J. clin. Path., 1970, **23**, 127-134

Studies on iron metabolism in sickle cell anaemia, sickle cell haemoglobin C disease, and haemoglobin C disease using a large volume liquid scintillation counter

BELA RINGELHANN, FELIX KONOTEY-AHULU, AND SILAS R. A. DODU
From the Departments of Chemical Pathology and Internal Medicine, Ghana Medical School, Accra

SYNOPSIS Iron absorption as measured by a faecal recovery method in young adult males living in a tropical zone was high, even in the absence of anaemia. There was an inverse relation between the iron absorption and the packed cell volume. The highest absorption was found in sickle cell anaemia patients, where the packed cell volume is the lowest. The incorporation of iron was also the fastest and greatest in this group. In the controls the iron absorbed accumulated in the marrow and the spleen on the first day; in the sickle cell anaemia group the spleen has an insignificant role in iron storage. The growing radioactivity in the liver parallels that of the heart in the group of sickle cell anaemia patients; however, it remains low in the spleen in the same group, implying a diminution of splenic blood flow. In the sickle cell haemoglobin C and the haemoglobin C patients, the liver and spleen have an intermediate position between that of the sickle cell anaemia group and the control group.

Only a few papers on the absorption and distribution of iron in abnormal haemoglobin disease have been published (Dubach, Callender, and Moore, 1948; Erlandson, Walden, Stern, Hilgartner, Wehman, and Smith, 1962; McCurdy, 1962; Malamos, Belcher, Gyftaki, and Binopoulos, 1963; Movitt, Mangum, and Porter, 1963; Movitt, Pollycove, Mangum, and Porter, 1964); however, no such study has been reported from tropical countries. Haemoglobinopathies have different natural histories and features in tropical and non-tropical countries due to differences in climate, nutrition, bacterial infections, and parasitic infestations. Iron turnover is also influenced by these factors.

The aim of this study was to examine the absorption of iron, its incorporation into red cells, and, finally, its distribution in the main iron storage organs—the bone marrow, liver, and spleen—in patients with haemoglobinopathies. A large volume liquid scintillation counter[1] was used in this study.

[1]Armac Pacard Co.

Received for publication 20 June 1969.

Material and Methods

Twelve patients with a diagnosis of sickle cell anaemia, five with sickle cell haemoglobin C disease, and three with haemoglobin C disease were selected from the Sickle Cell Clinic, Korle Bu Teaching Hospital, Accra. As controls, inpatients were chosen from the same hospital. Criteria for the selection of the controls were as follows: AA haemoglobin type; patients with no anaemia or any abnormality in haematological status, no apparent infection or malignant disease, and those of a similar age and socio-economic status as the patients with haemoglobinopathies.

To exclude the influence of physiological iron loss and the variable iron balance of menstruating females only male patients were investigated. All individuals were in hospital during the observation period, and were not put on any special diet.

On the first day, between 8 am and 9 am, blood was withdrawn from the fasting patient for baseline determinations and a mixture containing

5 μCi of ^{59}Fe (ferric citrate, Amersham), 5 mg of ferrous sulphate, and 100 mg of ascorbic acid dissolved in 200 ml of water was given orally. Three standards were prepared, one for blood, one for faecal, and one for surface body counting. The patients fasted for four hours after taking the mixture, and blood was collected after one, two, and four hours on the first day and once daily from the second day onwards. After centrifugation the radioactivity of the plasma and red cells was measured in a well-type scintillation counter with a sodium iodide crystal. The standard was counted together with the daily samples.

With the large-volume liquid scintillation counter, it was not necessary to concentrate the faecal sample. Faeces were collected daily in a cylindrical container, 15 cm long by 10 cm in diameter, manufactured for the purpose from non-wettable paper. The standard was counted simultaneously in a bottle of similar size to the faeces container, filled to a depth of about 2 in (5 cm) with the radioactive solution. This was approximately the average height of the specimen in the container. The scintillation detection by diameter photomultiplier (15 cm × 7·5 cm) was fairly uniform. Serial counts to check the efficiency were made by placing a ^{59}Fe solution in 1·5 cm diameter test tube in different parts of the chamber.

Surface body counting, over the spleen, liver, heart, and sacrum, was carried out in four control patients, seven sickle cell anaemia cases, five sickle cell haemoglobin C, and three haemoglobin C patients, on the first and fourth hour on day one, and once daily on the following days for eight to ten days, using a 2·54 × 17·4 cm sodium iodide crystal. The standard was counted simultaneously.

