Use of diethylenetriamine penta-acetic acid (D.T.P.A.) in the clinical assessment of total body iron stores

L. W. POWELL AND M. J. THOMAS

*From the Medical Professorial Unit and the Department of Pathology,
Royal Brisbane Hospital, Brisbane, Australia*

SYNOPSIS As a quantitative test of iron storage the six-hour urinary excretion of iron was measured after the intravenous infusion of the iron-chelating agent diethylenetriamine penta-acetic acid (D.T.P.A.). The value of this test in determining the presence and magnitude of the total body iron stores was assessed in patients with diseases of iron storage. In patients with idiopathic haemochromatosis and in their first-degree relatives with increased iron stores as measured by hepatic biopsy, the result of the D.T.P.A. test fell progressively as iron stores were reduced by venesection therapy. There was a significant correlation between the result of the D.T.P.A. test and the excess iron storage as measured retrospectively by the effects of venesection therapy, and also between the D.T.P.A. test and the unsaturated iron-binding capacity (U.I.B.C.) in these patients.

In patients with excess iron stores due to diseases other than idiopathic haemochromatosis (*e.g.*, alcoholic cirrhosis, haemochromatosis associated with refractory anaemia) the D.T.P.A. test demonstrated the presence of excess iron stores, but the results were very variable and did not correlate closely with the U.I.B.C. or the degree of hepatic haemosiderin deposits. Unexpectedly high results for the D.T.P.A. test were found in some patients with iron-deficiency anaemia.

The demonstration that iron-chelating agents promote increased urinary excretion of iron in patients with excess iron stores has led to the use of these agents in the study of iron storage disease. Both diethylenetriamine penta-acetic acid (D.T.P.A.) and desferrioxamine have been used in simple quantitative tests in which the urinary iron excretion following the injection of a known dose of chelating agent is measured at six or 24 hours (Walsh, Perkins, Blackburn, Sanford, and Cantrill, 1963; Keberle, 1964; Lloyd, Powell, and Thomas, 1964; Ploem, de Wael, Verloop, and Punt, 1966; Fielding, O'Shaughnessy, and Brunström, 1966). However, both agents have limitations when used to assess quantitatively the degree of excess storage iron.

Desferrioxamine has more than one metabolic pathway; a variable proportion of the ferrioxamine formed *in vivo* is used for haemoglobin synthesis and is not excreted in the urine (Bannerman, Callender, and Williams, 1962). This can be allowed for if desferrioxamine is injected together with a known dose of ferrioxamine labelled with ^{59}Fe , and

the six-hour excretion of ferrioxamine and ^{59}Fe measured (Fielding, 1965). Desferrioxamine also chelates iron released from the breakdown of haem in addition to storage iron and thus high chelation values are obtained in haemolytic conditions and in disorders of haem metabolism (Fielding, 1965; O'Shaughnessy, Brunström, and Fielding, 1966; Smith, Studley, and Williams, 1967).

D.T.P.A. is a less specific chelating agent for iron than desferrioxamine; small quantities of other cations such as copper, magnesium, lead, and zinc are chelated in addition (Tripod, 1964). However, the metabolic pathway of D.T.P.A. following intravenous injection appears to be relatively simple. It is distributed widely in the extracellular compartment; 80 to 90% of the administered dose is recovered unchanged in the urine in the first four hours; it does not participate in normal iron exchange between effete red cells, reticuloendothelial cells, and transferrin; and it appears that only tissue iron is chelated (Stevens, Rosoff, Weiner, and Spencer, 1962; Cleton, Turnbull, and Finch, 1963). These properties suggest that D.T.P.A. should be a valuable chelating agent for use in a simple quantitative

test of storage iron, and this has been confirmed by the findings of Walsh *et al.* (1963) in patients with idiopathic haemochromatosis.

A previous study (Powell, 1966a) has shown that, in rabbits loaded with iron-dextran, the results of a quantitative test using D.T.P.A. correlated with the total body iron excess when the test was performed at a constant time interval after iron loading. The present study was undertaken to assess the value of such a test in determining the presence and magnitude of increased total body iron stores in patients with various diseases of iron storage. An indication of the state of the iron stores in these patients was also obtained by measurement of the serum-iron, total iron-binding capacity (T.I.B.C.), the extent of haemosiderin deposition in hepatic biopsy specimens and bone marrow aspirates, and where possible, by means of repeated venesection.

