Differential ferrioxamine test in idiopathic haemochromatosis and transfusional haemosiderosis

J. FIELDING, M. C. O'SHAUGHNESSY, AND GILLIAN M. BRUNSTRÖM

From Paddington General Hospital, London

SYNOPSIS The differential ferrioxamine test measures the amount of body iron as ferrioxamine (Fv) chelated by a standard dose of desferrioxamine.

Five patients with untreated, uncomplicated idiopathic haemochromatosis and one with transfusion haemosiderosis gave Fv in the range 1,948 to 2,462 $\mu\text{g./kg.}$ (normal 110 to 500). One case of transfusion haemochromatosis with haemolytic anaemia and renal failure gave an Fv value of 8,019 $\mu\text{g./kg.}$ Four patients with idiopathic haemochromatosis after therapeutic venesection gave Fv values of 212 to 885 $\mu\text{g./kg.}$ One relative with a value for Fv of 776 $\mu\text{g./kg.}$ was shown to have early cirrhosis by liver biopsy. Serial Fv measurement after venesection in this patient provided a preliminary assessment of the relationship between Fv values and available iron stores up to about 2,000 mg. iron. This relationship applies only when red cell survival is normal. Approximate figures for the range of available storage iron in 31 healthy men are deduced, namely, 200 mg. to 1,000 mg. (3 to 14 mg./kg.).

The test should prove useful in the diagnosis of iron overload, in the screening of relatives for early haemochromatosis, and in the management of iron storage diseases.

The introduction of chelating agents of low molecular weight which bind iron *in vivo* and result in the excretion of an iron chelate in the urine has suggested several procedures as aids to diagnosis and control of therapy in haemochromatosis and allied conditions. Diethylenetriamine penta acetic acid (DPTA) in doses of 1 g. has been infused intravenously over 30 minutes and the urinary output of iron measured in the subsequent five or six hours (Walsh, Perkins, Blackburn, Sanford, and Cantrill, 1963; Lloyd, Powell, and Thomas, 1964). Desferrioxamine, from 500 to 1,000 mg., has been injected intramuscularly with measurement of the 24-hour urinary iron output (Keberle, 1964; Ploem, Verloop, and Wael, 1964). Sometimes comparison with a 24-hour urinary iron output without a chelator has been advised.

The differential ferrioxamine test (Fielding, 1965) measures the amount of body iron chelated by desferrioxamine. It has some advantages over previously described tests: some biological variables have been reduced by using test doses adjusted to body weight and expressing the results in terms of body weight; the chemical estimation determines

urinary ferrioxamine by a simple and reproducible method, avoiding the often variable estimation of urinary iron, the data permitting not only the proportion excreted in an arbitrary time to be calculated but also body iron chelated by the test dose. The timing of collecting specimens or urine is not critical and the measurement of iron chelation is possible in the presence of renal failure.

Desferrioxamine is a more specific chelator of trivalent iron and seems less toxic when used in long-term therapy than other chelators. Tests based on desferrioxamine are therefore preferred since they offer a guide to its therapeutic efficiency in promoting iron excretion in each case.

The present paper describes the results of the test in certain patients with iron storage disease.

METHODS

The differential ferrioxamine test is based on the chelation of body iron by desferrioxamine (Fig. 1). Briefly, it consists of an intravenous injection of a standard body-weight dose, 500 mg./60 kg., of desferrioxamine methane sulphonate (Desferal, Ciba), together with a body-weight dose of ferrioxamine labelled with 2 $\mu\text{C}^{59}\text{Fe}$. A single six-hour specimen of urine is collected. The proportion of

FIG. 1. The chelation of iron by desferrioxamine.

injected ferrioxamine excreted in six hours (Fex%) is estimated as ^{59}Fe and is also expressed as the amount of injected ferrioxamine excreted (Fex). The total amount of ferrioxamine in the urine (Ft) is estimated chemically (Fielding and Brunström, 1964). The estimations are simple and take only some minutes. The difference, $\text{Fd} = \text{Ft} - \text{Fex}$, measures the amount of ferrioxamine excreted which has been derived from chelation of body iron. Fd (mg.) corresponds to the amount of ferrioxamine (or of iron) excreted in six hours after a standard intravenous dose of desferrioxamine alone, and therefore is a measure of the therapeutic effectiveness of the drug in the case under investigation.