Iron incorporation into the red cells was also calculated on the seventh or eighth day, using

Case No.	Age	PCV	Serum Iron (μg/100 ml)	TIBC (μg /100 ml)	Percentage ^{59}Fe Absorbed	Fe Incorporated	Stool Parasites (in pcp)	Spleen	Liver
Control group									
1	15	42	140	403	28·9	20·9	Hookworm 10	—	—
2	32	41	75	241	85·1	46·0	Hookworm 4	—	—
3	35	36	60	239	78·4	52·0	Hookworm 6	—	—
4	26	37	110	421	58·6	45·0	Hookworm 20	—	—
5	54	43	90	284	4·9	—	Hookworm 4	—	—
6	50	41	50	—	15·0	—	No ova or parasites	—	—
7	41	42	120	—	67·0	—	No ova or parasites	—	—
8	35	43	50	315	59·7	—	*Entamoeba coli*	—	—
9	23	39	—	—	62·0	—	No ova or parasites	—	—
10	43	44	106	256	44·6	—	Hookworm 10	—	—
11	24	40	146	265	60·9	—	No ova or parasites	—	—
12	23	45	—	—	59·5	—	No ova or parasites	—	—
Mean	33	41	94·7	303	52·8	40·9			
Sickle cell anaemia group									
1	10	18	118	239	92·5	80·0	No ova or parasites	—	+
2	14	24	20	346	86·2	—	No ova or parasites	+	+
3	17	27	34	241	69·5	—	No ova or parasites	—	—
4	17	25	—	—	94·5	—	No ova or parasites	—	—
5	30	22	60	241	41.3	—	No ova or parasites	—	—
6	30	24	96	220	66·9	60·0	No ova or parasites	—	—
7	16	17	80	241	93·9	—	Hookworm 15	—	—
8	18	29	70	265	27·2	6·2	No ova or parasites	+	—
9	18	17	170	242	71·5	60·0	No ova or parasites	+	—
10	17	15	270	316	62·4	—	No ova or parasites	—	—
11	25	26	126	285	98·0	70·3	No ova or parasites	—	—
12	24	26	100	316	86·0	83·0	No ova or parasites	—	—
Mean	19·6	22·5	104	268	74·1	60·0			
Sickle cell haemoglobin C group									
1	30	30	60	571	81·3	64·0	No ova or parasites	—	—
2	13	31	80	403	70·4	60·0	No ova or parasites	—	—
3	21	31	100	269	42·2	36·0	No ova or parasites	+	+
4	19	35	220	271	53·3	38·6	Hookworm 6	—	—
5	13	28	80	330	86·4	46·0	No ova or parasites	—	—
Mean	19	31	108	369	66·5	48·8			
Haemoglobin C group									
1	20	35	50	385	56	38·0	No ova or parasites	+	—
2	35	36	60	119	38	30·0	Ascaris 2	+	—
3	42	31	80	301	76·5	58·0	No ova or parasites	+	—
Mean	32	34	63	268	57·0	42·0			

Table I *Iron absorption and incorporation in all four groups*

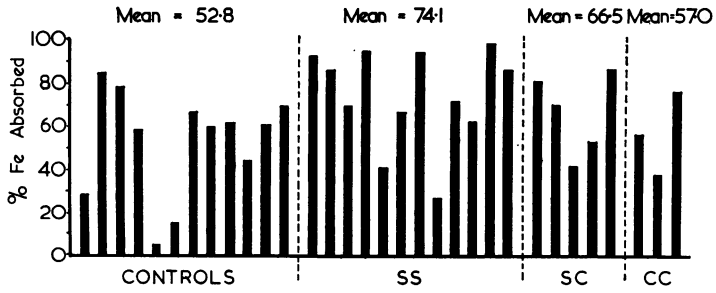

Fig. 1 *The percentage of the iron absorbed measured by faecal recovery in the 35 patients studied. (SS-sickle cell anaemia patients, SC sickle cell haemoglobin C patients, and CC-haemoglobin C patients).*

the equation:

$$\frac{\text{counts per minutes in total red cells}}{\text{total counts per minute administered}}.$$

Blood volume was calculated on a basis of 80 ml/kg body weight. Serum iron was measured by Ramsay's method (Ramsay, 1953) modified by that of Ringelhann (1956). The total iron-binding capacity was determined by Ramsay's (1957) method.