METHODS

SERUM IRON AND URINARY IRON The serum-iron level was estimated by the method of Trinder (1956) and the total iron-binding capacity (T.I.B.C.) by the method of Ramsay (1957). Urinary iron was measured by a wet-ashing technique. To 5 ml. of urine was added 0.2 ml. concentrated sulphuric acid and 0.5 ml. concentrated perchloric acid. During the final stage of digestion 0.1 ml. of hydrogen peroxide (30% w/v) was added and the mixture heated to dryness. After cooling, 2 ml. of water was added and the contents heated to boiling point and again allowed to cool before adding 1 drop of thioglycollic acid and 2.0 ml. of 0.2% α , α -dipyridyl in buffer (2.0 M sodium acetate/acetic acid, pH 5.0). After mixing and standing for 10 minutes the solutions were read in a spectrophotometer with appropriate standards and blanks which had been treated in the same manner as the test samples.

D.T.P.A. TEST (LLOYD *et al.*, 1964) The patient's bladder was emptied and the urine discarded. A solution containing 1 g. of D.T.P.A. in 500 ml. of physiological saline was then infused intravenously over exactly one hour. All urine was collected for the six-hour period after the start of the infusion.

Aspiration liver biopsy was performed with a Menghini needle. Tissue was fixed in acid formalin for 48 hours before processing. Sections were stained with haematoxylin and eosin and for free iron by Perls' technique. Iron deposition was graded according to Scheuer, Williams, and Muir (1962).

CRITERIA FOR VENESECTION Weekly venesections of 500 ml. were performed on those patients with cirrhosis and grade 4 iron deposition in liver biopsy specimens. In each case the presence of excess body iron stores was confirmed by the failure of the haematocrit or haemoglobin concentration to fall by 25% or more after seven weekly venesections (Haskins, Stevens, Finch, and Finch, 1952; Finch and Barnett, 1957). Weekly or fortnightly

venesections each of 500 ml. were then continued until there was evidence of iron depletion as judged by a subnormal haemoglobin concentration, a low serum-iron level and high total iron-binding capacity. First degree relatives with evidence of increased iron stores in liver biopsy specimens and the D.T.P.A. test were also submitted to venesection therapy as above. The other patients in this study did not undergo regular venesection.

RESULTS

NORMAL SUBJECTS (33) The mean values for serum iron, T.I.B.C., and urinary iron excretion in the D.T.P.A. test are shown in Table I. The serum iron was significantly higher in males than females ($P < 0.05$) but no sex difference was found in the D.T.P.A. test. The 95% confidence limit for the D.T.P.A. test was 0.29 mg.

TABLE I

MEAN VALUES $\pm$ S.D. IN NORMAL CONTROLS

Subjects	Serum Iron ($\mu\text{g./100 ml.}$)	T.I.B.C. ($\mu\text{g./100 ml.}$)	Percentage Saturation of T.I.B.C.	D.T.P.A. Test (mg.)
Males (14) ¹	125 $\pm$ 33.3	327 $\pm$ 46	37 $\pm$ 9	0.16 $\pm$ 0.06
Females (19)	101 $\pm$ 32.3	334 $\pm$ 68	31 $\pm$ 10	0.15 $\pm$ 0.08
Total (33)	—	—	—	0.15 $\pm$ 0.07

¹Total number in parentheses.

