Gut, 1969, **10**, 697-704

Differential ferrioxamine test in haemochromatosis and liver diseases

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SUMMARY The effect of desferrioxamine is examined in more than 100 patients with liver disease, including haemochromatosis, using the differential ferrioxamine test.

The procedure gives a reasonably accurate estimate of the size of the iron stores, as determined by multiple venesection, in patients with idiopathic haemochromatosis. Since desferrioxamine is not specific for storage iron, unequivocally abnormal results are not obtained unless the iron load exceeds about 2·3 g.

In other forms of liver disease the effect of desferrioxamine is generally increased compared with that in controls. The results show no correlation with the serum iron level or the degree of hepatic siderosis. High values are usual in the presence of jaundice and overlap the range found in untreated haemochromatosis, adding to other evidence that desferrioxamine can derive iron from a hyperchelatable source unrelated to the stores.

It is concluded that in liver diseases other than haemochromatosis the results of the test do not reliably reflect body storage iron content.

Although many workers have shown that the iron excretion produced by the chelating agent desferrioxamine reflects the size of the iron stores in haemochromatosis, conflicting results have been obtained in other forms of liver disease. Verloop (1964) and Vannotti (1964) considered that desferrioxamine could be used to distinguish between cirrhosis with siderosis and idiopathic haemochromatosis, and Smith, Studley, and Williams (1967), using the differential ferrioxamine test, agreed with this view. Walsh, Mass, Smith, and Lange (1965), however, found that alcoholic cirrhosis with siderosis could not be separated from idiopathic haemochromatosis with desferrioxamine, and Schnack and Wewalka (1964) concluded that the increased iron excretion observed in half their large series of cirrhotics was more closely related to poor liver function that to siderosis.

We have used the differential ferrioxamine test (Fielding, 1965) to measure the effect of desferrioxamine in more than 100 patients with various forms of liver disease. Our results indicate that the procedure can be used to measure, with considerable accuracy, the size of the iron stores in idiopathic haemochromatosis. In other forms of liver disease, however, high results are commonly obtained in the absence of any evident disorder of iron metabolism.

MATERIALS AND METHODS

DIFFERENTIAL FERRIOXAMINE TEST The procedure of Fielding (1965 and 1967) was followed in detail. In principle, desferrioxamine (8·33 mg/kg), labelled with a trace quantity of ^{59}Fe-ferrioxamine, is given intravenously. The percentage of the injected label excreted in a six-hour urine is determined; the total urinary iron excretion is estimated colorimetrically as ferrioxamine. These values permit the total quantity of iron chelated *in vivo* to be calculated. This result (F_V) is expressed as μg ferrioxamine formed/kg body weight.

LABORATORY METHODS Iron-free reagents and receptacles were employed (Barry, 1968). Ferrioxamine in aqueous solution and in urine was estimated by the method of Fielding and Brunström (1964), using the modifications of Barry and Cartei (1968) for jaundiced urine, and for the preparation of standard ferrioxamine solutions; the results were expressed as ferrioxamine base. Serum iron and total iron-binding capacity (TIBC) were determined by AutoAnalyzer (Young and Hicks, 1965), our normal range for the serum iron being 60 to 205 μg/100 ml. Liver biopsy specimens were stained for iron by Perls' stain and the degree of parenchymal siderosis graded on a 1 to 4 scale (Scheuer, Williams, and Muir, 1962).

PATIENTS Control subjects and patients with primary and secondary haemochromatosis, cholestatic jaundice,

TABLE I

SUMMARY OF FINDINGS IN DIFFERENT LIVER DISEASES (MEAN ± SEM VALUES)