Results

IRON ABSORPTION AND INCORPORATION

Table I contains the data on age, packed cell volume (PCV), serum iron, total iron-binding capacity, the percentage of iron absorbed as measured by the faecal recovery method, the percentage of the iron incorporated into the red cells, and the degree of liver and splenic enlargement. From Table I and Fig. 1, a gradual increase of the iron absorption can be seen. The lowest average absorption was found in the control

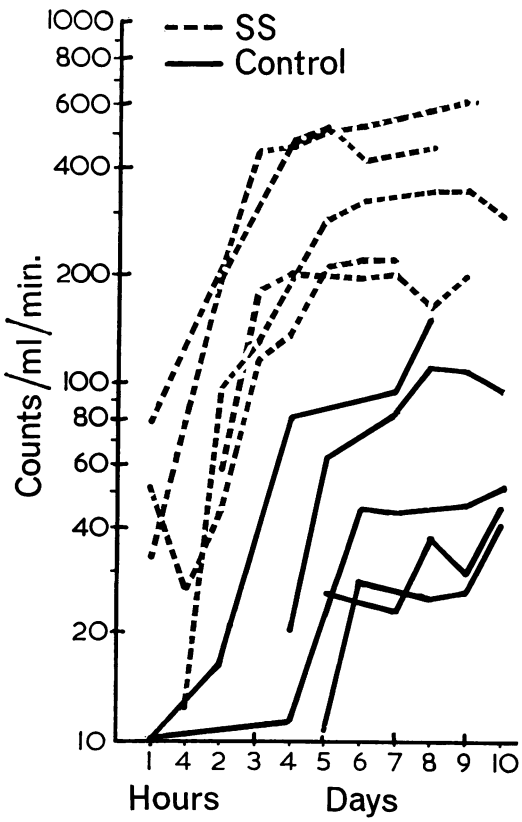

Fig. 2 *Radioactivity in the blood in the sickle cell anaemia patients (SS) and the control group.*

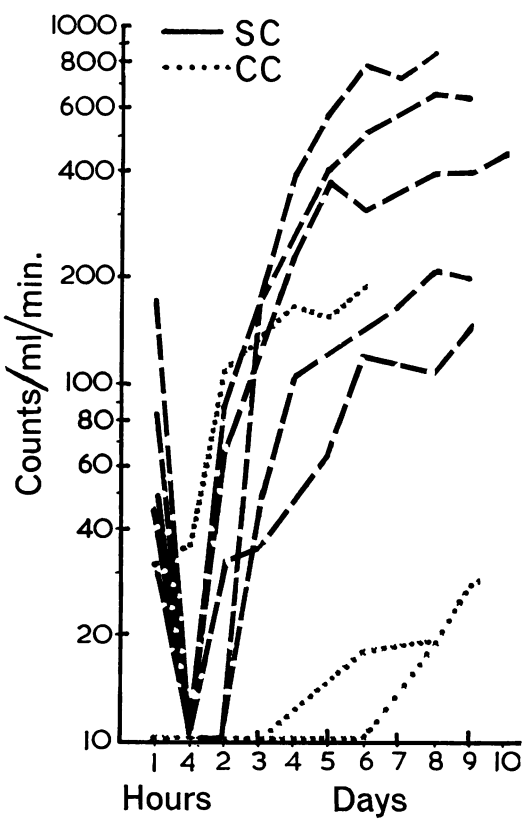

Fig. 3 *Radioactivity in the blood in the sickle cell haemoglobin C (SC) and haemoglobin C (CC) patients.*

group, 52·8% (4·9-85·1), a slightly higher absorption in patients with haemoglobin C disease, 57%, an even higher absorption in the sickle cell haemoglobin C group, 66·5% (42·2-86·4), and the highest absorption in the sickle cell anaemia patients, 74% (27·2-94·5). There was a very large scatter around the mean in all four groups and the values were not statistically significant.

The average percentage of the iron incorporated into the red cells was lowest in the control group and highest in the sickle cell anaemia patients (Figures 2 and 3). The quantity of iron in the red cells was less than the values found by the faecal recovery of unabsorbed iron.

BODY COUNTS

The counting rates in all four groups were highest over the bone marrow four hours after the ingestion of iron, except in two control individuals and one patient with sickle cell haemoglobin C disease in whom there was a further increase on the second day (Figure 4). Except in these three patients, values on the second day were lower and the decline in radioactivity was continuous. However, in the sickle cell anaemia and sickle cell haemoglobin C groups the decline was less steep than in the other two groups. Between the eighth and tenth days, the percentage of radioiron in the marrow of the sickle cell anaemia patients was slightly higher (the mean being about 40%) than in the sickle cell haemoglobin C group (30%) and the haemoglobin C group (20%).

