Plasma and Red Cell Iron Turnover in Protein Calorie Malnutrition

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The term protein calorie malnutrition, first introduced by Jelliffe in 1959, is at present accepted to include kwashiorkor and marasmus, together with a wide spectrum of intermediate conditions.

Different morphological features of the anaemia associated with kwashiorkor were reported by various authors. Altmann and Murray (1948) and Gómez *et al.* (1955) described it as normochromic normocytic, whereas Chaudri (1950) and Stransky and Dauis-Lawas (1950) reported it to be hypochromic microcytic. A macrocytic type, however, was also described by Adams (1954), Kondi *et al.* (1963), and Pereira and Baker (1966). Furthermore, bone-marrow studies demonstrated normoblastic, macronormoblastic, and megaloblastic reactions (Trowell, Davies, and Dean, 1954; Woodruff, 1955; Mehta and Gopalan, 1956). An erythroid hypoplasia was observed by Walt *et al.* (1962) and an erythroblastopenia with giant proerythroblasts was reported by Lien-Keng (1957).

The present paper deals with a study of plasma and red cell iron turnover in kwashiorkor and marasmus using ^{59}Fe .

Material and Methods

Nine infants were included in this study; 3 with kwashiorkor, 4 with marasmus, and 2 normal controls. The first 3 patients presented with typical manifestations of kwashiorkor, showing retarded growth and weight, oedema, hair and skin changes, hepatomegaly, and clinical signs of vitamin deficiencies. The 4 marasmic infants were shorter than normal and showed marked loss of weight. The liver was felt in 2 of them, but there was no oedema, nor skin changes. The tuberculin test was negative, and the urine and stools were normal in all infants investigated.

The infants were only investigated before treatment. It was thought advisable to avoid exposing them to more than one series of radioactive iron studies.

Serum iron estimation was done using the method of Ramsay (1954). Serum proteins were determined by

the method of Phillips *et al.* (1950). Haemoglobin concentration, haematocrit, reticulocytic count, blood and bone-marrow pictures, and red cell indices were all studied using standard laboratory procedures.

Radioactive iron studies were done using ^{59}Fe obtained from the Radiochemical Laboratory of Amersham, Bucks, as $^{59}\text{FeCl}$ with a specific activity of 11.4 mCi/mg. Fe.

Six microcuries of radioiron were mixed with 2.5 ml. of the patient's plasma for half an hour. A syringe containing approximately 2 ml. of the labelled plasma was accurately weighed, and the plasma was injected intravenously, the mass of the injected dose being determined by reweighing the syringe. An aliquot of the labelled plasma was also carefully weighed and diluted to 250 ml. with water and kept as a standard solution.

The following measurements were made.

(1) The plasma ^{59}Fe disappearance curve over the first 4 hours after the injection period. The plasma volume was determined from the zero-time intercept of plasma activity using the dilution principle.

(2) The ^{59}Fe red cell uptake over 14 days after the injection.

(3) The sacral marrow uptake was determined by surface counting over the upper half of the sacrum using a collimated 2.5 cm. scintillation detector. All counts were corrected for physical radioactive decay and expressed as $\frac{\text{CPM}_t}{\text{CPM}_0}$, where CPM_t is the corrected count rate obtained at any time t after the injection, and CPM_0 is the initial count rate obtained immediately after injection.

The following parameters were also calculated:

(a) The apparent plasma iron turnover rate was calculated as:

$$\frac{K \times \text{plasma iron mass}}{\text{body weight (kg.)}} \text{ mg./day per kg.,}$$

where $K = \frac{0.693}{T_{\frac{1}{2}}}$; $T_{\frac{1}{2}}$ being the half-time of the ^{59}Fe plasma disappearance curve. The plasma iron mass was obtained from the plasma volume and serum iron concentration.

(b) The apparent red cell iron turnover rate was calculated as follows:

$$\text{plasma iron turnover rate} \times f \text{ mg. iron/kg. per day,}$$

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FIG. 1.—The plasma ^{59}Fe disappearance over the first 4 hours after injection in a normal control (a), and in one infant with kwashiorkor (b).

where f = the maximal fraction of ^{59}Fe incorporated into circulating red cells (Bothwell and Mallett, 1955).

(c) The apparent daily Hb synthesis rate in g. per day was calculated as:

$$\frac{\text{Apparent red cell iron turnover rate (mg./kg. per day)} \times \text{body weight (kg.)}}{3.33}$$

(d) The apparent red cell survival in days was calculated as:

$$\frac{\text{Total intravascular Hb mass (g.)}}{\text{Daily Hb synthesis (g./day)}}$$

Results

Results (Fig. 1–6) of the present study show that a hypochromic anaemia was present in all infants

investigated. This was associated with a low MCV, MCH, and MCHC. The reticulocyte count was normal and the serum iron concentration was low. The serum protein values were low in kwashiorkor and in one marasmic infant but normal in the others (Tables I and II).

In kwashiorkor the bone-marrow picture showed diminished cellularity, with diminution in the normoblasts. Similar findings were present in one marasmic infant. In another the marrow megaloblasts constituted 8% of the count (Table III).