Group (No. of Cases)	*Hb (g/100 ml)*	*Serum Iron (μg/100 ml)*	*TIBC (μg/100 ml)*	*Serum Bilirubin (mg/100 ml)*	*F_v (μg ferrioxamine/kg)*
Control males (15)	15·0 ± 0·3	119 ± 11	340 ± 11	—	217 ± 18
Control females (8)	13·9 ± 0·3	122 ± 16	366 ± 23	—	217 ± 39
Idiopathic haemochromatosis					
Before treatment (7)	14·7 ± 0·7	260 ± 31	286 ± 21	1·0 ± 0·4	1,721 ± 271
After treatment (6)	11·6 ± 0·7	71 ± 21	396 ± 16	<0·8	122 ± 17
Secondary haemochromatosis (4)	9·2 ± 0·8	226 ± 22	234 ± 21	3·1 ± 1·6	3,245 ± 353
Chronic cholestasis (22)	11·8 ± 0·5	99 ± 7	408 ± 20	7·6 ± 1·3	834 ± 75
Alcoholic cirrhosis (20)	12·5 ± 0·5	118 ± 13	332 ± 15	4·4 ± 1·5	584 ± 135
Cryptogenic cirrhosis (27)	12·3 ± 0·3	141 ± 13	310 ± 14	3·0 ± 1·1	482 ± 47
Portacaval anastomosis (14)	13·1 ± 0·4	150 ± 16	286 ± 21	3·5 ± 0·1	899 ± 231
Acute hepatitis (16)	13·3 ± 0·3	212 ± 25	421 ± 20	10·6 ± 3·1	892 ± 143

TABLE II

RESULTS IN PATIENTS WITH IRON OVERLOAD DURING VENESECTION

Case No.	*Sex Age*	*Serum Iron (μg/100 ml)*	*Serum TIBC (μg/100 ml)*	*Hb (g/100 ml)*	*Iron Removed by Venesection (g)*	*Stainable Liver Iron (Grade)*	*F_v (μg ferrioxamine/kg)*	
Idiopathic haemochromatosis								
1	M, 43	265	315	15·6	nil	3	1,161	Before treatment
		295	318	14·0	5·12	2	825	—
		66	405	12·4	10·76	0	56	Treatment complete
2	F, 52	230	255	15·1	nil	3	1,508	Before treatment
		185	195	13·4	4·12	—	1,093	—
		202	240	13·7	8·12	3	755	3·15 g iron subsequently removed
3	M, 43	238	273	14·0	nil	3	1,880	Before treatment
		254	345	13·5	2·73	—	1,303	7·65 g iron subsequently removed
4	M, 39	292	330	15·4	nil	3	1,133	9·10 g iron subsequently removed
5	M, 35	424	435	17·7	nil	4	1,303	8·53 g iron subsequently removed
6	M, 48	271	324	14·6	13·77	—	797	—
		160	318	13·2	20·80	0	87	Treatment complete
7	M, 54	180	394	12·8	10·59	—	439	—
		66	582	14·0	11·90	—	363	—
		34	417	10·4	12·73	0	148	Treatment complete
8	M, 58	250	300	15·4	9·80	0	201	0·83 g iron subsequently removed
9	M, 58	320	345	16·8	nil	3	865	Before treatment
		310	360	14·5	4·48	3	494	1·60 g iron subsequently removed
10	M, 27	285	372	16·0	nil	1	342	Before treatment brother of case 1
		115	372	14·3	1·69	—	106	Treatment complete
Haemochromatosis secondary to hereditary spherocytosis								
11	F, 40	279	306	14·5	19·0	2	448	—
		23	441	9·3	21·3	0	167	Treatment complete
Cirrhosis with siderosis								
12	F, 57	110	190	10·8	nil	3	366	1·70 g iron subsequently removed

virus hepatitis, alcoholic and cryptogenic cirrhosis, and a portocaval anastomosis were studied. The details of these groups are summarized in Table I. Selection of the controls was based on a normal haemoglobin concentration, serum iron, and TIBC; four were healthy volunteers, and the remainder were fully informed patients in a medical ward whose clinical record indicated that disordered iron metabolism or erythropoiesis were unlikely to be present.

