Significance for the diagnosis of iron overload of histochemical and chemical iron in the liver of control subjects

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SYNOPSIS Storage iron was examined in surgical liver biopsy specimens in 43 haematologically normal and otherwise healthy adult individuals. These patients had no history of unphysiological iron losses nor of unphysiological iron intake. Histochemical iron was estimated in parenchymal and Kupffer cells and graded from 0 to 4+. Stainable iron of grade 1+ or more was present in parenchymal cells in 23 of the 27 men. Six of them had a 3+ grade. In nine cases iron was also visible in Kupffer cells. Visible iron was absent in most of the menstruating women. The mean total nonhaemin iron concentration for the male group was 80·2 (19·4 to 227·0), for the postmenopausal women 50·7 (19·3 to 106·6), and for the menstruating women 23·5 (5·5 to 65·9) mg./100 g. dry weight. The mean value for the women was significantly lower than the mean value for the men. There was a significant correlation between the histochemical grades of iron and chemically determined nonhaemin iron, but the degree of overlapping was considerable.

The presence of stainable iron in the parenchymal liver cells is a normal finding. The significance of the present results with reference to familial studies of haemochromatosis is discussed.

Most authors consider that the pathogenic mechanism in idiopathic haemochromatosis is an inherited abnormality of iron metabolism resulting in increased iron absorption from normal dietary iron (Sheldon, 1927; 1935). To demonstrate the validity of this concept several authors have studied asymptomatic relatives of patients with haemochromatosis and found an increased frequency of histochemically visible iron in liver biopsy specimens (Bothwell, Cohen, Abrahams, and Perold, 1959; Brick, 1961; Frey, Milne, Johnson, and Ebaugh, 1961; Williams, Scheuer, and Sherlock, 1962; Johnson and Frey, 1962; Balcerzak, Westerman, Lee, and Doyle, 1966). Some of these authors regarded even the presence of small amounts of stainable iron in parenchymal liver cells as a criterion. MacDonald and his associates, however, have advanced the concept that idiopathic haemochromatosis is a variant of portal cirrhosis with iron overload resulting from excess dietary iron ingested, particularly in the form of wine (MacDonald, 1961; 1963; 1964). MacDonald also points out that the

significance of stainable iron in liver biopsy specimens from relatives of patients with haemochromatosis is hard to evaluate since controls are lacking.

Data about the histochemical and chemical content of liver iron reported during the last years (MacDonald, Becker, and Pechet, 1963; MacDonald, 1964; Isacson Seftel, Keeley, and Bothwell, 1961; French, Pechet, Levy, and MacDonald, 1965) derive from necropsies of patients with various illnesses, and factors affecting the size of iron stores, such as blood loss, oral or parenteral iron administration, blood transfusions, or anaemia not due to iron deficiency could not always be evaluated. Furthermore due distinction was not made between men and between menstruating and non-menstruating women. It is, however, of prime importance to pay attention to the difference between sexes when cases of presumed iron overload are compared with controls (Weinfeld, 1964).

The aim of this study is to give an account of the histochemical and chemical iron in surgical liver biopsy specimens of healthy individuals from the area of Göteborg, Sweden.

MATERIAL AND METHODS

The subjects included in this study were essentially healthy and had no history of unphysiological blood loss. Their diet was the average one of this country containing 12 to 15 mg. of iron a day at a high caloric intake. They had never had blood transfusions or parenteral injections of colloidal iron. Some of the women had received oral iron during several months of pregnancy. There was no abuse of alcohol and the consumption of wine was low in all the subjects examined. The lower limit for haemoglobin concentration for men was 13·5 g. per 100 ml. and for women 11·5 g. per 100 ml., and no haematological abnormalities were found. They were admitted to the surgical department of the hospital for either uncomplicated cholelithiasis or uncomplicated gastric ulcer. At the time of operation no subject had signs of infection. All cases examined who fulfilled the above criterias were included in the study with the exception of those who at histological examination revealed pathological changes of liver architecture.

The series comprised 43 adults, 27 men and 10 postmenopausal and six menstruating women (Tables I and II).

The liver biopsy specimen was taken at the start of operation, and usually weighed 0·5 to 1·0 g. Acid-washed utensils, iron-free water, and reagents of analytical purity were used throughout the study.