Measurements over the spleen four hours after the iron intake in all four groups showed lower counting rates than over the marrow, except in two control individuals and in two patients with sickle cell haemoglobin C disease. In the following days (Fig. 5), the radioactivity in the control group fell swiftly, except in one case. In the patients with sickle cell haemoglobin C disease and haemoglobin C disease the decline was, with one or two exceptions, slower. As a group the sickle cell anaemia patients showed a fairly uniform behaviour; in five out of seven there were only slight changes over the spleen.

In the control group, radioactivity over the liver declined from the fourth hour onwards until the fourth and fifth days then, after remaining at the same level for a time, it started to rise again on the seventh and eighth days (Fig. 6), showing a triphasic shallow U-shaped curve. In the sickle cell haemoglobin C group, in four patients there was no appreciable change. In one case (case 3), after a sharp fall, the values re-

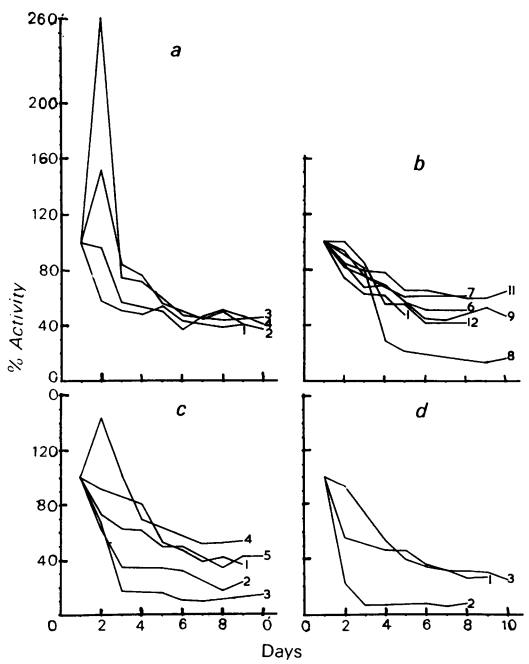

Fig. 4 *Surface body counts*[1] *over the bone marrow.*

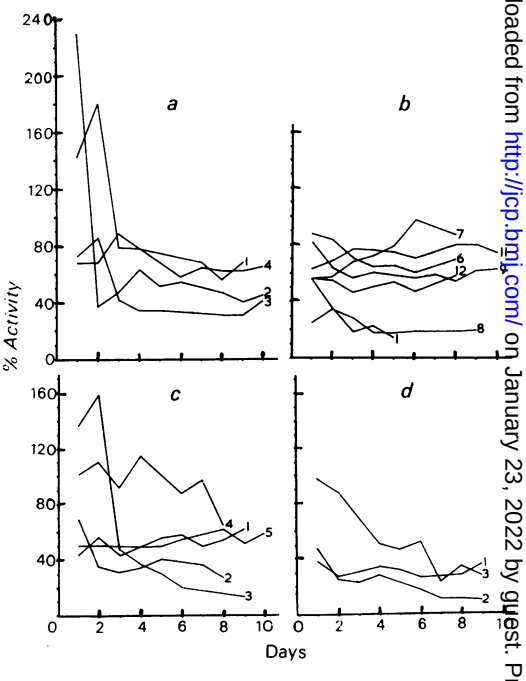

Fig. 5 *Surface body counts over the spleen.*

[1]In figures 4-8 the values of the count are given in the percentage of bone marrow uptake at four hours. The counts for each group are identified as follows: the control group (a), the sickle cell anaemia group (b), the sickle cell haemoglobin C group (c), and the haemoglobin C group (d).