VENESECTION TECHNIQUE To determine the correlation between F_v and body storage iron content 12 patients with iron overload (Table II) were studied prospectively in relation to multiple venesection therapy. Blood was taken at a measured rate of 500 to 600 ml/week, except in the final stages when venesections were spaced at two- to four-week intervals. The completion of treatment was indicated by a fall in serum iron to normal or low levels and by inability to restore the haemoglobin concentra-

tion within two weeks of a venesection; absent stainable liver iron was confirmed by biopsy after treatment in eight cases. At this stage the size of the iron stores before the start of treatment, or at any time during its course, was calculated from the quantity of haemoglobin-iron that had been subsequently removed (Balcerzak, Westerman, Lee, and Doyle, 1966); the calculation involves a correction for circulating haemoglobin deficit. Predicted blood volume for height and weight was calculated by the formulae of Nadler, Hidalgo, and Bloch (1962).

RESULTS

CONTROLS The findings are summarized in Table I. F_V values ranged from 57 to 419 μg ferrioxamine/kg. Only two subjects had values below 100 μg/kg, defined as the lower limit of normal by Fielding, O'Shaughnessy, and Brunström (1965): one had formerly been a regular blood donor and the other had failed to take iron supplements during her second pregnancy six months previously. The 99% upper confidence limit for the combined results of both sexes was 465 μg/kg.

HAEMOCHROMATOSIS F_V values ranged from 1,130 to 3,190 μg/kg in seven patients with untreated idiopathic haemochromatosis, and from 2,424 to 3,835 μg/kg in four with secondary haemochromatosis (due to sideroblastic anaemia in three cases and hereditary spherocytosis in one). In six patients tested one to 12 weeks after the completion of treatment the range was 56 to 167 μg/kg.

In 12 patients with iron overload studied before and during venesection therapy a highly significant correlation was found between F_V and the quantity of haemoglobin-iron subsequently removed (Fig. 1).

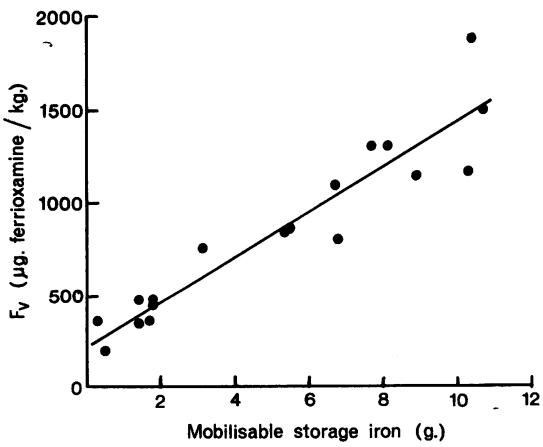

FIG. 1. *Relation between F_V and body storage iron, as determined by venesection. $y = 230 + 121x$, $r = 0{\cdot}94$.*

The standard error of the mean estimated for storage iron on F_V was 0·30 g. The regression intercept differed significantly from the origin ($t = 3{\cdot}42$, $P < 0{\cdot}01$) implying that iron was also chelated from a tissue source other than the stores.

Serum iron showed no tendency to fall until the terminal stages of venesection. The relation between F_V and stainable liver iron is shown in Figure 2.

