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PURE RED-CELL APLASIA IN MARASMUS AND KWASHIORKOR TREATED WITH RIBOFLAVINE*

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During an investigation of the anaemias in marasmus and kwashiorkor it was found that a considerable proportion of patients developed pure red-cell aplasia or hypoplasia during treatment.

So far selective red-cell aplasia has not been reported as a complication of marasmus and kwashiorkor, perhaps because critical haematological work and bone-marrow cytology have not been extensively done in these conditions, and serial bone-marrow punctures are not commonly performed on small infants. As the aplasia does not usually develop until two weeks or more after admission, a single marrow examination done at that stage, when red-cell precursors are usually high, will not reveal a hypoplasia that is yet to appear.

From the present investigation it seems that this type of aplasia is a fairly common accompaniment of marasmus and kwashiorkor in Kenya, since it developed in nine instances during the treatment of 23 cases; of these, five were thoroughly investigated.

Red-cell aplasia probably has a number of different causes among which are thymomas (Chalmers and Boheimer, 1954; Jacobs *et al.*, 1959; Freeman, 1960), toxic agents, allergy, iso-immunization, and infections (Foy and Kondi, 1953).

The case of pure red-cell aplasia previously reported (Foy and Kondi, 1953) and successfully treated with riboflavine induced us to try this vitamin in the present cases, and the results are reported here.

Material and Methods

Typical cases of marasmus and kwashiorkor attending the King George VI Hospital, Nairobi, were diagnosed clinically by the criteria of Dean and Schwartz (1953), Scrimshaw *et al.* (1957), and Jelliffe and Dean (1959). On admission complete haematological and parasitological examinations were done, together with *x*-ray examination to discover any chest infection.

All cases were then admitted to the children's ward, where dietary treatment consisted of "casilan," skimmed milk, and vegetable oil mixture (Jelliffe and Dean, 1959). They were covered during the first week with either tetracycline or penicillin and streptomycin. Intercurrent infections, such as hookworm, giardiasis, malaria, measles, diarrhoea, and respiratory complaints, when present, were appropriately treated. As most of the patients had low haemoglobin (Hb) and mean corpuscular haemoglobin concentration (M.C.H.C.) levels, oral iron was given in appropriate doses.

Blood was taken from the internal jugular vein, and bone-marrow from the lumbar spinous processes. Blood and marrow examinations were done on admission and thereafter fortnightly, or more often, for from two to four months. Marrow iron was estimated by the direct unstained method (Rath and Finch, 1948; Wallerstein and Pollycove, 1958) and graded accordingly. For sideroblast estimations marrow smears were fixed with 10% formalin in absolute alcohol, washed in water, and the slide flooded with warm potassium ferrocyanide in 5N HCl, again washed in water and counterstained with 1% aqueous safranine. In searching for the red-cell precursors marrow particles were most carefully examined after staining with Leishman and Giemsa. Haemoglobin was determined by the cyanomethaemoglobin method, and the packed cell volume (P.C.V.) by the microhaematocrit. The proteins were estimated by the biuret method (Gornall *et al.*, 1949); fractionation was done by using 27.2% sodium sulphate, to avoid carrying over α_1-globulin to the albumin and thereby giving falsely high albumin and low globulin values. Separation of the globulins was done by low-voltage paper electrophoresis at constant *p*H to reduce the albumin trail. Since there were great variations in the protein content of the serum, sometimes falling as low as 3 g./100 ml. of total protein, the amount of serum seeded on to the paper was calculated by the formula

$$\mu l. = \frac{35}{T.P.}$$

Case Histories: Treatment and Results

Case 1

A 12-months-old Kikuyu girl entered hospital on April 30, 1960, with typical marasmus/kwashiorkor and a three-months history of diarrhoea. Stools contained giardia; treated with mepacrine. Hb, 9.5 g./100 ml.; P.V.C., 33%;

*Riboflavine used in this work was kindly supplied by Roche Products Ltd.

W.B.C., 9,900/c.mm.; total proteins (T.P.), 3 g./100 ml.; albumin (A), 1.04 g./100 ml.; marrow haemosiderin (Fe), lumps grade 4; sideroblasts (sids.), 70%; giant stab-cells (G.S.C.), for which folic acid and vitamin B_{12} were given; red-cell precursors (R.C.P.), 17%.