It is also clear from Table IV that plasma and blood volume were increased in marasmus and kwashiorkor compared to normal controls.

The plasma iron turnover was low in 2 infants with kwashiorkor and within normal range in the

TABLE I
Blood Picture and Red Cell Indices in Studied Cases

Diagnosis	Hb (g./100 ml.)	RBC millions/cu.mm.	MCV (cu μ)	MCH ($\mu\text{g.}$)	MCHC (%)	Haematocrit (%)	Reticulocytes % RBC
Normal controls	12.5	4.5	88.8	27.7	31.2	40	1.4
	12.5	4.4	88.6	28.4	34.3	39	1.6
Kwashiorkor	6.5	2.7	92.5	24	26	25	0.3
	5.5	3.4	76.4	16	21.1	26	0.5
	5	3.2	75	18.7	21	23	0.7
Marasmus	6	3.1	80.6	20	24	25	0.5
	6	1.4	150	42.8	28.5	21	0.8
	7	3.9	74.3	21.7	24.1	29	0.5
	6.5	3.6	69.4	18	26	25	0.5

FIG. 2.—The plasma ^{59}Fe disappearance over the first 4-hour period after injection, in a typical marasmic infant (a), and in a marasmic infant with megaloblastic bone-marrow reaction (b).

whole group of marasmus. The red cell iron uptake was slightly subnormal in kwashiorkor and in one marasmic infant. The apparent red cell iron turnover rate was increased and the corresponding apparent red cell survival was diminished in kwashiorkor and marasmus. The latter was, however,

shortest in the marasmic infant showing a megaloblastic bone-marrow reaction (Table V). The sacral bone marrow uptake in this infant showed an incomplete release. Fig. 1–6 show graphic representations of the radioactive iron studies performed.

FIG. 3.—The ^{59}Fe red cell uptake over the 14-day period after injection, in a normal control (a), and in an infant with kwashiorkor (b).

FIG. 4.—The ^{59}Fe red cell uptake over the 14-day period after injection in a typical marasmic infant (a), and in a marasmic infant with megaloblastic bone-marrow reaction (b).

Discussion

In all the cases of kwashiorkor and marasmus studied, an iron deficiency anaemia presenting with hypochromia, subnormal serum iron concentration, and low MCV, MCH, and MCHC,

was present. In one marasmic infant, however, there was an associated megaloblastic reaction. In a previous control study on 73 infants with protein calorie malnutrition, the incidence of megaloblastic anaemia was 48% (M. Khalil, A. Tanios, and M. Hafez,

FIG. 5.—Surface counts over the sacral marrow in a normal control (a), and in an infant with kwashiorkor (b).

FIG. 6.—Surface counts over the sacral marrow, in a typical marasmic infant (a), and in a marasmic infant with megaloblastic bone-marrow reaction (b).

TABLE II
Serum Proteins and Serum Iron

Diagnosis	Serum Proteins (g./100 ml.)	Serum Iron (μ g./100 ml.)
Normal controls ..	6.6	147.2
	6.5	163.4
Kwashiorkor ..	3.8	69.9
	4.2	48.4
	4.1	56.9
Marasmus ..	5.1	71.3
	6.3	63.7
	6.2	62.6
	6.4	67.4

TABLE IV
Blood Volume, Plasma Volume, and Red Cell Mass

Diagnosis	Blood Volume (ml./kg.)	Plasma Volume (ml./kg.)	Red Cell Mass (ml./kg.)
Normal controls	69.1	41.2	27.9
	70	42.5	27.5
Kwashiorkor ..	79.9	62.7	17.2
	87.8	65	22.8
	85.1	61	24.1
Marasmus ..	92.8	72.3	20.5
	94.1	69	25.1
	94.1	68.5	25.6
	73	55	18

TABLE III
Bone-marrow Findings

Diagnosis	Total Nucleated Cells/cu.mm.	Normoblasts/ cu.mm.	M/E Ratio	Other Cells	Sacral Surface Counting
Normal controls	86,000	17,200	4/1	—	Normal
	72,000	16,000	3.5/1	—	Normal
Kwashiorkor ..	25,000	5000	4/1	—	Hypoplastic curve
	19,000	6300	2/1	—	Hypoplastic curve
	18,000	5100	2.5/1	—	Hypoplastic curve
Marasmus ..	32,000	7000	3.5/1	—	Subnormal
	186,000	46,500	3/1	14,880 megaloblasts	Incomplete release
	75,000	12,500	5/1	—	Normal
	68,000	12,360	4.5/1	—	Normal