Since the excretion from a known intravenous dose of ferrioxamine (F) is known, the total amount of ferrioxamine formed from body iron (Fv) is deduced by simple proportion.

$$\text{Fv} = \frac{\text{Fd}}{\text{Fex}} \times \text{F mg.} = \frac{\text{Fd}}{\text{Fex}} \times 833 \mu\text{g./kg.}$$

When simplified in terms of the observations made, this expression becomes

$$\text{Fv } \mu\text{g./kg.} = \left[\frac{\text{ferrioxamine in 6-hr. urine (mg.)} \times ^{59}\text{Fe in test dose (cpm/ml.)}}{^{59}\text{Fe in 6-hr. urine (cpm/ml.)}} \right] - 833$$

Serum iron was determined by the method of Kok and Wild (1960) and serum total iron-binding capacity was determined by the absorption of excess ferric chloride with magnesium carbonate (Ramsay, 1957).

The grading of stainable iron in the liver follows that of Scheuer, Williams, and Muir (1962).

In one patient (V.L.) with a minor degree of iron overload, the amount of storage iron that could be mobilized was calculated during repeated venesection following the principle of Haskins, Stevens, Finch, and Finch (1952). The calculation made the following assumptions: blood volume, 70 ml./kg.; iron content of haemoglobin, 0.34g%; menstrual blood loss, 30 mg. iron per month; available iron stores nil when $\text{Fv} = 88 \mu\text{g./kg.}$ (Fielding, O'Shaughnessy, and Brunström, 1965).

CLINICAL MATERIAL

Twelve patients with iron storage disease were tested; five

with untreated idiopathic haemochromatosis; four with idiopathic haemochromatosis who had been venesected for varying periods of time; one patient with a minor degree of iron overload identified in a family study of a patient with idiopathic haemochromatosis and two patients with defective erythropoiesis who had been transfused over many years resulting in an iron overload of not less than 40 g. iron. One of the latter patients (J.W.) was in chronic renal failure, possibly due to pyelonephritis, with a blood urea level of 100 to 150 mg./100 ml. The anaemia in this patient was complicated by a marked haemolytic element, with mean red cell survival of 20 days.

The results in these patients are compared with those in 31 healthy men (medical students and hospital employees) previously described (Fielding *et al.*, 1965).

RESULTS

FV IN NORMAL MEN In 31 healthy men, Fv, the amount of ferrioxamine formed *in vivo* by chelation of body iron after a standard dose of desferrioxamine ranged from 109 to 498 $\mu\text{g./kg.}$ There is a suggestion of bimodal distribution among these normal men, one subgroup with an Fv value of 110 to 200 $\mu\text{g./kg.}$ and another subgroup 250 to 500 $\mu\text{g./kg.}$

FV IN UNTREATED IDIOPATHIC HAEMOCHROMATOSIS In five patients Fv values varied from 1,992 to 2,462 $\mu\text{g./kg.}$ (Table I). Since ferrioxamine contains approximately one tenth its weight of iron, this is equivalent to the mobilization of about 0.2 to 0.25 mg. iron per kg. body weight. Figure 2 shows the pattern of excretion in four of these cases which were studied by observations on two-hourly urine specimens.

FV IN VENESECTED HAEMOCHROMATOSIS Of four patients with idiopathic haemochromatosis who had undergone therapeutic venesection, two had Fv values above the normal range, 885 and 777 $\mu\text{g./kg.}$; of these only one had a high serum iron level and fully saturated binding capacity. In two patients the Fv value was in the normal range, with low serum iron levels and less than 20% saturation of binding capacity. Only one patient among the venesected patients had a successful needle liver biopsy at the time of the test ($\text{Fv} 346 \mu\text{g./kg.}$), which showed an excess of haemosiderin in the fibrous tissue of a cirrhotic liver but without parenchymal haemosiderin.