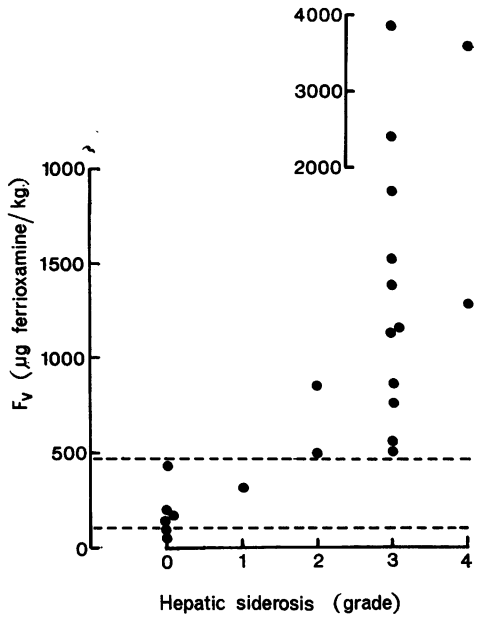

FIG. 2. *Relation between F_V and stainable liver iron in haemochromatosis.*

CHRONIC CHOLESTASIS The results were elevated in 19 of the 22 cases, and overlapped the range found in untreated idiopathic haemochromatosis (Fig. 3). Thirteen of these patients had large duct obstruction due to gallstones, traumatic stricture, or primary carcinoma, and five had primary biliary cirrhosis. None had a raised serum iron level, and hepatic siderosis was absent in the 17 in whom tissue was available for examination. The F_V values showed no correlation with the serum bilirubin or alkaline phosphatase levels. A mild hypochromic normocytic anaemia was commonly present but a reticulocyte count >2% was unusual. The highest F_V values were obtained in patients with jaundice of most recent onset (Fig. 4) in whom the haemoglobin concentration was normal.

CIRRHOSIS Approximately half the patients with cirrhosis, irrespective of aetiology, had F_V values above the normal range (Fig. 3). Although the means for the alcoholic and cryptogenic groups did not

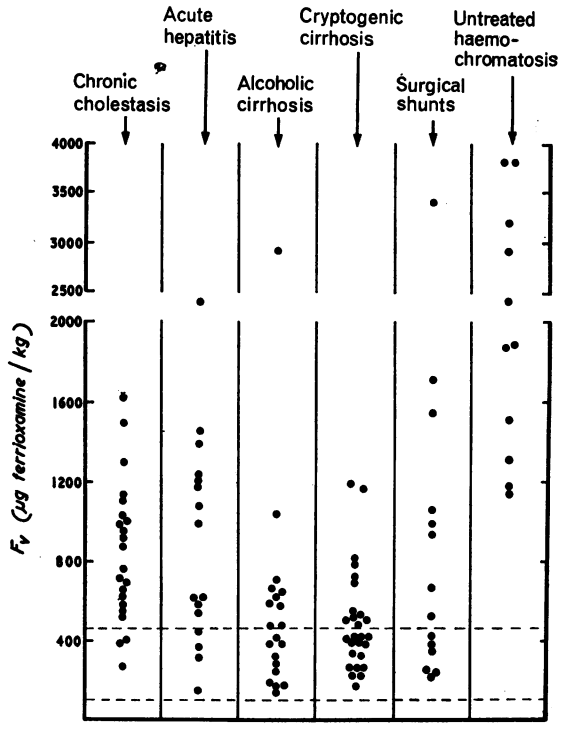

FIG. 3. *F_v in liver diseases. Horizontal broken lines define the normal range.*

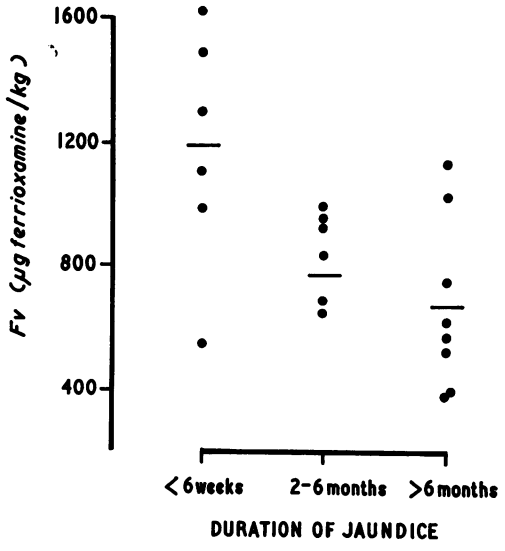

FIG. 4. *Relation between F_v and duration of jaundice in patients with chronic cholestasis.*

differ significantly from each other (Table I) both were significantly different from the control value ($P < 0.01$ and < 0.001 respectively).

The relationship between F_V and clinical status was examined for the clinical categories shown in Table III, in which the results for the alcoholic and cryptogenic groups have been combined. Values above the normal range were obtained in only three patients with good liver function, arbitrarily defined by a serum bilirubin level of < 2 mg/100 ml and absent portal systemic encephalopathy; however, the mean for this group was significantly higher than the control value ($t = 2.55$, $P < 0.02$). The mean for the group with jaundice was higher than that for the non-jaundiced group, the difference being significant ($t = 2.06$, $P < 0.05$). The findings in the patients with variceal haemorrhage and with encephalopathy varied according to the presence of jaundice, but the tendency to higher values in those with siderosis could not be attributed to this effect.