IDIOPATHIC HAEMOCHROMATOSIS The details of venesection therapy in patients in this group are given in Table II. In five patients with untreated haemochromatosis the urinary iron excretion in the D.T.P.A. test ranged from 4.1 mg. to 12.4 mg. (mean 8.6 mg.). A further nine such patients had received treatment by repeated venesection but their serum transferrin had remained almost fully saturated; the result of the D.T.P.A. test in these patients ranged from 1.2 mg. to 16.0 mg. (mean 6.3 mg.). In five patients who had been treated by weekly venesection until the haemoglobin concentration and serum iron levels were low, the results of the D.T.P.A. tests were normal, ranging from 0.003 mg. to 0.29 mg. (mean 0.18 mg.). In two patients with idiopathic haemochromatosis and in four first-degree relatives with increased iron stores, the D.T.P.A. test was repeated at intervals of three to six months during treatment by weekly or twice weekly venesection (see Figures 1 and 2). In each case the urinary iron excretion in the D.T.P.A. test fell progressively as the excess storage iron was reduced by therapy. Table III shows the result of the initial D.T.P.A. test in each subject compared with the total body iron stores as measured by venesection therapy until evidence of iron depletion occurred, and in one patient (case 14), by chemical estimation of iron in tissues obtained at necropsy. Although the number of subjects studied

TABLE II

CLINICAL AND PATHOLOGICAL FINDINGS IN PATIENTS WITH DISEASES AFFECTING IRON METABOLISM

Patient	Sex	Age	Serum Iron ($\mu\text{g./100 ml.}$)	T.I.B.C. ¹ ($\mu\text{g./100 ml.}$)	Liver Biopsy (grade iron) ²	Marrow Aspiration Test (mg.) (grade iron) (0-4)	D.T.P.A. Test (mg.)	Remarks
<i>Idiopathic haemochromatosis: untreated</i>								
1	M	54	281	281	4	—	12.4	5 g. iron removed after D.T.P.A. test: venesection continuing
2	F	52	217	228	4	—	11.9	15.5 g. iron removed after D.T.P.A. test: venesection continuing
3	M	65	190	228	4	—	8.8	3.2 g. iron removed: patient then refused further treatment.
4	M	40	242	284	4	—	6.0	Total of 13.2 g. iron removed after D.T.P.A. test
5	M	65	230	290	4	—	4.1	Died from hepatoma 2 months later.
<i>Idiopathic haemochromatosis: incompletely treated</i>								
6	F	64	254	266	4	—	2.5	6.25 g. iron removed before D.T.P.A. test: venesection discontinued because of anginal pain
7	M	65	264	288	4	—	1.2	16.5 g. iron removed before D.T.P.A. test: venesection continuing
8	M	62	—	—	4	—	3.5	7.5 g. iron removed before D.T.P.A. test: venesection continuing
9	M	44	335	335	4	—	9.4	7.8 g. iron removed before D.T.P.A. test and continuing
10	M	47	267	298	4	—	5.5	12.0 g. iron removed before D.T.P.A. test and continuing
11	M	65	211	235	4	—	5.3	> 8.0 g. iron removed before D.T.P.A. test: irregular attender
12	F	44	233	264	4	—	7.2	15.0 g. iron removed after D.T.P.A. test
13	F	39	251	272	4	—	6.0	8.5 g. iron removed after D.T.P.A. test
14	M	58	330	342	4	—	16.0	Iron content of tissues at necropsy = 37.4 g. ³
<i>Idiopathic haemochromatosis: completely treated⁴</i>								
15	M	63	78	356	0	—	0.003	20 g. iron removed before D.T.P.A. test.
16	M	46	44	412	0	—	0.2	18.5 g. iron removed before D.T.P.A. test
4 (see above)	M	40	38	430	—	—	0.25	13.2 g. iron removed before D.T.P.A. test
12 (see above)	F	44	45	300	1	—	0.18	19.0 g. iron removed before D.T.P.A. test
13	F	39	28	296	—	—	0.29	11.5 g. iron removed before D.T.P.A. test
<i>Relatives of patients with haemochromatosis: completely treated⁴</i>								
17	M	42	210	—	3	—	4.9	11 g. iron removed after D.T.P.A. test
18	M	46	156	288	Fibrosis + 2	—	0.51	2.5 g. iron removed after D.T.P.A. test
19	F	41	112	280	2	—	0.46	2.1 g. iron removed after D.T.P.A. test
20	F	35	121	274	2	—	0.55	1.9 g. iron removed after D.T.P.A. test
<i>Secondary haemochromatosis</i>								
21	M	8	198	250	3 Fibrosis +	4	9.6	Refractory anaemia. Approx. 15 g. iron given parenterally
22	F	36	399	412	—	4	9.4	Sideroblastic anaemia > 50 g. iron given parenterally. Figlu test 42 mg./hr.
23	M	9	294	294	—	4	4.6	Thalassaemia major

¹T.I.B.C. = total iron-binding capacity²Grading after Scheuer *et al.* (1962)³Reported in detail elsewhere (Tyrer, Powell, and Burnett, 1966)⁴Treatment by venesection, considered complete when patient developed a low haemoglobin concentration, low serum iron level, and high total iron-binding capacity.