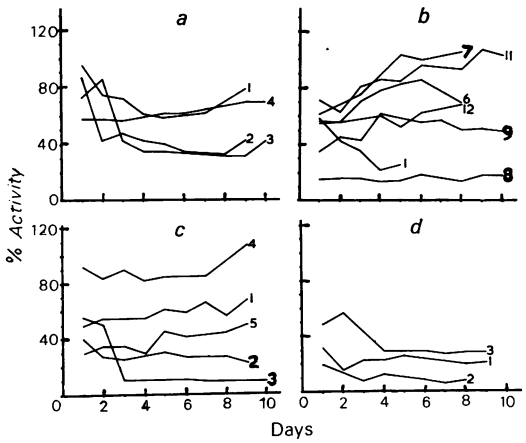

Fig. 6 *Surface body counts over the liver.*

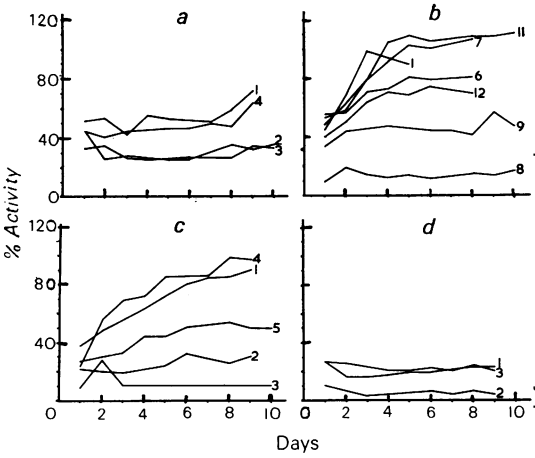

Fig. 7 *Surface body counts over the heart.*

mained at a low level. There was no change in the haemoglobin C group after a slight decrease. In the sickle cell anaemia patients, there was no decline after four hours and in five out of seven cases a definite rise began on the second day.

Although there were large individual variations in each group, it seemed that the splenic and liver uptake (calculated from the heart/spleen and heart/liver ratios) was lowest in the sickle cell anaemia group in the fourth hour after the iron intake (Table II).

Only slight changes in the counting rate over the heart (Fig. 7) occurred in the control group on the seventh and eighth days, when there was a small rise. Similarly, there was no change over the heart in the haemoglobin C patients. However, a fairly steep rise in the counting rate appeared in the sickle cell haemoglobin C and sickle cell anaemia groups after the second and third days; the values reached as high as 70 to 100% above the initial counting rate and a plateau was formed between the third and fourth days.

Figure 8 shows one illustrative case of each of the four groups showing the distribution of iron in the body measured by surface body counting.

Case No.	*Heart/Spleen*	*Heart/Liver*	*Remarks*
Control group			
1	0·36	0·53	
2	0·19	0·49	
3	0·44	0·26	
4	0·67	0·78	
Mean	0·42	0·52	
Sickle cell anaemia group			
1	0·81	0·76	Liver +
6	0·58	1·00	
7	0·96	0·88	
8	0·44	0·67	Spleen ++++
9	1·58	0·94	Spleen ++
11	0·76	0·67	
12	0·70	0·72	
Mean	0·83	0·80	
Sickle cell haemoglobin C group			
1	0·76	0·77	
2	0·32	0·56	
3	0·06	0·15	Spleen ++++
4	0·23	0·25	
5	0·62	0·90	
Mean	0·40	0·53	
Haemoglobin C group			
1	0·67	0·83	Spleen ++
2	0·21	0·54	Spleen ++
3	0·27	0·55	Spleen ++++
Mean	0·38	0·64	

Table II *Heart/spleen and heart/liver ratio counts (per minute, at four hours)*

Discussion

CHOICE OF METHOD AND PROBLEMS OF TECHNIQUE

The recovery of ingested and unabsorbed radioiron in stools as a technique for measuring iron absorption (Dubach *et al*, 1948; Bothwell, Mallett, Oliver, and Smith, 1955; Chodos, Ross, Apt, Pollycove, and Halkett, 1957; Bonnet, Hagedorn, and Owen, 1960) has been criticized, because discrepancies were found between this and the two other isotope methods, whole body counting and double isotope technique (Pitcher, Williams, Parsonson, and Williams, 1965; Lunn, Richmond, Simpson, Leask, and Toghill, 1967; Heinrich and Bartels, 1967). The divergencies are mainly due to inadequate faecal collection. Although this point is worth considering, the other two isotope methods also have their critics (Giblett, Coleman, Pirzio-Biroli, Donohue,

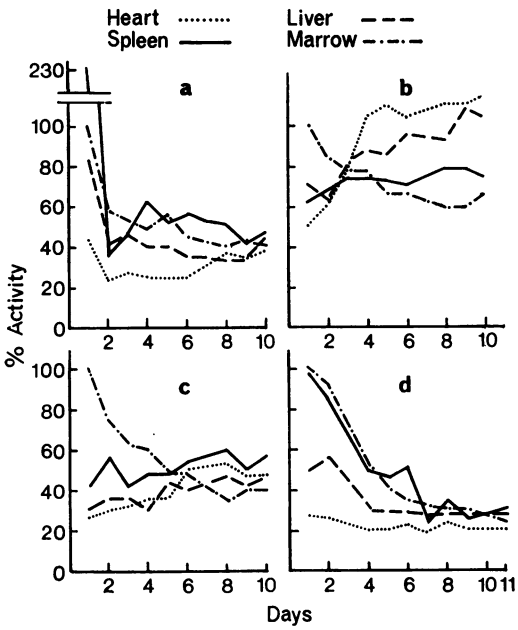

Fig. 8 *An illustrative case of surface body counts in one patient from each of the four groups.*

Motulsky, and Finch, 1956; Schiffer, Price, Cuttner, Cohn, and Cronkite, 1964; Pollack, Balcerzak, and Crosby, 1966). In patients with fluctuating increased erythrocyte production and destruction, the measurement of unabsorbed iron probably offers more advantages than those obtained by incorporation of radioiron into the red cells (Erlandson *et al*, 1962), provided that the collection of faeces and the technique of counting are good. As we were first interested in the differences of iron absorption in the four groups of patients, we thought that this method would yield useful data for comparison within the groups. The advantage of the use of a large volume liquid scintillation counter for measuring unabsorbed iron in stools has been mentioned.