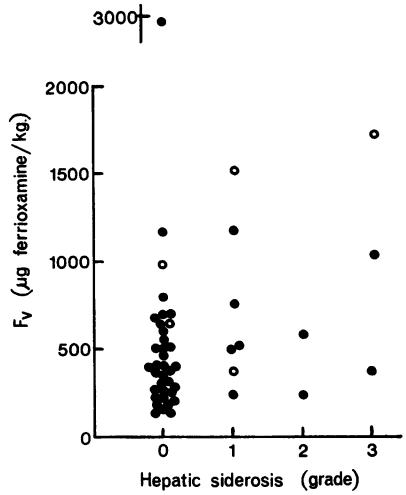

FIG. 5. *Relation between F_v and stainable liver iron in patients with cirrhosis (●) and with a portacaval shunt (○).*

There was no correlation with the serum iron ($r = 0.18$), which was raised in only five cases, or with the degree of hepatic siderosis (Fig. 5).

PORTOCAVAL ANASTOMOSIS F_V values were high in eight of the 14 cases (Fig. 3). The mean of 899 ± 231 μg/kg for the group differed significantly ($P < 0.02$) from the value for cirrhotics with good liver function shown in Table III, but not from those with jaundice. As shown in Table IV the patients with raised F_V values tended to have a higher serum iron and bilirubin concentration, and a markedly lower TIBC, than those with normal F_V values.

TABLE III

MEAN (± SEM) VALUES IN PATIENTS WITH CIRRHOSIS SUBDIVIDED ACCORDING TO CLINICAL STATUS

Clinical Status	*No. Alcoholic*	*No. Cryptogenic*	*Hb (g/100 ml)*	*Serum Iron (μg/100 ml)*	*TIBC (μg/100 ml)*	*Serum Bilirubin (mg/100 ml)*	*F_v (μg ferrioxamine/kg)*
Good liver function	9	9	12·9 ± 0·4	130 ± 12	368 ± 12	1·2 ± 0·2	347 ± 51
Jaundice	9	13	11·9 ± 0·4	135 ± 13	290 ± 15	7·3 ± 1·4	639 ± 117
Recent haematemesis	5	6	11·5 ± 0·4	112 ± 16	357 ± 20	1·9 ± 0·7	362 ± 60
Encephalopathy	1	7	12·7 ± 0·7	163 ± 26	286 ± 21	2·3 ± 0·4	441 ± 57
Hepatic siderosis	2	6	11·7 ± 0·9	175 ± 28	269 ± 22	2·2 ± 0·7	648 ± 113

TABLE IV

COMPARISON OF GROUPS OF CASES WITH PORTACAVAL SHUNT WITH NORMAL AND HIGH F_v VALUES

Group	*n*	*F_v (μg/kg)*	*Serum Iron (μg/100 ml)*	*TIBC (μg/100 ml)*	*Hb (g/100 ml)*	*Serum Bilirubin (mg/100 ml)*	*Years Since Operation*
Normal F_v	6	300 ± 34	116 ± 29	345 ± 20	12·4 ± 0·5	2·0 ± 0·6	5·9 ± 0·8
High F_v	8	1,348 ± 324	175 ± 14	241 ± 23	13·7 ± 0·7	4·7 ± 1·7	2·6 ± 0·8
p[1]			>0·1	<0·02	>0·1	>0·1	<0·01

[1]Wilcoxon's test.

Although this suggested that both iron excess and poor liver function were operative factors it was not possible in most cases to differentiate between the role of each. The serum iron level was above normal in only one patient. Hepatic siderosis was present in three of the five cases biopsied (Fig. 5). F_v tended to be lowest in those in whom the shunt had been present longest, possibly reflecting the fact that the longest survivors were those with the best liver function.