TABLE II—continued

CLINICAL AND PATHOLOGICAL FINDINGS IN PATIENTS WITH DISEASES AFFECTING IRON METABOLISM								
Patient	Sex	Age	Serum Iron ($\mu\text{g./100 ml.}$)	T.I.B.C. ¹ ($\mu\text{g./100 ml.}$)	Liver Biopsy (grade iron) ²	Marrow Aspiration (grade iron) (0-4)	D.T.P.A. Test (mg.)	Remarks
<i>Alcoholic cirrhosis with haemosiderosis</i>								
24	M	51	107	298	3	—	0.18	Wernicke's encephalopathy
25	M	50	250	364	2	—	0.19	Hepatomegaly 18 cm.
26	M	62	217	—	2	—	3.1	Serum amylase 530 Somogyi units
27	M	60	255	282	2	1	0.39	Macrocytic anaemia
28	F	57	144	414	2	—	0.09	—
29	M	56	97	307	2	—	0.44	Indurated pancreas at laparotomy
30	M	42	104	320	2	—	1.4	Abdominal pain: urinary diastase = 80 Wohlgemuth units
31	F	43	—	—	3	—	0.16	Macrocytic anaemia
32	M	64	97	342	3	—	0.19	—
33	M	26	122	290	2	—	0.7	Hypogammaglobulinaemia
34	M	41	255	282	2	—	0.39	—
<i>Alcoholic cirrhosis without haemosiderosis</i>								
35	M	51	72	—	0	—	0.26	Previous haematemeses
36	F	48	129	416	0	—	0.27	Wernicke's encephalopathy, peripheral neuritis
37	M	54	72	404	0	—	0.22	—
<i>Chronic alcoholism without cirrhosis</i>								
38	M	55	40	264	0	—	0.23	Cardiomyopathy
39	M	37	105	376	1	—	0.15	—
40	M	53	32	326	0	—	0.28	—
41	M	50	—	—	1	—	0.29	—
42	M	52	96	322	1	—	0.2	Duodenal ulcer, previous haematemesis
43	M	70	126	340	0	0	0.15	—
44	M	48	112	330	0	—	0.28	—
45	M	45	—	—	0	—	0.25	—
<i>Cryptogenic cirrhosis</i>								
46	F	44	92	278	0	—	0.32	Haematemeses
47	F	47	67	428	0	—	0.25	Haematemeses, sister of patient 40
48	M	22	56	364	0	—	0.19	Ulcerative colitis
49	F	64	96	312	0	—	0.15	—
<i>Haemosiderosis</i>								
50	M	67	248	262	—	3	6.3	Aplastic anaemia
51	M	60	212	282	—	3	2.4	Aplastic anaemia
52	M	54	226	256	—	4	0.98	Untreated pernicious anaemia
53	M	65	196	284	—	2	0.2	Untreated pernicious anaemia
54	F	64	95	388	—	1	0.1	Treated pernicious anaemia
55	M	55	—	—	—	1	0.3	Treated polycythaemia vera
56	M	58	157	298	—	2	0.3	Treated chronic myeloid leukaemia
<i>Iron deficiency</i>								
57	F	45	44	396	—	0	0.22	Previous menorrhagia
58	F	21	36	416	—	0	0.3	Aspirin-induced melaena
59	F	38	16	520	—	0	0.8	Previous menorrhagia
60	F	20	18	354	—	0	0.94	Melaena
61	F	52	56	360	0	0	0.21	Melaena
62	F	80	26	432	—	0	0.1	Dietary
63	F	56	32	370	0	0	0.18	Haematemeses
64	F	33	28	416	—	0	0.2	Previous menorrhagia
65	M	78	50	524	—	0	0.1	Duodenal ulcer
66	M	49	—	—	—	0	4.5	During oral iron therapy
67	F	56	117	330	—	0	0.17	Three months after cessation of iron therapy
<i>Chronic pancreatitis</i>								
68	F	36	89	364	—	0	0.17	—
69	F	66	—	—	—	—	0.14	—
70	F	60	56	176	—	—	0.5	Diabetes mellitus

