

Iron storage in human disease

Fractionation of hepatic and splenic iron into ferritin and haemosiderin with histochemical correlations

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SYNOPSIS The hepatic and splenic storage iron, including the relative distribution between ferritin and haemosiderin, was estimated in 130 necropsies including normal accident cases and cases with a variety of diseases. The storage iron was also examined histochemically. It was found that in the normal subjects, on the average, approximately 400 mg. of iron was stored in these two organs, somewhat more than half being present as ferritin iron. Increased storage iron was found in some cases of infection, malignancy, and in blood and hepatic diseases, while low stores were present in other cases with malignancy and in polycythaemia vera. Although there was a slight tendency in infections and malignant diseases for more of the storage iron to be present as haemosiderin than normally, the most important factor affecting the distribution of iron between ferritin and haemosiderin was the total storage iron concentration. With total storage iron less than 500 μ g. per gram of tissue, more iron was stored as ferritin than haemosiderin, and with values above 1,000 μ g. per gram more was stored as haemosiderin. The behaviour of storage iron in this respect was very similar both in the liver and in the spleen. Although the histological and chemical estimates of the storage iron showed a general agreement there was much variation and histological examination of the tissues gave only a very approximate idea of the storage iron levels.

Iron is stored in the body as the two non-haem compounds, ferritin and haemosiderin. Although several workers have studied their chemical relationships (Granick, 1946, 1951; Richter, 1958; Wöhler, 1960; Shoden and Sturgeon, 1960, 1961) and the physiological relationships in experimental animals (Shoden, Gabrio, and Finch, 1953; Konitzer, 1957; Kaldor, 1958; Shoden and Sturgeon, 1958, 1959; Wöhler and Zoll, 1960; Morgan, 1961a and b, 1962), few have investigated the storage of iron in man (Shoden *et al.* 1953; Meier, Beneke, and Ahlert, 1959; Wöhler and Otte, 1961; Wöhler, Otte, and Fulgraff, 1961).

In our study the relative distribution of storage iron between ferritin and haemosiderin was measured chemically in the liver and spleen of normal humans and in others dying from a variety of diseases. In addition tissue sections were examined histochemically for demonstrable iron as a comparison with the chemical findings. It was hoped that these results would provide useful information on the pathophysiology of iron storage.

MATERIALS

Specimens of liver and spleen were obtained at necropsy from 130 subjects during a one-year period at the Royal Perth Hospital, Western Australia. They were selected to include as many cases of diseases of the blood, liver, and reticulo-endothelial system as available, as well as a number of each of the more common diseases seen at necropsy (cardiovascular disease, malignant disease, and infection). The results were arranged (Tables I and II) according to the major pathological changes found at necropsy or in a group labelled as 'diabetes', where diabetes mellitus had been present for many years before death and where at necropsy, atheroma and its sequelae were predominantly associated. The 26 cases of cardiovascular disease included 19 in which death was due to myocardial infarction, five with cerebrovascular accidents, and one each with hypertensive cardiac disease and pulmonary embolism. Of the 32 cases with infections, 19 had died of pneumonia and the others of peritonitis, meningitis, septicaemia, and infection of the genitourinary tract. The malignant diseases were, with the exception of one subject with cerebral tumour, all cases of carcinoma in its more common sites, usually with extensive metastases. In addition to the tissues from these subjects, specimens of liver and spleen were obtained

from 21 normal subjects who had been in good health until their death by accident and at necropsy showed no evidence of disease other than the effects of trauma.

METHODS

ESTIMATION OF STORAGE IRON FRACTIONS From the time of necropsy until analysis the tissues for the chemical estimation of storage iron were stored at -16°C . They were then thawed and aliquots cut, weighed, and analysed in duplicate for total storage iron and water-soluble storage iron (ferritin iron) by the methods of Kaldor (1954, 1958). Gabrio, Shoden, and Finch (1953) proved the validity of using differences in water solubility for the estimation of ferritin and haemosiderin iron, and Kaldor (1958) showed that the sum of the ferritin iron and haemosiderin iron equalled the total storage iron. In the present work, therefore, the water-soluble storage iron has been called ferritin iron, and the haemosiderin iron values have been calculated as the difference between the total storage iron and ferritin iron.