TABLE V
Ferrokinetic Data

Diagnosis	Plasma Fe		Red Cell Fe			
	Total serum Fe (mg./kg.)	Serum Fe Turnover Rate (mg./kg. per day)	RBC Fe Mass (mg./kg.)	% RBC Uptake	RBC Fe Turn-over Rate (%)	Apparent RBC Survival (days)
Normal controls ..	62.8	0.77	28.9	87.8	2.1	47.6
	69.3	0.91	29.2	91.2	2.4	41.6
Kwashiorkor ..	43.1	0.83	17.2	72.1	3.9	25.6
	31.3	0.57	15.9	75.5	3.3	30.3
Marasmus	34.6	0.58	17.6	81.2	3.2	31.2
	44.6	0.81	27.4	75.1	3.7	27
	58	1.01	18.9	82.6	4.4	22.7
	42.6	0.80	29.5	83.9	3.9	25.6
	36.5	0.81	15.6	83.7	4.0	25

unpublished). These findings agree with those of other authors who have reported a variable incidence of megaloblastosis in protein calorie malnutrition (Majaj *et al.*, 1963; Lien-Keng and Odang, 1959). Such variations are expected in view of the existence of a variety of deficiencies, infections, and parasitic infestations that differ from one country to the other. This megaloblastosis was found to be due to folic acid deficiency (Walt, 1959; MacDougall and Ross, 1960).

In the present study on kwashiorkor a diminished erythropoietic activity of the bone-marrow was shown. Total erythropoiesis was below normal, as shown by diminished cellularity of the bone-marrow and diminution in the number of marrow normoblasts (Finch and Noyes, 1961). Subnormal plasma iron turnover was also present in 2 cases. Effective erythropoiesis was similarly diminished, as shown by the low reticulocyte count in the peripheral blood and the low red cell ^{59}Fe uptake. The low sacral marrow ^{59}Fe uptake is additional evidence of diminished erythropoietic activity.

It is highly probable that protein deficiency is an underlying factor in this erythropoietic insufficiency. The observed low serum protein concentration is a reflection of such protein depletion. Whipple and Robscheit-Robbins (1940), using standard anaemic dogs, found convincing evidence of the importance of adequate protein supply in the synthesis of haemoglobin. Similarly Mertz *et al.* (1948) and Nizet and Lambert (1954) reported that proteins of high biological value were important for normal erythropoiesis. Their deficiency may be responsible for certain enzymatic defects or inability in the provision of factors essential for globin synthesis due to absence of some essential amino acids.

It was suggested by Foy, Kondi, and MacDougall (1961) that marrow hypoactivity was related to

riboflavine or vitamin C deficiency. Walt *et al.* (1962), on the other hand, reported 9 patients with kwashiorkor who developed erythroid hypoplasia while under treatment with riboflavin and other vitamins.

In the marasmic group total and effective erythropoiesis were normal in 3 and diminished in only one patient. The sacral marrow in the latter showed a low ^{59}Fe uptake curve. This may suggest a coexisting protein deficiency interfering with active erythropoiesis, and can be confirmed by the finding of a low serum protein concentration. The serum protein values in the other 3 patients were normal. The marrow uptake curve in the marasmic infant with megaloblastic reaction showed an incomplete release, suggesting that iron had accumulated in the marrow and was only gradually released in small amounts into the peripheral blood (Harris, 1963).

The apparent red cell iron turnover rate in kwashiorkor and marasmus was consistent with a short red cell survival. This agrees with the findings reported by Lanzkowsky *et al.* (1967). Capsular and extracapsular factors were suggested to be responsible for the shortened survival as shown by ^{51}Cr labelled red cells. After protein feeding they reported a considerable improvement in red cell survival, suggesting that protein depletion was principally responsible. Jandl (1955), Sheehy *et al.* (1960), and Pitcher and Williams (1963) also found that, in patients with liver disease, there was a similar acquired erythrocyte defect resulting in shortening of its life span. These observations suggest that in addition to protein deficiency there may be factors causing shortened red cell survival which are common to patients with protein calorie malnutrition and hepatic dysfunction. Fatty infiltration of the liver is a constant finding in kwashiorkor and is not infrequent in marasmus.

As mentioned before, the apparent red cell survival was shortest in the marasmic infant with a megaloblastic reaction. A similar finding was reported by Hamilton, Sheets, and deGowin (1958) who explained it by intracapsular and extracapsular haemolytic mechanisms. Harris (1963) furthermore described 'stillborn' erythrocytes which in megaloblastic anaemias are destroyed in the marrow cavity before being delivered to the peripheral blood.

The findings in this investigation, of an increased blood and plasma volume in protein calorie malnutrition, agree with those of other workers (Smith, 1960; Shah, 1961). Dean (1965) considers the increase as a representation of water shift into the blood from the extravascular compartments. Using the Evans blue method he found a 10 to 20% increase in blood volume in patients with kwashiorkor.

Summary

Turnover studies with ^{59}Fe were performed in kwashiorkor and marasmus. An iron deficiency anaemia was present in all infants investigated.

In kwashiorkor a diminished erythropoietic activity of the bone-marrow caused by protein depletion was demonstrated. Effective erythropoiesis, however, was normal in marasmus. In a marasmic infant with megaloblastic reaction the bone-marrow uptake curve showed an incomplete release.

In kwashiorkor and marasmus the red cell survival was shorter than normal. Blood and plasma volume in the two disorders were more increased than in normal controls.

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