dogs and rats have also suggested that hepatic siderosis results from the presence of the shunt *per se*, and that it may develop in the absence of cirrhosis or detectable abnormality on light microscopy (Doberneck, Nunn, Johnson, and Chun, 1963; Rubin, Kohan, Tomita, and Jacobson, 1964); both the type and the size of the shunt appear to be important in these animals. The development of a haemochromatosis-like syndrome in man has been reported after end-to-side portacaval anastomosis (Tuttle *et al*, 1959; Hoffbauer, 1960; Grace and Balint, 1966), splenorenal anastomosis (Tisdale, 1961; Schaefer *et al*, 1962), and in association with spontaneous collaterals (Nixon, 1966). The present results suggest that the occurrence of iron excess in such patients may not be as rare as has been believed although in but one case (the only alcoholic) was iron chelation in the range observed in propositi with untreated idiopathic haemochromatosis. Grace and Balint (1966) have also noted that the tissue iron levels in a patient with post-shunt siderosis were far less than in idiopathic haemochromatosis, although the distinction was not possible on pathological grounds. The pathogenesis of iron excess after portacaval anastomosis remains obscure; in our patients the administration of iron or blood did not emerge as a likely factor, and the chelation values showed no correlation with the time since operation. Serial iron absorption measurements combined with quantitative determination of tissue iron stores may resolve this problem.

There was a general correlation between iron chelation and stainable liver iron content. Although the correlation was better in haemochromatosis than in other forms of cirrhosis, in both groups the range of chelation values for each histochemical grade was wide and the overlap between different grades considerable. A similar relationship has been found between non-haem liver iron concentration and stainable liver iron (Morgan and Walters, 1963; Weinfeld, Lundin, and Lundvall, 1968; Lundvall *et al*, 1969) and it is apparent that iron stores may be normal despite the finding of considerable siderosis.

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