HISTOLOGICAL METHODS Blocks ($1.5 \times 1.5 \times 0.5$ cm.) of liver and spleen were fixed in 10% buffered formalin (pH 7) (Lillie, 1954) for 36 hours, processed, and embedded in paraffin. Sections were cut at 5μ and stained first by celestin blue-haematoxylin and eosin and secondly by Perls's prussian blue reaction (Culling, 1957). A qualitative estimation of the extent of the visible haemosiderin deposits was made according to an arbitrary scale, increasing in intensity from 0 to 4.

RESULTS

The storage iron values for the main disease groups are shown in Table I. In the normal and the diabetic group the histological iron gradings ranged from

0 to 3 and the other groups from 0 to 4. The storage iron values for the normal subjects were somewhat higher between the ages of 20 and 50 years than below or above this age range. However, no changes occurred in the proportion of storage iron present as ferritin and haemosiderin with respect to age.

The mean storage iron values for the liver in the disease groups differed little from the normal values. A statistically significant difference between disease and normal groups was found only for the percentage of storage iron in the ferritin fraction in diabetic subjects ($P < 0.01$). If the means of the normal cases ± 3 standard deviations are taken as the normal range of values, then only two subjects with infections and two with malignant disease showed abnormally high storage iron concentrations, while in three with infections and two with malignant disease relatively less of the storage iron was present as ferritin than normal.

In the case of the spleen, however, the differences between normal and disease groups were more marked. In the cardiovascular group the mean total storage iron concentration was significantly increased ($P < 0.001$), six subjects having values above the normal range. The mean total storage iron values were still higher in infections and malignant disease and the differences from the normal were again significant ($P < 0.001$ and $P < 0.04$, respectively). Values were abnormally high in 19 of the subjects with infection and in 10 with malignant diseases. A significant alteration in the relative distribution of storage iron between ferritin and haemosiderin was found only in the malignant group ($P < 0.05$), although two of the subjects

TABLE I
HEPATIC AND SPLENIC TOTAL STORAGE IRON AND PERCENTAGE OF TOTAL STORAGE IRON IN FERRITIN FRACTION IN NORMAL SUBJECTS AND SUBJECTS WITH CARDIOVASCULAR DISEASE, INFECTIONS, MALIGNANT DISEASES, AND DIABETES MELLITUS¹

Diagnosis	Number of Subjects	Liver			Spleen		
		Storage Iron Concentration ($\mu\text{g. per g.}$)	Storage Iron Content (mg.)	Percentage in Ferritin Fraction	Storage Iron Concentration ($\mu\text{g. per g.}$)	Storage Iron Content (mg.)	Percentage in Ferritin Fraction
Normal	21	244.5 \pm 32.10 ¹ 67-758	391.1 \pm 75.74 107-1,213	66.76 \pm 2.143 47.90	149.1 \pm 16.21 69-203	22.24 \pm 2.59 10-57	65.28 \pm 2.575 44-88
'Normal range'		0-677	0-1,432	37-96	0-372	0-59	30-100
Cardiovascular disease	26	228.3 \pm 37.01 21-641	389.4 \pm 87.50 64-1,321	67.00 \pm 2.469 37-94	288.1 \pm 29.80 19-1,263	48.12 \pm 7.69 5-158	62.26 \pm 2.706 37-91
Infections	32	280.2 \pm 11.01 60-874	481.2 \pm 71.55 84-1,487	60.18 \pm 2.904 29-92	479.9 \pm 63.12 81-1,425	67.33 \pm 10.89 8-197	61.45 \pm 3.268 16-99
Malignant diseases	25	256.8 \pm 43.28 30-907	448.2 \pm 71.78 47-1,470	62.58 \pm 2.834 33-84	377.2 \pm 61.58 28-1,314	68.29 \pm 11.57 4-216	56.76 \pm 3.122 32-91
Diabetes mellitus	7	187.7 \pm 34.13 72-323	373.5 \pm 121.6 130-708	81.03 \pm 3.982 67-99	201.8 \pm 64.85 30-452	55.01 \pm 23.48 8-116	71.04 \pm 6.015 51-94

¹Mean values \pm standard error of the mean, and observed range of values. 'Normal range' mean ± 3 standard deviations

TABLE II
HEPATIC AND SPLENIC TOTAL STORAGE IRON AND PERCENTAGE OF TOTAL STORAGE
IRON IN FERRITIN FRACTION IN VARIOUS DISEASES