A representative piece of the biopsy specimen was fixed in neutral 10% formaldehyde, embedded in paraffin, and sections cut at 3μ which were subsequently stained for haemosiderin with potassium ferrocyanide according to the modification of Hutchison (1953). Iron was graded on an arbitrary scale of 0 to 4+, essentially in the same way as previously described for bone marrow sections (Lundin, Persson, and Weinfeld, 1964). The histochemical grading of stainable iron was made without reference to the chemical iron determination. The major part of the specimen was homogenized and aliquots of 100 to 300 mg. of wet liver tissue were taken for chemical analysis and for determination of dry weight. The analysis of total nonhaemin iron and ferritin iron (water-soluble iron) was according to the procedure described previously (Weinfeld, 1964). Haemosiderin iron was calculated from the difference between total nonhaemin iron and ferritin iron. The values are means of double determinations and expressed as milligram nonhaemin iron per 100 gram liver tissue dry weight.

The serum iron was determined according to the method of Laurell (1964) and total iron binding capacity (TIBC) according to Peters, Giovanniello, Apt, and Ross (1956). The haemoglobin was determined photometrically as cyanmethaemoglobin.

RESULTS

HISTOCHEMICAL IRON In parenchymal liver cells (Fig. 1) stainable iron of grade 1+ or more was present in 21 of the 27 male subjects (78%), and nine had stainable iron of grade 2+ and six grade 3+ (Table I). In Kupffer cells (Fig. 2) stainable iron was visible in nine cases (33%). All who had stainable iron in Kupffer cells had also haemosiderin deposits in the parenchymal cells. There was only one subject (G.G.) who had grade 3+ of stainable iron in the Kupffer cells. He also had the highest concentration of nonhaemin iron in the liver which was significantly different from the normal range of his group.

Of the nine postmenopausal women six had grade 1+ or more of stainable iron in the parenchymal liver cells (Fig. 1). Two of them had grade 1+ of haemosiderin in Kupffer cells (Fig. 2). Of the seven menstruating women only one had grade 1+ in the parenchymal liver cells and none had stainable iron in the Kupffer cells (Figs. 1 and 2). For both groups of women, stainable iron in parenchymal liver cells was found in seven subjects (44%). Table II shows the results for all women.

CHEMICALLY DETERMINED NONHAEMIN IRON In the men the mean total nonhaemin iron concentration
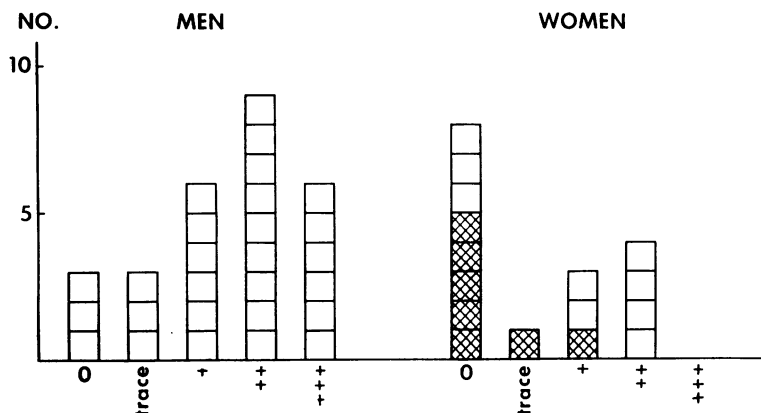

FIG. 1. *Distribution of the subjects according to the grade of histochemically demonstrable iron in parenchymal liver cells (hatched squares represent menstruating women).*