VIRUS HEPATITIS The F_v values in the acute phase were very similar to those in chronic cholestasis; only four of the 16 patients had values in the normal range (Fig. 3). These results showed no correlation with the serum iron level (Fig. 6); the higher values tended to occur in the more deeply jaundiced subjects (Fig. 7). In seven out of nine patients in whom repeated tests were performed the F_v fell progressively during the course of the illness, returning to

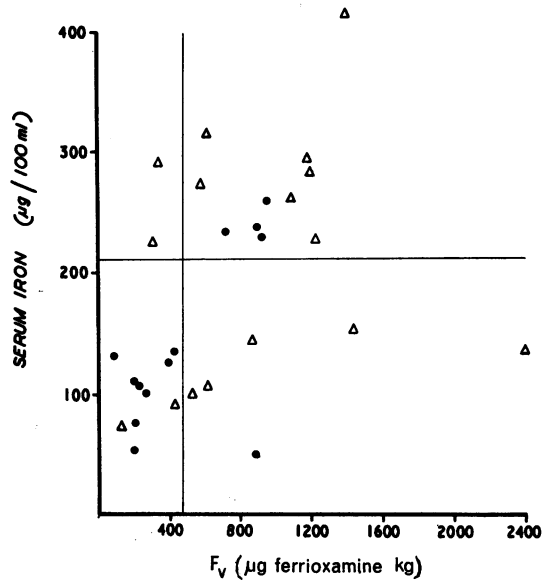

FIG. 6.

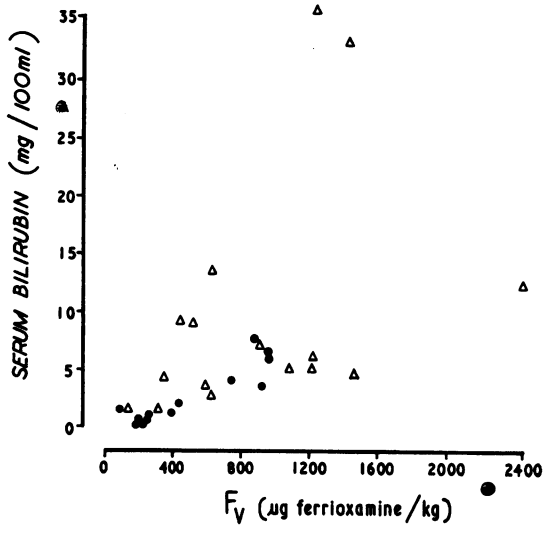

FIG. 7.

FIG. 6. *Relation between F_v and serum iron in acute hepatitis at first test* △, *and at follow-up tests* ●. *The upper limits of normal for F_v and serum iron are indicated.*

FIG. 7. *Relation between F_v and serum bilirubin in acute hepatitis at first test* △, *and at follow-up tests* ●.

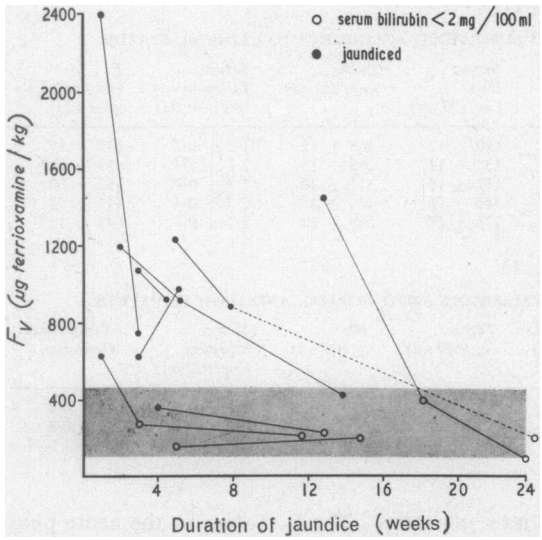

FIG. 8. *Results of repeated tests in nine patients with acute hepatitis. Shaded area indicates the normal range for F_V.*

In one case, indicated by the broken line, the final test was done 26 weeks after the onset of jaundice.