FV IN TRANSFUSION IRON OVERLOAD Both patients with defects of erythropoiesis gave high Fv values, 1,948 and 8,019 $\mu\text{g./kg.}$ The first is close to the values found in untreated haemochromatosis; the second patient (J.W.) had a short red cell life and was in

TABLE I

Case	Sex	Age	Diagnosis	Hb (g./100 ml.)	Serum Fe/Total Iron-binding Capacity (μ g./100 ml.)	Glucose Tolerance Test	Liver Biopsy		Fex (%)	Fv ³ (μ g./kg.)
							Fe Grade	Cirrhosis		
S.M.	M.	47	Untreated haemochromatosis	11.8	158/344	Diabetic	++++	+++	50	2,055
C.J.	M.	64	Untreated haemochromatosis	12.9	210/213	Diabetic	++++	+++	51	2,090
A.G.	M.	50	Untreated haemochromatosis	11.8	200/465	Diabetic	++++	+++	64	2,148
S.B.	M.	71	Untreated haemochromatosis	14.7	240/248	Diabetic	++++	+++	51	2,462
M.D.	M.	41	Untreated haemochromatosis	12.8	268/268	Prediabetic	+++++	+++	51	1,992
J.D.	M.	18	Transfusion haemosiderosis	6.9	305/330	Normal		N.D.	90	1,948
J.W.	F.	25	Transfusion haemochromatosis	5.0	335/342	Diabetic		N.D.	10 ^a	8,019
T.D. ¹	M.	57	Venesected haemochromatosis	16.4	280/284	Diabetic		N.D.	25	885
B.A. ¹	F.	52	Venesected haemochromatosis	12.6	114/390	Diabetic		N.D.	37	777
W.W. ¹	M.	66	Venesected haemochromatosis	13.4	56/374	Normal	+	+++	31	346
C.R. ¹	M.	45	Venesected haemochromatosis	12.2	53/324	Diabetic		N.D.	45	212
V.L.	F.	42	Early haemochromatosis	15.7	225/225	Normal	++	+	58	776

N.D. = not done

¹Liver biopsy at first diagnosis gave Fe grade + + + +, cirrhosis + + + ^aChronic renal failure ³Fv range in normal men, 110-500.

FIG. 2. Pattern of ferrioxamine excretion during differential ferrioxamine test. Solid dots = Ft, total ferrioxamine excretion by chemical assay; open circles = Fex, amount of ferrioxamine excreted from injected dose by ⁵⁹Fe assay. A two-hourly study was not made in patient J. D. (Table I).

TABLE II

SERIAL FV DURING PHLEBOTOMY

Date	Hb (g./100 ml.)	Serum Fe/Total Iron-binding Capacity ($\mu\text{g./100 ml.}$)	Liver Biopsy (Fe grade)	Fe Removed by Phlebotomy (mg.)	Fex (%)	Fv ($\mu\text{g./kg.}$)
28.10.64	15.7	225/225	++	0	58	776
8.2.65	15.4	272/272		1,040	46	576
14.4.65	13.8	155/390		2,333	44	190
27.5.65	13.1	65/414	+	2,960	44	88

TABLE III

FV AND STORAGE IRON

Date	Hb (g./100 ml.)	Fv ($\mu\text{g./kg.}$)	Fe Removed at Phlebotomy (mg.)	Hb Deficit as Fe (mg.)	Menstrual Loss of Fe (mg.)	Fe Removed from Stores ¹ (mg.)		Available Storage Fe (mg.)	
						A	B	A	B
28.10.64	15.7	776	0	0	0	0	0	2,060	1,644
8.2.65	15.4	576	1,040	53	90	771	567	1,289	1,077
14.4.65	13.8	190	2,330	333	150	1,649	1,315	411	329
27.5.65	13.1	88	2,960	456	180	2,060	1,644	0	0

¹A: Corrected for excess absorption over excretion of 3 mg. Fe per day; B, for 5 mg. Fe per day excess.

chronic renal failure. Both these factors could contribute to the exceptionally high chelation and are further discussed below. The pattern of excretion in these cases is also shown in Figure 2.