TABLE I

HISTOCHEMICAL AND CHEMICAL NONHAEMIN LIVER IRON IN THE MALE GROUP

Subject	Age	Hb (g./100 ml.)	Serum Iron (μg./100 ml.)	T.I.B.C. (μg./100 ml.)	E.S.R. (mm./hr.)	Liver Histology	Histochemical Estimation of Stainable Iron (grade 0 − 4 +) Parenchymal Cells	Kupffer Cells	Total Non-haemin Iron (mg./100 g. dry wt.)	Ferritin Iron (mg./100 g. dry wt.)	Haemosiderin Iron (mg./100 g. dry wt.)
Cholelithiasis											
B.G.	59	14·6	69	322	26	Normal	trace	0	54·5	40·4	14·1
G.G.	57	15·1	120	269	4	Normal	+++	+++	227·0	174·4	58·8
A.R.	37	16·1	187	293	3	Normal	++	+	50·4	31·6	18·9
R.N.	45	13·6	123	287	11	Normal	+	0	48·8	34·4	12·4
L.A.	40	13·5	126	238	6	Normal	++	++	124·4	85·8	38·5
P.G.	59	13·5	82	296	8	Normal	++	0	74·0	48·9	25·1
H.H.	59	17·2	118	309	2	Slight steatosis	++	0	96·9	61·7	35·2
G.H.	58	14·5	173	296	6	Normal	+	0	19·4	10·8	8·6
N.K.E.	23	15·4	123	421	2	Normal	+	0	61·2	34·4	26·8
B.P.	26	14·1	68	335	3	Normal	+	0	67·7	49·5	18·2
B.S.	27	14·9	82	411	2	Normal	trace	0	37·6	25·4	12·2
J.A.	49	14·1	145	378		Normal	+	0			
N.H.	63	14·0	137	350	5	Normal	trace	0			
N.P.	65	14·1	95	281	7	Normal	0	0			
P.A.	40	14·5	151	400		Slight steatosis	++	0			
Gastric ulcer											
F.T.	50	16·6				Slight steatosis	+	0	102·9	43·7	59·2
S.A.	38	15·3	80	345		Normal	+++	0	111·1	58·9	52·2
E.E.	32	13·8	103			Normal	++	0	113·5	62·9	60·7
H.A.	39	15·2	120	325		Normal	++	+	60·3	25·2	35·1
H.N.E.	53	16·8	60	230		Normal	0	0	30·4	13·0	17·4
A.H.W.	42	14·1	135	350		Normal	+++	+	69·7	31·6	38·1
O.K.	61	14·1	75	245		Normal	+++	++	141·2	67·8	73·4
E.H.	61	13·9	140	315	11	Slight steatosis	++	0	47·0	14·9	32·1
J.P.A.	62	14·0	151	305	9	Normal	++	+	55·4	37·4	18·0
F.G.	62	13·5	91	299	13	Slight steatosis	+++	+	120·7	66·7	54·0
B.S.	24	14·3	112	287	4	Normal	0	0	41·6	25·5	16·1
J.S.	44	14·0	183	336	6	Normal	+++	+	88·7	53·8	34·8