IRON ABSORPTION AND THE APPEARANCE OF THE RADIOIRON IN THE CIRCULATING BLOOD

Table III contains the normal values for iron absorption found by various authors using radioisotope methods.

Compared with these data our controls have high absorption values. In tropical zones, iron deficiency does not only affect the vulnerable population groups, females at the reproductive age, and especially pregnant women, but also adult men who are usually regarded as not vulnerable. In an agricultural population living in a tropical zone Layrisse (1966) found iron absorption as high as 60%, and this was not related to anaemia. He concluded that iron deficiency exists in practically all the subjects studied by him. Foy (1962) thinks that hookworm is the main cause of iron deficiency in the tropics, but in addition, tropical dietaries are very rich in phytates and phosphates which, when combined with iron, reduce its absorption. It is still not clear whether an increased loss of iron through excessive sweating could be a source of negative iron balance (Mitchell and Hamilton, 1949; Dubach, Moore, and Callender, 1955; Hussain and Patwardhan, 1959; Coltman and Rowe, 1966). Ethnic differences in distribution of glands producing apocrine and exocrine sweat might play a role in the variance of iron content of the sweat (Foy, 1962).

No. of Cases	*Sex*[1]	*Percentage of Iron Absorbed*		*Technique*	*Author(s)*
		Average	*Range*		
8	?	14·6	2-21	Faecal recovery	Dubach *et al* (1948)
8	M	39·8	17-69	Faecal recovery	Bothwell *et al* (1955)
2	F	23	18-28		
22	M	40	14-62	Faecal recovery	Bonnet *et al* (1960)
19	F	62	36-75		
24	M	4·7	0·2-10	Whole body counting	Hoek and Conrad (1961)
1	F	4·0			
4	?	16·2	5-27	Faecal recovery	Erlandson *et al* (1962)
10	M	15·9	5·7-24·7	Whole body counting	Price, Cohn, Wasserman, Reizenstein, and Cronkite (1962)
3	F+				
9	M	9·7	4-20	Whole body counting	Deller (1965)
1	F				
25	M	4·1	1-13		
13	F	6·5	1-11	Double isotope	Pitcher *et al* (1965)
10	F	22·9	10·9-37·2	Double isotope	Haurani, Green, and Young (1965)
108	M	18·7	4-35		
40	F	31·5	16-44	Whole body counting	Heinrich and Bartels (1967)
3	M	31	20-47	Faecal recovery combined with ^{131}Ba	Boender, Mulder, Ploem, de Wael, and Verloop (1967)

Table III *Various techniques marked for noting iron absorption*

[1]Children are marked '?', +' signifies postmenopausal.

The appearance of radioactivity in the plasma during iron absorption cannot be directly related to the quantity of iron absorbed (Bothwell *et al*, 1955), because the level in the plasma depends principally on three components, the influx from the gut to the intestinal cells, release from those cells to the plasma, and the removal from the blood by storage organs. A fourth regulating factor, the unsaturated iron-binding capacity, also participates. The radioiron absorption curve is, however, the only technique currently available which characterizes the movement of the absorbed iron from the mucosal cell into the plasma. The increase of radioactivity in the sickle cell anaemia and sickle cell haemoglobin C patients is very rapid compared with the increase in the control and haemoglobin C groups. In the sickle cell anaemia group, there is also a fairly rapid utilization of iron in the haemoglobin synthesis. In the sickle cell haemoglobin C patients utilization is less rapid but is higher than in the haemoglobin C and control groups. The iron incorporated into the total red cell volume was also highest in the sickle cell anaemia patients, the three other groups showing diminishing values, with the haemoglobin C group lying between the sickle cell haemoglobin C group (higher) and the control group (lower). There was no correlation between percentage absorption measured from the faeces and the incorporated iron, calculated from the total red cell volume. In haemolytic anaemia, the patient may absorb more iron than can be recovered in the peripheral blood, because the haemoglobin is being removed from the circulation at a rapid rate (Dubach *et al*, 1948). Ineffective erythropoiesis, with the reutilization of iron liberated from the non-viable new red cells in the marrow, may also lessen the amount of radioiron recovered in the red cells.