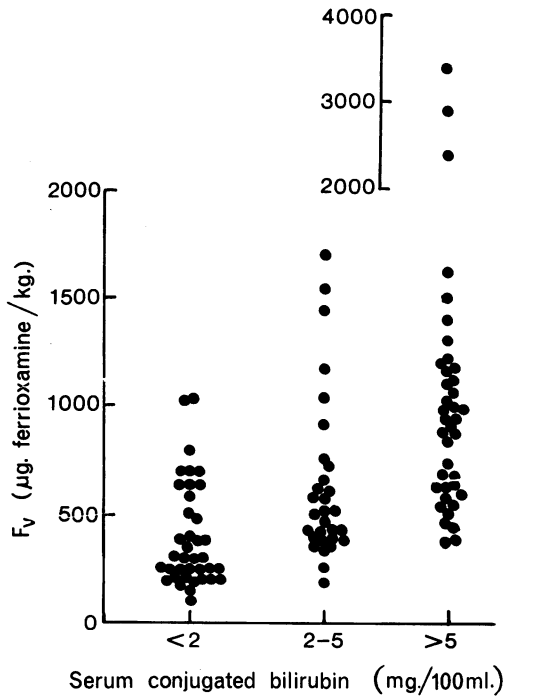

FIG. 9. *Relation between F_V and serum conjugated bilirubin in patients with liver disease, excluding haemochromatosis.*

normal by the convalescent phase (Fig. 8). The fall in F_V appeared to be less closely related to the serum iron than the decreasing serum bilirubin level.

RELATION BETWEEN F_V AND JAUNDICE As shown in Fig. 9, which includes the results for all patients with liver disease except those with haemochromatosis, there was a general correlation between F_V and the serum conjugated bilirubin concentration. Despite considerable overlap, the mean for the group with bilirubin levels of 2 to 5 mg/100 ml differed significantly from the means for the less and more deeply jaundiced groups ($P < 0.005$ in each case).

DISCUSSION

The results obtained with the differential ferrioxamine test in control subjects have been closely reproducible by different workers. Our normal range and mean value for control males agree almost exactly with the findings of Karabus and Fielding (1967). Although the normal range appears to be 100 to 500 µg ferrioxamine/kg (Fielding, Karabus, and Brunström, 1968) it has been general experience that relatively few controls give values above 350 µg/kg.

Our findings in untreated idiopathic haemochromatosis tended to be lower than those in other series (Fielding, O'Shaughnessy, and Brunström, 1966; Smith *et al*, 1967; Wardle and Israels, 1968). This may reflect earlier diagnosis. The F_V showed a general correlation with the duration of symptoms and some of the cases were symptom-free. The F_V also showed a high degree of correlation with the size of the iron stores, as determined by multiple venesection. Balcerzak, Westerman, Heinle, and Taylor (1968), measuring the simple iron excretion produced by desferrioxamine, obtained a similar relationship with storage iron. Hallberg, Hedenberg, and Weinfeld (1966) found that iron excretion after desferrioxamine was linearly related to non-haem liver iron concentration in control subjects.

A finding common to all these studies relating the effect of desferrioxamine to precise estimates of storage iron has been that the calculated regression line deviates significantly from the origin, indicating that chelation also occurs from a source unrelated to the stores. As a consequence of this additional variable the normal range for desferrioxamine is inappropriately wide for its action for storage iron, the upper limit of normal for F_V corresponding to an iron store content of 2·3 g, according to our results. Thus, while the test provides a reasonably accurate estimate of gross iron overload in haemochromatosis, subjects with minor degrees of excess have F_V values

within the normal range, so that ambiguous results may be obtained in mildly affected relatives (Smith *et al*, 1967).