TABLE II

HISTOCHEMICAL AND CHEMICAL NONHAEMIN LIVER IRON IN MENSTRUATING AND NON-MENSTRUATING WOMEN

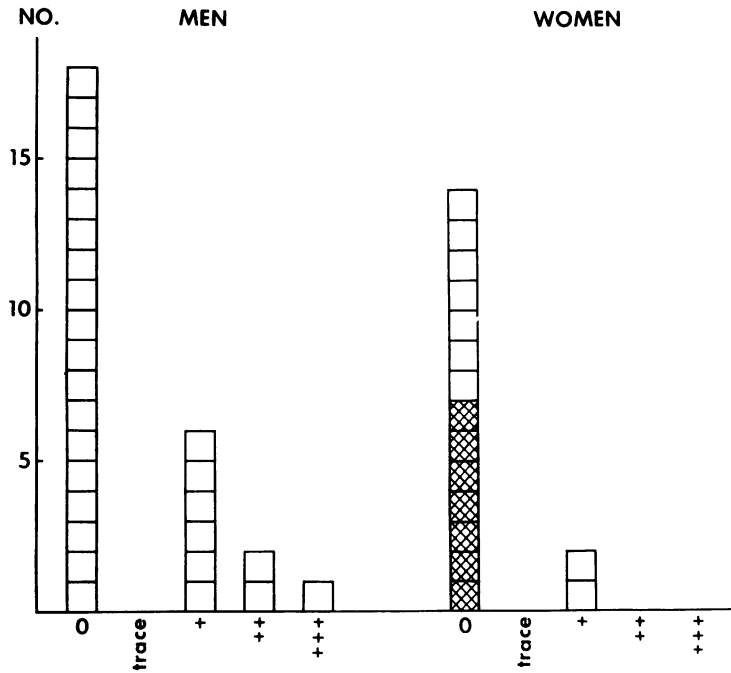| Subject	Age	Hb (g./100 ml.)	Serum Iron (μg./100 ml.)	T.I.B.C. (μg./100 ml.)	E.S.R. (mm./hr.)	Liver Histology	Histochemical Estimation of Stainable Iron (grade 0 − 4 +) Parenchymal Cells	Kupffer Cells	Total Non-haemin (mg./100 g. dry wt.)	Ferritin Iron (mg./100g. dry wt.)	Haemosiderin Iron (mg./100 g. dry wt.)
Non-menstruating women: cholelithiasis											
F.H.	69	14·4	94	343	15	Slight steatosis	0	0	19·3	19·1	0·2
O.M.	78	16·0	137	233		Normal	++	+	46·6	33·3	13·3
B.V.	51	11·9	113	229	9	Normal	++	+	50·7	39·8	10·9
S.E.	58	13·0			5	Normal	++	0	106·6	64·3	42·3
D.E.	59	12·7	60	256	32	Slight steatosis	+	0	32·1	28·6	3·5
N.S.	45	12·6	151	305	15	Normal	++	0	73·3	40·2	33·1
G.E.	56	12·8	82	353	26	Normal	0	0	26·2	21·3	4·9
N.H.	55	13·4	114	259		Slight steatosis	+	0			
S.E.	66	12·9			32	Slight steatosis	0	0			
Menstruating women: cholelithiasis											
J.U.	25	13·7	65	375	10	Normal	0	0	13·0	13·0	0·0
M.M.	46	13·7	108	433		Normal	0	0	5·5	4·6	0·9
S.K.	35	12·4	123	311	11	Normal	0	0	14·0	14·0	0·0
W.E.	23	11·8	249	351	12	Normal	Trace	0	26·9	20·9	6·0
F.S.	35	13·0	198	305	5	Normal	0	0	31·1	23·7	7·3
J.B.	40	12·2	88	413	33	Normal	0	0	8·1	4·8	3·3
Gastric ulcer											
J.B.	43	15·3	177	299	17	Normal	+	0	65·9	44·3	21·6

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FIG. 2. *Distribution of the subjects according to the grade of histochemically demonstrable iron in Kupffer cells (hatched squares represent menstruating women).*
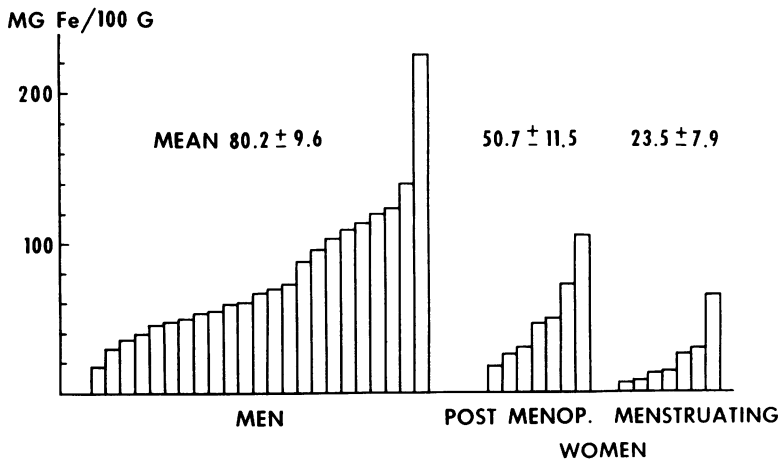

FIG. 3. *Distribution of chemical non-haemin iron concentrations in liver biopsy specimens.*

in the liver was 80.2 ± 9.6 mg./100 g. dry weight with a range of 19·4 to 227·0 mg./100 g. (Table I and Fig. 3). If the highest value of subject G.G., which differed from the normal range of this group, is eliminated, the mean value would be 73.5 ± 7.2 mg./100 g. dry weight. The mean value of the 11 subjects who had a cholecystopathy (78.4 ± 17.2) was not different from the mean of the 12 subjects who had uncomplicated gastric ulcer (81.9 ± 10.4).