Another factor might be the iron deficiency prevalent in tropical countries. The serum iron and total iron-binding capacity did not indicate iron deficiency in any of our four groups. Nevertheless an inverse correlation between the iron absorption and the PCV value was found, the iron absorption being highest where the PCV was lowest; increased iron aborption was therefore correlated with the intensity of the anaemia.

DISTRIBUTION OF IRON IN THE MARROW, LIVER, SPLEEN, AND HEART

Previously this was usually studied in haemoglobinopathies during iron-kinetic studies, following the intravenous injection of radioactive iron (Dubach *et al*, 1948; Erlandson *et al*, 1962; McCurdy, 1962; Malamos *et al*, 1963; Movitt *et al*, 1963; Movitt *et al*, 1964). Walsh, Cantrill, and Sanford (1963), in rats, found a slight difference in the distribution of deposits of iron depending on the route of administration. As yet, there is no indication that in man such a difference exists. In our sickle cell anaemia patients, the radioactivity in the bone marrow declined slowly because there was a very active and persistent marrow hyperfunction combined with ineffective erythropoiesis. In sickle cell haemoglobin C patients, the uptake and release into the marrow was intermediate between the normal and the sickle cell anaemia patients; and in the haemoglobin C group, bone marrow activity was similar to that in the control group.

The splenic counts indicated that the spleen had a storage function in the control individuals on the first and second days. In the sickle cell anaemia patients, there were no changes of radioactivity over the spleen, most probably because it had lost this temporary storage function. The spleen in sickle cell haemoglobin C patients seemed to have a more active role in storage. In our haemoglobin C group, we found a less characteristically slow decline of the radioiron content over the enlarged spleen.

In our sickle cell anaemia patients, the liver uptake showed a different pattern from that of the controls. We think that the increasing radioiron content of the liver in patients with sickle cell anaemia and sickle cell haemoglobin C disease at the early stage does not mean that iron is deposited in the liver, but is a reflection of the high concentration of radioactivity in the circulating blood. The lack of correlation between spleen and heart could be attributed to the diminished splenic blood flow ('physiological splenectomy') which occurs in sickle cell anaemia patients in consequence of frequent splenic haemorrhages and subsequent fibrosis. Presumably, this is also the reason for the low initial uptake of iron indicated by the heart/spleen ratio (Table II). Some diminution of the splenic blood flow could also be seen in the sickle cell haemoglobin C patients.

Our study shows that although there are common characteristics of iron metabolism in each group, there are also individual variations so that individual curves may resemble the pattern of another group more closely than that typical of the diagnosis.

We are grateful to Mr E. A. Badoe for giving us permission to study some of his patients and Dr H. Miller, Regional Physics Department, Sheffield, for his advice and criticism. This work was supported by a research grant from the International Atomic Energy Agency.

References

Boender, C. A., Mulder, E., Ploem, J. E., de Wael, J., and Verloop, M. C. (1967). Iron absorption and retention in man. *Nature (Lond.)*, **213**, 1236-1237.

Bonnet, J. D., Hagedorn, A. B., and Owen, C. A. (1960). A quantitative method for measuring the gastrointestinal absorption of iron. *Blood*, **15**, 36-44.

Bothwell, T. H., Mallett, B., Oliver, R., and Smith, M. D. (1955). Inability to assess absorption of iron from plasma radioiron curves. *Brit. J. Haemat.*, **1**, 352-357.

Chodos, R. B., Ross, J. F., Apt, L., Pollycove, M., and Halkett, J. A. E. (1957). The absorption of radioiron labeled foods and iron salts in normal and iron-deficient subjects and in idiopathic hemochromatosis. *J. clin. Invest.*, **36**, 314-326.

Coltman, C. A., Jr., and Rowe, N. J. (1966). The iron content of sweat in normal adults. *Amer. J. clin. Nutr.*, **18**, 270-274.

Deller, D. J. (1965). Iron absorption measurements by whole-body counting: studies in alcoholic cirrhosis, hemochromatasis, and pancreatitis. *Amer. J. dig. Dis.*, **10**, 249-258.

Dubach, R., Callender, S. T. E., and Moore, C. V. (1948). Studies in iron transportation and metabolism. VI. Absorption of radioactive iron in patients with fever and with anemias of varied etiology. *Blood*, **3**, 526-540.

Dubach, R., Moore, C. V., and Callender, S. (1955). Studies in iron transportation and metabolism. IX. The excretion of iron as measured by the isotope technique. *J. Lab. clin. Med.*, **45**, 599-615.