The F_V values obtained in other forms of liver disease appeared to bear little relation to the size of the iron stores. High results were usual in jaundiced patients, the values in obstructive jaundice and acute hepatitis sometimes being indistinguishable from those for haemochromatosis. There was some evidence that recency of onset, as well as depth, of jaundice was a factor. Increased chelation in acute hepatitis has been thought to be related to the frequently present high serum iron level (Schnack and Wewalka, 1964). Desferrioxamine is unable to capture transferrin iron *in vivo* (Hallberg and Hedenberg, 1965a) and it has been suggested that in hepatitis there may be chelation of circulating ferritin iron (Scuro and Dobrilla, 1967). However, Reissman and Dietrich (1956) found that ferritinaemia occurred in less than half their patients with hepatitis and, even when present, comprised but a small part of the total serum iron elevation; hyperferraemia due to increased transferrin-iron was often found without demonstrable ferritin. In our patients F_V showed no correlation with serum iron, and at all stages of the disease appeared to be more closely related to the degree of jaundice.

Biliary iron excretion after desferrioxamine appears to be due to chelation within the hepatocyte, and is slight in the absence of excess iron (Figueroa and Thompson, 1968; Harker, Funk, and Finch, 1968). The increased urinary iron excretion in jaundiced patients was too great to be explained by spillover from the obstructed biliary tract.

The effect of desferrioxamine was generally increased in cirrhosis with respect to controls. The difference was least, though still significant, when liver function was well preserved; in jaundiced subjects the F_V values almost invariably exceeded the 99 % upper confidence limit of the normal range. Hallberg *et al* (1966) found that the response to desferrioxamine in two cirrhotics deviated widely from the relationship with non-haem liver iron found in controls. In our cases abnormal values were seldom associated with elevated serum iron levels or hepatic siderosis, and F_V showed no correlation with these parameters. The high results appeared to be a non-specific accompaniment of jaundice, essentially confirming the conclusion of Schnack and Wewalka (1964). Our inability to relate F_V to iron store status in cirrhosis was particularly disappointing in the case of the patients with a portocaval shunt; the high values in these were generally associated with a serum iron in the upper part of the normal range and a markedly reduced TIBC, suggesting some degree of iron excess, but all were also clinically jaundiced and it was not possible to differentiate between the role of the two factors.

There is much clinical evidence that desferrioxamine can derive iron from a hyperchelatable source unrelated to the stores. Observations in pernicious anaemia have implicated a highly labile iron pool closely connected with erythropoiesis (Hallberg, 1964; Fielding, 1965; Balcerzak *et al*, 1968). Raised F_V values have been obtained in sideroblastic, megaloblastic, and inconstantly, in haemolytic anaemias (Karabus and Fielding, 1967), following fractures (O'Shaughnessy, Brunström, and Fielding, 1966), and in some patients with rheumatoid arthritis and neoplasms (Wardle and Israels, 1968). Karabus and Fielding (1967) have postulated the existence of a hyperchelatable derivative of haem catabolism located within the reticuloendothelial cell, but there have been reservations about this view (Balcerzak *et al*, 1968). Although shortened red cell survival is a usual accompaniment of jaundice (Pitcher and Williams, 1963) it is questionable whether the findings with desferrioxamine are related to this. Hallberg and Hedenberg (1965b) obtained only an inconstant and minor increase in the effect of desferrioxamine despite a 15-fold increase in peripheral red cell destruction, and we have found normal F_V values in hereditary spherocytosis and elliptocytosis at times of active haemolysis (unpublished observations). A mild hypochromic normocytic anaemia tends to develop with prolonged jaundice, and there is evidence that red cell production and haemoglobin synthesis are relatively deficient in liver disease (Jandl, 1955; Kimber, Deller, Ibbotson, and Lander, 1965). Whether the enhanced effect of desferrioxamine in jaundice is related to abnormal handling of iron within the red cell precursor, perhaps through an increase in the soluble non-haem stronal fraction, or whether it is due to ineffective erythropoiesis, or to some other factor remains speculative.

We are grateful to Dr P. J. Scheuer for the liver biopsy interpretations and to Dr D. M. Burley of CIBA Laboratories for supplies of desferrioxamine and ferrioxamine. M.B. was in receipt of a Saltwell scholarship of the Royal College of Physicians.

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