The mean concentration of ferritin iron (water-soluble iron) was 47·8 mg./100 g. dry weight and the

range 10·8 to 174·4. The mean haemosiderin iron concentration was 33·0 mg./100 g. dry weight with a range of 8·6 to 60·7.

The mean total nonhaemin iron concentration of the non-menstruating women was 50.7 ± 11.5 mg./ 100 g. dry weight with a range of 19·3 to 106·6. The mean ferritin iron concentration was 35·2 and ranged from 19·3 to 64·3. The mean haemosiderin iron concentration was 15·5 with a range of 0·2 to 42·3 mg./100 g.

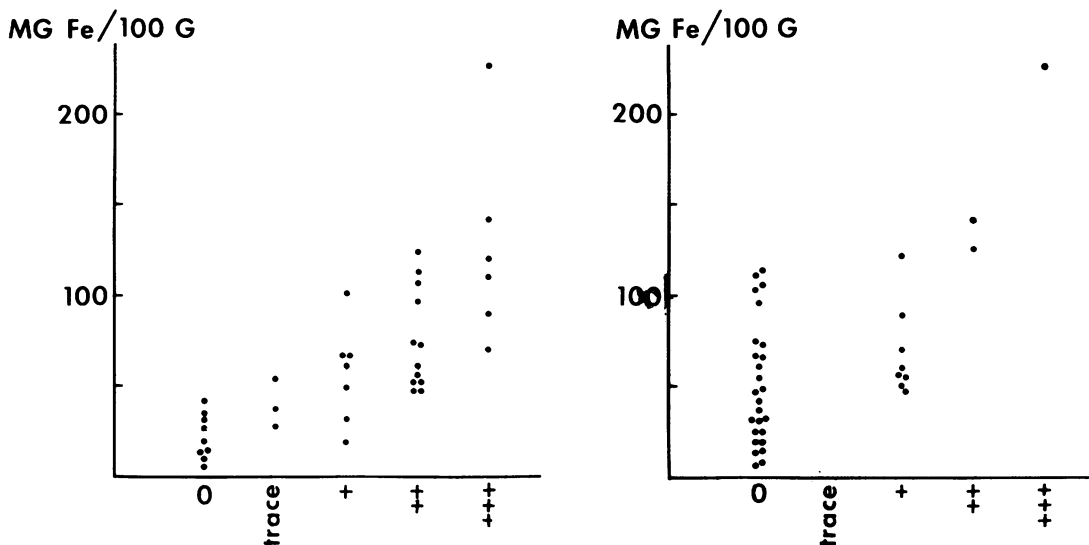The mean total nonhaemin iron concentration for

FIG. 4. *Relationship between total nonhaemin iron concentration and histochemically stainable iron in parenchymal cells (left) and in Kupffer cells (right).*

the group of menstruating women was 23.5 ± 7.9 mg./100 g. dry weight with a range of 5·5 to 65·9. The mean ferritin iron concentration was 17·9 with a range of 4·6 to 44·3. The mean haemosiderin iron concentration was 5·6 with a range of 0·0 to 21·6 mg./100 g.

The mean total nonhaemin iron concentration for both groups of women was 37.1 ± 7.7 mg./100 g. dry weight and was significantly lower than the mean value of the males.

RELATION BETWEEN IRON ESTIMATED HISTOCHEMICALLY AND NONHAEMIN IRON DETERMINED CHEMICALLY As shown in Fig. 4, the mean total nonhaemin iron concentration increased as the amount of histochemically demonstrable iron in parenchymal liver