FIG. 1. Serial tests in a patient with idiopathic haemochromatosis during venesection therapy. Note the progressive fall in the result of the D.T.P.A. test during therapy whilst the serum-iron and haemoglobin concentration remained virtually unchanged, but the U.I.B.C. rose. (Interrupted lines indicate the upper limits of normal for females for haemoglobin concentration serum-iron and D.T.P.A. tests.)

TABLE III

RELATION BETWEEN URINARY IRON EXCRETION IN THE D.T.P.A. TEST AND BODY IRON STORES IN FOUR PATIENTS WITH HAEMOCHROMATOSIS AND FOUR RELATIVES WITH INCREASED IRON STORES

Patient	Initial D.T.P.A. Test (mg.)	Iron Removed by Venesection after D.T.P.A. Test (g.)
4	6.0	13.2
12	7.2 (after 4 g. iron removed)	15
13	6.0 (after 3 g. iron removed)	8.5
14	16.0	37.4 ¹
17	4.9	11
18	0.51	2.5
19	0.46	2.1
20	0.55	1.9

¹Iron content of major organs of iron storage measured on tissues obtained at necropsy (Tyrer *et al.*, 1966).

is small the correlation is significant ($r = 0.984$, $P < 0.001$).

In these patients studied serially the response to D.T.P.A. appeared to depend on the degree of unsaturation of the iron-binding capacity of the serum, although the relationship was not a simple linear one (Fig. 3).

Figure 2 demonstrates an observation which was made on three patients when treatment by venesection had been suspended because the serum iron, the haemoglobin concentration, and the result of the D.T.P.A. test had all fallen to subnormal levels. After a further two to three months the serum iron and the result of the D.T.P.A. test had returned to abnormally high levels, and a further 1.5 g. of iron could be removed by venesection before the levels fell again to normal or subnormal values.

SECONDARY HAEMOCHROMATOSIS (TABLE II) In the three patients with secondary haemochromatosis and chronic anaemia (one each with refractory

FIG. 2. Serial tests in a patient with idiopathic haemochromatosis. Venesection therapy was withheld in March 1965 for eight weeks. The serum iron and result of the D.T.P.A. test returned to abnormally high levels and a further 1.5 g. of iron was then removed. (Interrupted lines indicate the upper limits of normal for haemoglobin concentration, serum-iron and D.T.P.A. tests.)

anaemia, sideroblastic anaemia, and thalassaemia) the serum iron levels were elevated with increased saturation of the T.I.B.C. and there was excess haemosiderin in bone marrow aspirates. In these patients the increase in urinary iron excretion in the D.T.P.A. test (9.6, 9.4, and 4.6 mg. respectively) was similar to that in patients with untreated haemochromatosis although the total iron stores probably varied widely (see discussion).

ALCOHOLIC CIRRHOSIS (TABLE II) Fourteen patients with alcoholic cirrhosis were studied. In 11 of these stainable iron was present in the liver biopsy but the degree of siderosis (maximum grade 3, mean grade 2) was never as gross as in the cases of haemochromatosis (grade 4). None had been treated by repeated venesection. This group was distinguished from idiopathic haemochromatosis on the basis of the degree of iron excess and by the fact that family studies had failed to reveal excess iron storage in a first-degree relative. In six of these 11 patients the urinary iron excretion in the D.T.P.A. test was increased. However, the mean excretion (0.93 mg.)

was much lower than that of patients with haemochromatosis and the D.T.P.A. test did not show the correlation with the degree of hepatic iron deposition which was found in patients with untreated idiopathic haemochromatosis (Powell, 1965). In the three patients with alcoholic cirrhosis without haemosiderosis the result of the D.T.P.A. test fell within the normal range.