No.	Age	Sex	Diagnosis	Liver				Spleen			
				Histological Grading	Storage Iron Concentration ($\mu\text{g./g.}$)	Storage Iron Content (mg.)	Percentage in Ferritin Fraction	Histological Grading	Storage Iron Concentration ($\mu\text{g./g.}$)	Storage Iron Content (mg.)	Percentage in Ferritin Fraction
1	64	M	Polycythaemia vera	0	46	91	78	0	58	46	57
2	61	M	Polycythaemia vera	0	102	236	82	1	163	126	44
3	52	F	Aplastic anaemia	4	665	1,276	51	4	805	121	54
4	73	M	Myelofibrosis	1	146	260	72	2	142	80	85
5	62	F	Multiple myeloma	4	2,173	7,260	27	4	8,575	4,410	23
6	14	M	Chronic lymphatic leukaemia	3	686	1,958	44	4	462	1,250	79
7	53	F	Chronic lymphatic leukaemia	3	725	1	28	2	319	1	45
8	41	M	Acute myeloid leukaemia	3	1,819	3,384	31	0	305	276	47
9	62	F	Reticulum cell sarcoma	1	239	448	54	0	104	109	79
10	50	F	Active chronic hepatitis	4	517	403	50	1	127	23	82
11	45	M	Hepatic cirrhosis	3	394	461	99	2	483	72	82
12	46	M	Hepatic cirrhosis	4	1,150	2,725	43	2	349	248	55
13	69	M	Hepatic cirrhosis	1	123	207	75	0	66	36	55
14	69	M	Hepatic cirrhosis, portocaval shunt	4	1,344	2,010	42	1	208	52	35
15	39	M	Haemochromatosis	4	9,000	15,110	13	2	536	133	29
16	39	M	Acute hepatitis	2	149	1	70	3	248	1	77
17	31	M	Chronic glomerulo-nephritis	1	321	717	65	3	1,270	209	43
18	67	M	Pemphigus vulgaris	3	355	692	62	4	660	59	73
19	75	F	Post-gastrectomy malabsorption, splenectomy	1	60	84	57				

¹Liver and spleen weights not available.

TABLE III
STORAGE IRON CONCENTRATIONS ARRANGED ACCORDING TO HISTOLOGICAL GRADING¹

Histological Grading	Total Storage Iron Concentration ($\mu\text{g. per g.}$)		Ferritin Iron Concentration ($\mu\text{g. per g.}$)		Haemosiderin Iron Concentration ($\mu\text{g. per g.}$)	
	Liver	Spleen	Liver	Spleen	Liver	Spleen
0	132.67 \pm 14.69	110.67 \pm 27.24	85.43 \pm 9.18	78.28 \pm 22.48	47.24 \pm 6.44	32.39 \pm 8.72
1	228.06 \pm 16.96	148.57 \pm 13.80	144.06 \pm 8.77	85.46 \pm 6.62	84.00 \pm 11.41	63.11 \pm 7.71
2	315.33 \pm 29.32	283.36 \pm 30.01	217.07 \pm 27.48	174.31 \pm 16.18	98.26 \pm 9.42	109.05 \pm 17.81
3	594.59 \pm 87.22	611.42 \pm 124.91	297.76 \pm 26.42	315.36 \pm 31.58	296.82 \pm 71.95	296.06 \pm 41.98
4	1,836.75 \pm 737.5	1,269.29 \pm 565.9	425.33 \pm 79.92	504.50 \pm 115.4	1,411.42 \pm 665.5	756.78 \pm 454.5

¹ Mean values \pm standard error of the mean.

with infections did have abnormally low values.

In Table II are shown the results of a variety of diseases not included in Table I. It can be seen that the total storage iron concentrations were low in the two cases of polycythaemia vera and increased in patients with aplastic anaemia, multiple myeloma, leukaemia, and in two of the four with cirrhosis of the liver. Low values were found in the cases of rheumatic heart disease and with malabsorption complicating gastrectomy. It had been demonstrated during life that the latter had deficient iron absorption. The highest storage iron value found in any subject was in the liver of the patient with haemochromatosis. This man had been treated by repeated venesection during the last year of his life.

Marked alteration in the relative distribution of storage iron between ferritin and haemosiderin was

found only in subjects with high storage iron concentrations, where the proportion present as ferritin was small, and in the liver of one case of portal cirrhosis where this proportion was abnormally high.