TABLE III

CHEMICAL NONHAEMIN IRON CONCENTRATIONS RELATED TO HISTOLOGICAL GRADING OF STAINABLE IRON

Grade of Stainable Iron in Parenchymal Cells	n	Total Non-haemin Iron (mg./100 g. dry wt.)	Haemosiderin Iron (mg./100 g. dry wt.)
0	9	21·0 ± 3·7 (5·5 − 41·6)	5·6 ± 2·3 (0·0 − 17·4)
Trace	3	39·7 ± 8·0) (26·9 − 54·5)	10·8 ± 2·5 (6·0 − 14·1)
+	7	56·9 ± 10·3 (19·4 − 102·9)	21·5 ± 7·0 (3·5 − 59·2)
+ +	12	74·9 ± 8·2 (46·6 − 124·4)	30·3 ± 4·0 (10·9 − 60·7)
+ + +	6	126·4 ± 22·5 (69·7 − 227·0)	51·9 ± 5·8 (34·8 − 73·4)

cells increased. The difference between the mean of the group in which stainable iron was not demonstrable and that of the group in which at least grade 1+ of stainable iron was found was significant (Table III). Grade 2+ of stainable iron in parenchymal cells was the most frequent finding in the male group and in the postmenopausal group. The mean total nonhaemin iron concentration of livers with 2+ of stainable iron was similar to the mean of the male group.

The range of values found chemically for each of the histochemical gradings was, however, large and overlapped considerably with the values in the other gradings. The practical conclusion which can be drawn from a histochemical examination in the individual case is that if there is a grade 2+ or more of stainable iron in the parenchymal cells, the total nonhaemin iron concentration will probably be higher than the range of nonhaemin iron concentration encountered in the histochemical group with grade 0.

COMMENT

Early workers in pathology distinguished between haemosiderosis in which the iron is primarily located in the reticuloendothelial cells and haemochromatosis in which the iron is mainly present in parenchymal cells. Some authors still regard the presence of haemosiderin in parenchymal liver cells as a pathological finding caused either by intrinsic

liver disease or iron storage disease (Masshof, 1954; Kautzsch, 1959). A similar view is held by some authors who studied the problem of inheritance of iron storage disease by examination of stainable iron in liver biopsy specimens of asymptomatic relatives of patients with haemochromatosis. Thus Bothwell *et al.* (1959) found varying degrees of stainable iron in half of investigated siblings but they do not state which degree of iron deposition is regarded as an iron overload. Johnson and Frey (1962) regard even a minimal deposition of haemosiderin in parenchymal liver cells as haemosiderosis. In the extensive study of Williams *et al.* (1962) 28 of 46 examined asymptomatic relatives of propositi of haemochromatosis had stainable iron in liver cells. Of the male subjects 79% and of the females 29% had visible haemosiderin. The authors assume that the finding of free iron in liver cells is a sign of the disease.

The results of the present study have shown that haemosiderin was present in parenchymal liver cells in 78% of healthy male subjects with average dietary habits. The most frequent finding was grade 2+ but an appreciable number had a grade 3+ of stainable iron. The amount of stainable iron was lower in women, and in menstruating women histochemically visible iron was seldom encountered. Accordingly many subjects which in the above mentioned familial studies are regarded as haemosiderotics would fall within the normal range of the present series. Thus the presence of stainable iron in parenchymal liver cells does not deserve the term 'haemosiderosis', which implies a pathological state. On the contrary the absence of haemosiderin in parenchymal liver cells indicates low iron stores.

Since our controls show a large range of non-haemin iron concentrations in the liver and the histological estimations an appreciable degree of overlapping with the chemical values, it seems unlikely that a moderate deviation from normal in relatives of haemochromatotics could be demonstrated histochemically. We suggest that familial studies of haemochromatosis should be extended by chemical determinations of nonhaemin iron in needle biopsy specimens, determinations of desferri-oxamine-induced urinary iron excretion (desferal test), and iron absorption studies in relation to the iron stores (Balcerzak *et al.*, 1966).

Histochemical studies of liver iron by different authors have given varying results. Thus Zimmerman, Chomet, Kulesh, and McWhorter (1961) in Chicago and Scheuer, Williams, and Muir (1962) in London found stainable iron in about 15% of consecutive liver biopsy specimens. However, Pechet and MacDonald (1962) in Boston and MacDonald *et al.* (1963) in Johannesburg found parenchymal liver iron in about 60% of unselected necropsies without liver disease. The nature of these discrepancies does not need to be geographical, it might depend entirely on a heterogeneity of the materials studied. For evaluation of the normal range of histochemically visible iron in the liver all factors which might alter a normal iron balance must be considered, and it should be remembered that iron stores are rebuilt slowly and that results can therefore be influenced by blood losses which occurred years before the investigation.

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