SERIAL FV DURING VENESECTION V.L. is the sister of S.M., a patient with untreated haemochromatosis. Her serum iron level was 225 $\mu\text{g./100 ml.}$ and the binding capacity was fully saturated; a liver biopsy at this time showed early cirrhosis with 2+ histochemical iron, which was distributed in parenchyma, in reticulo-endothelium, and in periportal fibrous tissue. The Fv value, 776 $\mu\text{g./kg.}$, was above the normal range. She was venesected over a period of seven months during which 2,960 mg. iron was removed, and the Fv value fell progressively to 88 $\mu\text{g./kg.}$ (Table II). By this time the haemoglobin had fallen from 15.7 to 13.1 g./100 ml., serum iron and total iron-binding capacity were characteristic of iron deficiency, and liver biopsy showed no reticulo-endothelial haemosiderin, minimally in the parenchyma, but there remained an excess of haemosiderin in fibrous tissue assessed as 1+.

Storage iron was calculated from the venesection data (Table III) and is compared with the corresponding Fv values in Figure 3.

EXCRETION OF FERROXAMINE (Fex %) The proportion of an intravenous dose of ⁵⁹Fe-ferrioxamine excreted in the urine in six hours in 59 out of 61 normal men and women ranged from 30 to 53%; in two normal men, aged 63 and 76, it was 20%, and in these older subjects some degree of renal insufficiency may well have been present (Fig. 4).

In seven cases of untreated iron storage disease Fex % ranged from 50 to 90%; in four patients who had undergone therapeutic venesection Fex % ranged from 25 to 45%; in one patient with transfusional haemochromatosis in chronic renal failure, Fex % was 10%. Thus in untreated, uncomplicated iron storage disease, Fex % ranges from high normal

FIG. 3. V.L., woman aged 42. Fv values and storage iron during venesection. A, calculation corrected for excess absorption over excretion of 3 mg. Fe per day; B, corrected for 5 mg. Fe per day excess. The horizontal interrupted lines show the limits of Fv in normal men.

FIG. 4. Six-hour urinary excretion of ferrioxamine as a percentage of standard intravenous dose. In centre column, solid dots = untreated haemochromatosis, open circles = venesected haemochromatosis, $\triangle$ = transfusion haemochromatosis with renal failure.

to as high as 90%, and falls as iron stores are reduced. This contrasts with iron-deficiency anaemia in which Fex% ranges in the opposite direction from normal to as low as 10%. The significance of these patterns of excretion is discussed below.

DISCUSSION

A striking feature of the differential ferrioxamine test in six patients with uncomplicated untreated haemochromatosis is the narrow range of Fv values. The ratio of the highest, 2,462 $\mu\text{g./kg.}$, to the lowest, 1,948 $\mu\text{g./kg.}$, is 1.26. This compares with a high to low ratio of 4.0 in the DPTA test on a similar number of patients (Walsh *et al.*, 1963). Since it is improbable that all untreated patients with haemochromatosis have the same amount of storage iron per kilogram body weight, it is likely that in iron overloading to this extent the dose of desferrioxamine used, 8.33 mg./kg., is chelating to its maximum capacity *in vivo*, when the course of iron presented to it is ferritin-haemosiderin. Under these conditions 0.2 to 0.25 mg. iron per kilogram body weight is mobilized by the test dose of desferrioxamine, *i.e.*, about a quarter of its maximum capacity *in vitro*.

The narrow range of Fv in uncomplicated haemochromatosis suggests that the test should be a useful diagnostic procedure.

Iron may also be mobilized by desferrioxamine from sources other than or in addition to ferritin-haemosiderin stores. It has been shown that high chelation values are also obtained in pernicious anaemia and some haemolytic anaemias, in which the main source of chelated iron is considered to be iron newly released from haem (Fielding, 1965).