Erlandson, M. E., Walden, B., Stern, G., Hilgartner, M. W., Wehman, J., and Smith, C. H. (1962). Studies on congenital hemolytic syndromes. IV. Gastrointestinal absorption of iron. *Blood*, **19**, 359-378.

Foy, H. (1962). Some haematological problems in tropical medicine. In *Proceedings of the Symposium on the Use of Radioisotopes in the Study of Endemic and Tropical Disease*, pp. 73-85. International Atomic Energy Agency, Vienna.

Giblett, E. R., Coleman, D. H., Pirzio-Biroli, G., Donohue, D. M., Motulsky, A. G., and Finch, C. A. (1956). Erythrokinetics: quantitative measurements of red cell production and destruction in normal subjects and patients with anaemia. *Blood*, **11**, 291-309.

Haurani, F. J., Green, D., and Young, K. (1965). Iron absorption in hypoferremia. *Amer. J. med. Sci.*, **249**, 537-547.

Heinrich, H. C., and Bartels, H. (1967). Bestimmungsmethoden und Normalbereiche der intestinalen Eisenresorption beim menschen. Ein kritische Ubersicht. *Klin. Wschr.*, **45**, 553-558.

Hoek, R., and Conrad, M. E. (1962). Iron absorption: measurement of ingested iron by a human whole body liquid scintillation counter. *J. clin. Invest.*, **40**, 1153-1159.

Hussain, R., and Patwardhan, V. N. (1959). Iron content of thermal sweat in iron-deficiency anaemia. *Lancet*, **1**, 1073-1074.

Layrisse, M. (1966). In *XIth Congress of the International Society of Haematology, Sydney*, pp. 95-101.

Lunn, J. A., Richmond, J., Simpson, J. D., Leask, J. D., and Toghill, P. J. (1967). Comparison between three radioisotope methods for measuring iron absorption. *Brit. med. J.*, **3**, 331-333.

Malamos, B., Belcher, E. H., Gyftaki, E., and Binopoulos, D. (1963). Simultaneous radioactive tracer studies of erythropoiesis and red cell destruction in sickle-cell disease and sickle-cell haemoglobin thalassaemia. *Brit. J. Haemat.*, **9**, 487-498.

McCurdy, P. R. (1962). Erythrokinetics in abnormal hemoglobin syndromes. *Blood*, **20**, 686-699.

Mitchell, H. H., and Hamilton, T. S. (1949). The dermal excretion under controlled environmental conditions of nitrogen and minerals in human subjects with particular reference to calcium and iron. *J. biol. Chem.*, **178**, 345-361.

Movitt, E. R., Mangum, J. F., and Porter, W. R. (1963). Sickle-cell hemoglobin C disease: quantitative determination of iron kinetics and hemoglobin synthesis. *Blood*, **21**, 535-545.

Movitt, E. R., Pollycove, M., Mangum, J. F., and Porter, W. R. (1964). Hemoglobin C disease: quantitative determination of iron kinetics and hemoglobin synthesis. *Amer. J. med. Sci.*, **247**, 558-564.

Pitcher, C. S., Williams, H. S., Parsonson, A., and Williams, R. (1965). The measurement of iron absorption by the double isotope technique. *Brit. J. Haemat.*, **11**, 633-641.

Pollack, S., Balcerzak, S. P., and Crosby, W. H. (1966). Fe^{59} absorption in human subjects using total-body counting technic. *Blood*, **28**, 94-97.

Price, D. C., Cohn, S. H., Wasserman, L. R., Reizenstein, P. G., and Cronkite, E. P. (1962). The determination of iron absorption and loss by whole body counting. *Blood*, **20**, 517-531.

Ramsay, W. N. M. (1953). The determination of iron in blood plasma or serum. *Biochem. J.*, **53**, 227-231.

Ramsay, W. N. M. (1957). The determination of the total iron-binding capacity of serum. *Clin. chim. Acta*, **2**, 221-226.

Ringelhann, B. (1956). Egyszerü módszer a serumvas meghátarozására. *Orv. Hetil.*, **97**, 55-56.

Schiffer, L. M., Price, D. C., Cuttner, J., Cohn, S. H., and Cronkite, E. P. (1964). A note concerning the '100 per cent value' in iron absorption studies by whole body counting. *Blood*, **24**, 757-760.

Walsh, R. J., Cantrill, S., and Sanford, R. (1963). The distribution of iron following parenteral infection or oral ingestion. *Aust. J. exp. Biol.*, **41**, 385-394.