As shown in Table III the mean total storage iron concentration in both the liver and spleen increased as the amount of histologically demonstrable iron increased. However, the range of values found chemically for each of the histological gradings was large and overlapped greatly with the values found for the other gradings. This overlapping appears to be greater for the haemosiderin than for the total storage iron values. It can be seen, however, that with an histological grading of 0 and 1, only one total storage iron value each for the liver and spleen was greater than the upper limit of the

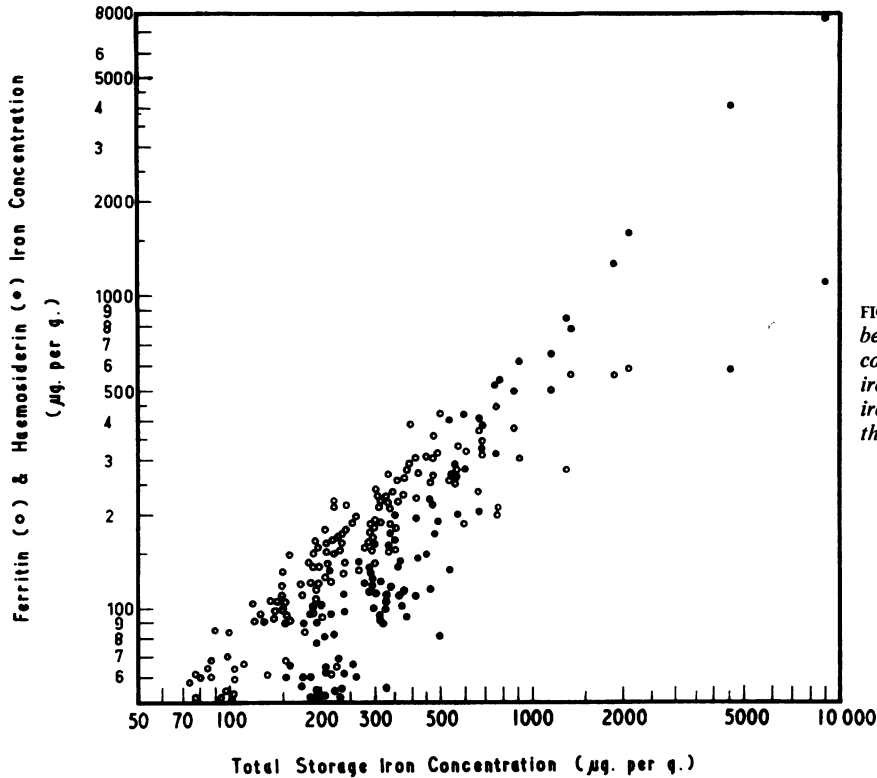


FIG. 1. Relationship between total storage iron concentration and ferritin iron (○) or haemosiderin iron (●) concentrations in the liver.

normal range. Also, with a grading of 3 or 4, and more especially in the spleen than the liver, the majority of the storage iron concentrations were greater than normal. It is worthy of note that stainable iron up to grade 3 was commonly found even though the haemosiderin iron concentration was less than 100 $\mu\text{g. per gram}$.

In Fig. 1 (liver) and Fig. 2 (spleen) the ferritin iron and haemosiderin iron concentrations have been plotted against the corresponding total storage values. In both organs, although being more marked in the liver than the spleen, when the total storage iron was below 500 $\mu\text{g. per gram}$, more of the iron was usually present as ferritin than as haemosiderin. Between 500 and 1,000 $\mu\text{g. per gram}$ the proportion in both fractions tended to be approximately the same, while above 1,000 $\mu\text{g. per gram}$ progressively more was found as haemosiderin.

DISCUSSION

The total storage iron concentrations found in normal subjects in the present work are similar to those previously reported (Tompsett, 1935; Brückmann and Zondek, 1939; Schairer and

Rechenberger, 1948a; Meier *et al.*, 1959). It is apparent, then, that the total storage iron concentration, high at birth, falls to low levels during infancy (Ramage, Sheldon, and Sheldon, 1933; Brückmann and Zondek, 1939; Smith, Rosello, Say, and Yeya, 1955) and that the level remains low until growth has ceased, when at about the age of 20 years it increases to higher values. These higher values may be maintained throughout adult life or may fall somewhat after the age of 50 or 60 years. There were only three females in the normal group and it is not possible to draw any conclusions from sex differences in iron storage. It is thought, however, that the iron stores are usually smaller in females than in males (Schairer and Rechenberger, 1948a; Roth, Jasiński, and von Bidder, 1951) especially during the reproductive period of life.