CHRONIC ALCOHOLISM WITHOUT CIRRHOSIS (TABLE II) In all eight patients with chronic alcoholism the D.T.P.A. test was normal. The serum iron level was reduced in two and normal in four others whilst the T.I.B.C. was within the normal range in all six tested. Liver biopsy showed no stainable iron in five, and in the remaining three mild haemosiderosis (grade 1) which is normal (Powell, 1966b).

CRYPTOGENIC CIRRHOSIS (TABLE II) The results of the D.T.P.A. test and the serum iron were within the normal range in all four patients in this group. In each case liver biopsy revealed an inactive cirrhosis with no stainable iron present.

HAEMOSIDEROSIS (TABLE II) Seven patients were studied in this group. All suffered from disorders of erythropoiesis commonly associated with excessive iron storage and in five patients excess haemosiderin deposition was demonstrated in bone marrow aspirates. In three of the seven patients the D.T.P.A. test was abnormal although the result showed no constant relation to the degree of haemosiderosis in bone marrow aspirates or to the serum iron level. There was a weakly significant correlation between the D.T.P.A. test and the U.I.B.C. in these patients ($r = -0.88$, $P < 0.05$). Liver biopsy was not performed on any patient in this group.

IRON DEFICIENCY (TABLE II) The D.T.P.A. test gave unexpectedly high results in three of the nine patients with untreated iron-deficiency anaemia. Only one of the three patients had suffered from menorrhagia and in this patient there was no vaginal blood loss at the time the test was performed. A further patient tested during oral iron therapy excreted 4.5 mg. iron in six hours in the D.T.P.A. test and in another patient tested three months after cessation of therapy the result was normal.

CHRONIC PANCREATITIS (TABLE II) In one of the three patients with chronic pancreatitis the D.T.P.A. test was increased to 0.5 mg. It is of interest that this patient had diabetes mellitus but there were no other clinical stigmata of haemochromatosis. She refused further investigation.

FIG. 3. Relation between D.T.P.A. test and unsaturated iron-binding capacity in the case illustrated in Figure 2. The correlation coefficient between X and $\log Y$ is $r = -0.927$ ($P < 0.01$).

DISCUSSION

This clinical study has confirmed the practical value of the D.T.P.A. test in demonstrating the presence of increased total body iron stores and in facilitating the quantitative assessment of excess body iron in patients with idiopathic haemochromatosis. In this condition the results showed a gradation from the high levels obtained in untreated patients down to the normal levels in those patients who had been treated until the serum iron and haemoglobin concentrations were below normal. These results agree closely with the findings of Walsh *et al.* (1963). In four patients with haemochromatosis and four immediate relatives, there was a significant correlation between the urinary iron excretion in the D.T.P.A. test and the amount of storage iron as measured by repeated venesection or, in one case, by chemical estimation of iron after necropsy (Table III). When serial D.T.P.A. tests were performed in six subjects during the period of treatment the fall in urinary iron excretion was not accompanied by a fall in serum iron or haemoglobin level until virtually all the storage iron had been removed (Figs. 1 and 2). Thus, during the period of treatment by venesection, the D.T.P.A. test gives a better indication of the amount of storage iron present than do the serum-iron and the haemoglobin

concentration. In these six patients studied serially the response to D.T.P.A. showed a significant negative correlation with the degree of unsaturation of the iron-binding capacity although the relationship was not a simple linear one (Fig. 3). Similar findings were observed with desferrioxamine by Walsh, Mass, Smith, and Lange (1965). However, this observation may not necessarily mean that the amount of iron chelated by D.T.P.A. is determined by the amount of circulating unsaturated transferrin. Walsh *et al.* (1963) showed that, in patients with treated haemochromatosis in whom the transferrin had become resaturated, the urinary excretion of iron provoked by D.T.P.A. was only slightly increased. In other words the serum iron was elevated and the T.I.B.C. fully saturated when there was a relatively small excess of body iron. Further increase in iron stores did not alter these values, but was shown by a further increase in urinary iron excretion in the D.T.P.A. test. In our patient A.W. (Fig. 2), resaturation of transferrin was associated with a substantial increase in response to D.T.P.A. In the present study, the result of the D.T.P.A. test in patients with alcoholic cirrhosis and hemosiderosis showed no constant relation to the serum iron or total iron-binding capacity.