These observations may explain the results in the patient J.W., who had defective erythropoiesis, transfusional haemochromatosis, haemolytic anaemia, and chronic renal failure. She also produced the exceptionally high value for Fv of 8,019 $\mu\text{g./kg.}$. This is about 85% of the maximum chelation capacity of the dose of desferrioxamine *in vitro*. Both iron overload and the haemolytic anaemia could provide excess of available iron for chelation and contribute to the high Fv value. Renal failure, by delaying excretion, may have prolonged high blood levels of desferrioxamine and also contributed to the high chelation obtained. Only 2.5% of the desferrioxamine dose was recovered from the urine in this case compared with 40 to 65% in normal subjects.

SIGNIFICANCE OF FEX% The two metabolic pathways of ferrioxamine which are known with certainty are urinary excretion and the utilization of a proportion of ferrioxamine iron for haemoglobin synthesis (Bannerman, Callender, and Williams, 1962; Hallberg, 1964). Although ferrioxamine has a high stability constant *in vitro*, the avidity of tissue receptors at erythropoietic sites is effective in detaching iron from the chelator. An inverse relationship is therefore to be expected between the amount of urinary excretion and the amount of erythropoietic uptake of ferrioxamine iron. Thus where erythroid avidity is high, as in iron-deficiency anaemia and in haemolytic anaemias, urinary excretion tends to be low. In iron overload marrow avidity for iron is replete from endogenous sources and therefore urinary excretion tends to be high. The highest urinary excretion of ferrioxamine observed was 90% in six hours, in a patient (J.D.) with erythropoiesis imperfecta in whom there were no erythropoietic receptor sites.

However, in most cases the total of urinary excretion and utilization of ferrioxamine iron does not account for all ferrioxamine injected and there is possibly a third metabolic pathway not yet defined.

FV AND STORAGE IRON In the patient V.L. with minor iron overload, Fv values were progressively reduced as storage iron was removed during the course of phlebotomy. Following the principles of Haskins *et al.* (1952) the amount of storage iron was calculated from the phlebotomy data. Two values were calculated: the first assumed an excess of ab-

sorption over excretion of 3 mg. a day (Haskins *et al.*, 1952), and since this patient almost certainly has early haemochromatosis, the second value assumed an excess of 5 mg. per day (Crosby, Conrad, and Wheby, 1963). These limits as calculated and the corresponding Fv values are shown in Figure 3. The shape of the curve suggests that at the dose of desferrioxamine used, a limiting value for Fv is reached as iron stores increase. This is consistent with the results in untreated, uncomplicated haemochromatosis which show a small scatter of high values.

Figure 3 also shows that storage iron corresponding to the Fv range in normal men varies over a five-fold range, from approximately 200 mg. to 1,000 mg. (3 to 14 mg./kg.). The limits are not well defined and this must be a provisional assessment, based on a single study; nevertheless it emphasizes that the range of storage iron in normal healthy men is much wider than hitherto reported and often approaches iron depletion. A similar conclusion was drawn from a comparison of the test in normal men and iron-deficiency anaemias (Fielding *et al.*, 1965).

CONTROL OF VENESECTION THERAPY Only five patients who had undergone therapeutic venesection have been investigated so far, and these are too few for assessing the value of the test in controlling therapy. In two patients, W.W. and V.L., needle liver biopsy, repeated after venesection, showed an excess of haemosiderin in fibrous tissue, although parenchymal and reticulo-endothelial haemosiderin were almost depleted. In these cases haemoglobin had fallen and serum iron was normal or low. The corresponding Fv values indicated normal to low storage iron levels, and thus gave a better assessment of available iron at the time of the test than the liver biopsy.

The criteria for the amount of therapeutic venesection in haemochromatosis have yet to be firmly established. After large amounts of iron have been removed, anaemia and the signs of iron deficiency

may be found when some parenchymal haemosiderin is still to be seen in the liver. This residual haemosiderin may be extremely difficult to mobilize (Frey, Milne, Johnson, and Ebaugh, 1961; Finch and Finch, 1955). The present findings indicate that histochemical haemosiderin of grade 2+ in the liver may mean the presence of no more than a total of 2 to 3 g. storage iron. It is doubtful therefore whether there is much to be gained by removing the last visible traces of liver haemosiderin, and, since Fv values provide a better guide to available iron than liver biopsy, it should also prove more useful in controlling venesection therapy. Further observations in venesected patients are required.

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