The total amount of storage iron present in the normal adult has been estimated to be 1.0 to 1.5 g. (Drabkin, 1951; Haskins, Stevens, Finch, and Finch, 1952; Granick, 1954). In the present work the mean total iron content of liver plus spleen was only 400 mg. The other major storage organ for iron is the bone marrow. If the total active marrow in the body is 3,000 g. (Whitby, 1954) and this has a

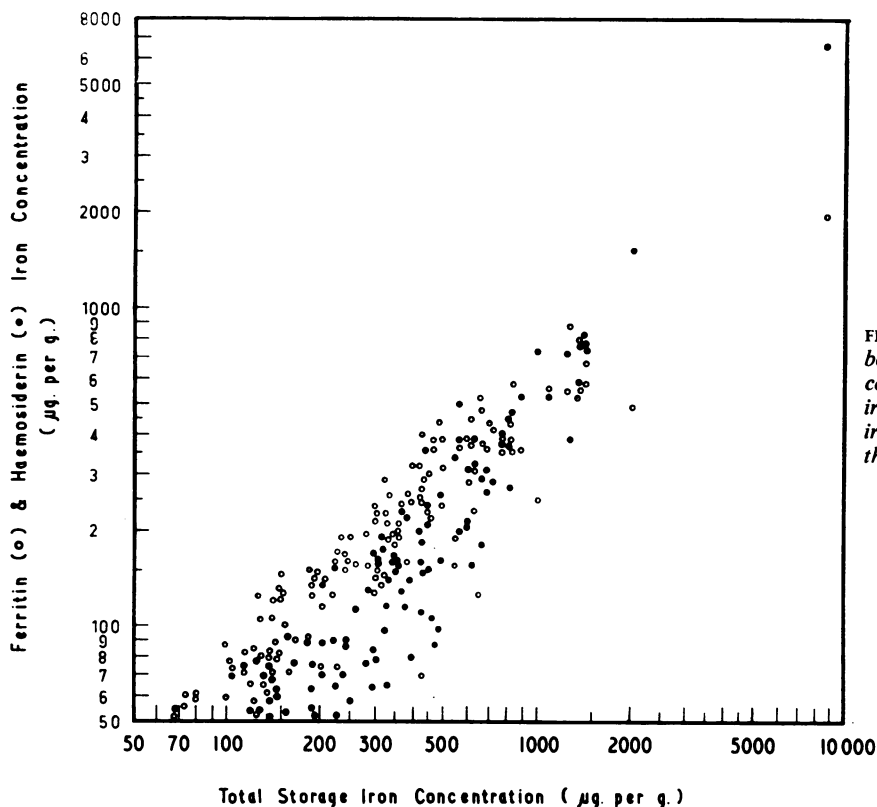


FIG. 2. Relationship between total storage iron concentration and ferritin iron (○) or haemosiderin iron (●) concentrations in the spleen.

storage iron concentration of about 100 µg. per gram (Hallgren, 1954) then the bone marrow would contain approximately 300 mg. storage iron. A considerable amount of storage iron still remains to be accounted for. Of the other organs of the body, the muscles, because of their great mass, contain the most non-haem iron, even though the concentration is low (Kooyman, 1949; Hallgren, 1954), and this may represent most of the remaining storage iron.

An increase in the mean total storage iron concentration was found in the spleen but not in the liver of subjects with infections and malignant diseases. This agrees with the results of Schairer and Rechenberger (1948b) although Gross, Sandberg, and Holly (1942), Roth *et al.* (1951), and Hallgren (1954) also found storage iron to be increased frequently in the liver. Some of the subjects in the present work showed an increase in storage iron in the liver. Mild to moderate anaemia with a reduction in the circulating haemoglobin mass is common in infections and malignant disease. The increase in iron stores can probably be completely accounted for by storage of the iron lost from the blood haemoglobin. Another factor which may alter the

iron stores in certain malignant conditions is haemorrhage with resultant iron deficiency. Of the seven subjects in the malignant group with lowest storage iron values (all with liver total storage iron concentrations below 100 µg. per gram) five had alimentary carcinomata, one renal and one hepatic haemangiosarcoma, all of which were complicated by haemorrhage.