The observation illustrated in Fig. 2 shows that not all storage iron has necessarily been removed by

venesection therapy when evidence of iron deficiency appears. During the period in which venesections were withheld the iron absorbed from the gut would probably not have exceeded 5 mg. per day (Haskins *et al.*, 1952). This is equivalent to 280 mg. over the eight-week period whereas 1,500 mg. was subsequently removed by venesection. It is probable that during this eight-week period the remaining iron, previously non-mobilizable and non-chelatable, was converted to a form which was both chelatable and readily mobilized. In case 12 (Fig. 1) a liver biopsy specimen obtained when the serum iron and result of the D.T.P.A. test had fallen to low levels showed some haemosiderin deposits still present.

Accurate measurement of body iron stores in patients with haemochromatosis and their relatives is difficult and there is debate about the best method currently used (Balcerzak, Westerman, Lee, and Doyle, 1966). Measurement of iron removed by means of repeated venesection is probably the most accurate method as almost all tissue iron is available for haemoglobin synthesis (Haskins *et al.*, 1952). However, this method provides only retrospective information. Liver biopsy is a simple, practical and relatively safe means of examining tissue iron stores by histochemical staining and also permits the assessment of liver damage. The amount of iron which stains histochemically, however, is not always directly proportional to the total body iron stores. In patients with untreated haemochromatosis the amount of iron which can be removed by a complete course of venesection may vary from 15 to 40 g. despite similar appearances (grade 4) of liver biopsy specimens (unpublished observations). In one patient with grade 3 iron deposition in the liver (Table II, patient 17) a total of 11 g. of iron was removed by venesection, and in three other patients with grade 2 iron deposition the amount of iron removed by venesection was 2.5, 2.1, and 1.9 mg. respectively. Patients with alcoholic cirrhosis may have moderate haemosiderosis of the liver (grades 2 to 3) with only a slight increase in chelatable body iron (Powell, 1965; Walsh *et al.*, 1965) and normal or slightly increased amounts (1.0 to 2.5 g.) of iron mobilizable by venesections (Conrad, Berman, and Crosby, 1962; Williams, Williams, Scheuer, Pitcher, Loiseau, and Sherlock, 1967). Quantitative tests of iron storage using iron-chelating agents can provide useful additional information about the degree of iron excess. The present study has demonstrated a relationship between the urinary iron excretion in the D.T.P.A. test and the amount of storage iron removed subsequently by venesections. Thus, the D.T.P.A. test is of value in the quantitative assessment of excess iron storage in patients with idiopathic haemochromatosis, but liver biopsy is

necessary to determine the degree of tissue damage, the presence of cirrhosis, and the distribution of storage iron within the liver.

In patients with diseases of iron storage other than idiopathic haemochromatosis (*e.g.*, alcoholic cirrhosis, secondary haemochromatosis associated with chronic anaemias such as sideroblastic anaemia and thalassaemia) the D.T.P.A. test demonstrated the presence of excess iron stores but the result showed no constant relation to the degree of iron excess as estimated by the serum iron, the T.I.B.C., and liver and marrow haemosiderin deposits. In such conditions the disorder of iron storage is complicated by ineffective erythropoiesis, increased iron absorption, and recent parenteral iron administration, each of which may affect iron chelation. However, as none of these patients was subjected to venesection therapy, firm conclusions cannot be drawn about the relationship between the D.T.P.A. test and body iron stores in these patients.

The results of the D.T.P.A. test in patients with iron deficiency were rather unexpected. Two of the three patients in whom the results were above normal had suffered from gastrointestinal bleeding but it is unlikely that contamination of urine with blood would account for the results obtained. Normal or high levels of urinary iron excretion after the administration of chelating agents have also been found by other workers in iron deficiency (Hwang and Brown, 1964). A possible explanation for this phenomenon is that in such patients a small amount of storage iron remained but that it was being rapidly mobilized and therefore was mostly in chelatable form.

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