The high storage iron values found in the cases with aplastic anaemia, multiple myeloma, and leukaemia (Table II) may have been due to redistribution of iron from the blood to stores, to blood transfusions, and to dietary iron absorption. The low values in polycythaemia vera were probably the result of utilization of the iron stores for the synthesis of the increased haemoglobin mass. The high hepatic storage iron values in two of the four cases with cirrhosis are of interest in relation to the aetiology of haemochromatosis, especially in the patient who had been treated with a porto-caval anastomosis. Four cases of excessive iron deposition into the tissues with the picture resembling haemochromatosis after porto-systemic shunts have been reported. These subjects had hepatic cirrhosis and little hepatic

storage iron at the time of operation (Tuttle, Figueroa, and Grossman, 1959; Hoffbauer, 1960; Tisdale, 1961). As there is no increase in hepatic storage iron in diabetes mellitus this suggests that diabetes is unlikely to be the cause of the increased storage iron found in haemochromatosis. Rather, as is generally believed, the excess deposition of iron in the pancreatic tissues probably causes the diabetes. Comparison of the results for stainable iron and chemically estimated storage iron (Table III) indicates that histological examination gives only an approximate indication of the storage iron level. If little or no stainable iron is found, then it may be reliably concluded that iron stores are not increased, while if iron is found up to grade 3 or 4 in the present work, then it is likely that the iron stores are increased. Other workers have also found an approximate agreement between histologically and chemically measured storage iron (Schairer and Rechenberger, 1948b; Emery and Hilton, 1960; Bradlow, Dunn, and Higginson, 1961). The tissue most frequently examined for stainable iron is the bone marrow, and Beutler, Robson, and Bittenwieser (1958) consider such an examination to be the most reliable way of assessing iron stores. However, the results of Kerr (1957) indicate that agreement between chemically and histologically demonstrable iron is no better in the bone marrow than found in the present work for liver and spleen. Furthermore, Hallgren (1954) found a good correlation between marrow and hepatic storage iron concentrations only when the values were relatively low. It is therefore apparent that histological examination of bone marrow for iron can only give an approximate estimate of iron stores.

The stainable iron grading correlates better with the total storage iron concentration than with the haemosiderin iron values. This is probably due to the fact that the iron of ferritin gives positive Prussian blue staining as does that in haemosiderin (Shoden and Richter, 1960).

In the diseases shown in Table I a significant reduction in the mean for the relative proportion of storage iron present as ferritin was found only in the spleen in the malignant group. In addition, in five individual instances (three infections, two malignant conditions) the values for the percentage of storage iron in the ferritin fraction were below the lower limit of the normal range. Only two of these subjects had abnormally high total storage iron values. It would appear, then, that although in some cases of infection and malignancy proportionately more storage iron may be deposited as haemosiderin than normally, this is not a constant or characteristic change in these diseases. Wöhler and Otte (1961) using different analytical methods found more

marked alterations in the storage iron distribution between ferritin and haemosiderin in infections and malignant disease than in the present work.

The most important factor affecting the relative distribution of iron between ferritin and haemosiderin appears to be the total storage iron concentration (Figs. 1 and 2). This may partly explain the low mean value for the percentage of storage iron in the ferritin fraction found in the malignant group (Table I) and probably also explains the low value found for this in several of the cases presented in Table II. It should also be noted that in the liver of the case with haemochromatosis the proportion of storage iron present as ferritin was no lower than would be expected from the general trend shown in Fig. 2. The progressively greater relative deposition of storage iron as haemosiderin than as ferritin in subjects with iron stores increasing above normal levels is in accordance with the results of Shoden *et al.* (1953). The biochemical basis for these changes is not known. It has been shown, however, that storage iron present both as ferritin and haemosiderin is readily available for use elsewhere in the body when required, as after haemorrhage (Konitzer, 1957; Morgan, 1961b, 1962), and both compounds should be considered normal forms of storage iron.

Considerable similarity was found in the behaviour of the storage iron in the liver and spleen (Figs. 1 and 2). This is probably in part due to the fact that much of this storage iron is in cells of the reticulo-endothelial system, these cells behaving in a similar way in both organs. It is also possible that iron is distributed between ferritin and haemosiderin in much the same way in hepatic parenchymal cells as in reticulo-endothelial cells.

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