May 20: Hb, 8.5 g./100 ml.; P.C.V., 31%; W.B.C., 6,700/c.mm.; T.P., 5.5 g./100 ml.; A., 2.04 g./100 ml.; marrow Fe, few lumps grade 2; sids., 0%; R.C.P., 38%.

May 23: Measles.

June 11: Child recovered from measles; severe diarrhoea; temperature 102° F. (38.9° C.); given sulphonamides, penicillin, and streptomycin. Hb, 9.4 g./100 ml.; P.C.V., 35%; W.B.C., 7,900/c.mm.; T.P., 6.5 g./100 ml.; A., 2.2 g./100 ml.; marrow Fe, big lumps grade 1; sids., 0%; R.C.P., 11%.

June 24: Diarrhoea better. Hb, 9.4 g./100 ml.; P.C.V., 35%; W.B.C., 8,400/c.mm.; T.P., 6.8 g./100 ml.; A., 3.5 g./100 ml.; marrow Fe, grade 1; sids., 0%; R.C.P., 32%.

July 11: Hb, 9.4 g./100 ml.; P.C.V., 35%; W.B.C., 6,600/c.mm.; T.P., 6.7 g./100 ml.; A., 3.1 g./100 ml.; retics., 0%; marrow Fe, lumps grade 1; sids., 0%; R.C.P., 0%.

July 18: Hb, 8 g./100 ml.; retics., 0%; marrow Fe, grade 1; sids., 0%; R.C.P., 0%. As there was complete red-cell aplasia of the marrow on two consecutive examinations, and the Hb was falling, riboflavine 5 mg. daily intramuscularly for six weeks was given.

July 25 and 28: Retics., 1% and 9%.

August 2: Hb, 8.8 g./100 ml.; P.C.V., 33%; W.B.C., 18,000/c.mm.; T.P., 6.8 g./100 ml.; A., 3.1 g./100 ml.; marrow Fe, nil; sids., 4%; R.C.P., 25%.

August 16: Hb, 10.5 g./100 ml.; P.C.V., 38%; W.B.C., 10,200/c.mm.; T.P., 7.8 g./100 ml.; A., 3.5 g./100 ml.; sids., 29%; R.C.P., 17%.

August 29: Hb, 11.7 g./100 ml.; P.C.V., 43%; discharged from hospital.

Case 2

A 1-year-old Kikuyu girl entered hospital on March 30, 1960, with typical marasmus/kwashiorkor, and a six-months history of diarrhoea and cough. *X*-ray examination showed miliary tuberculosis; treated with streptomycin and isoniazid. Hb, 9.4 g./100 ml.; P.C.V., 33%; W.B.C., 4,400/c.mm.; T.P., 5.6 g./100 ml.; A., 1.7 g./100 ml.; marrow Fe, nil; sids., 0%; R.C.P., 10%.

May 20: Hb, 10.4 g./100 ml.; P.C.V., 35%; W.B.C., 8,900/c.mm.; T.P., 6.3 g./100 ml.; A., 2.4 g./100 ml.; marrow Fe, nil; sids., 0%; R.C.P., 12%; G.S.C., for which folic acid was given.

June 4: Hb, 10.2 g./100 ml.; P.C.V., 35%; W.B.C., 9,900/c.mm.; T.P., 7.3 g./100 ml.; A., 2.4 g./100 ml.; marrow Fe, nil; sids., 40%; R.C.P., 17%.

June 21: Hb, 10 g./100 ml.; P.C.V., 35%; W.B.C., 10,000/c.mm.; T.P., 8.3 g./100 ml.; A., 2.6 g./100 ml.; marrow Fe, nil; sids., 0%; R.C.P., 9%. As there was no improvement in the Hb and P.C.V. for nearly a month and the R.C.P. was falling, oral riboflavine 3 mg. daily was given for two weeks.

June 30: Marrow Fe, nil; sids., 8%; R.C.P., 32%.

July 7: Two weeks after the first dose of riboflavine, Hb, 11.6 g./100 ml.; P.C.V., 40%; W.B.C., 12,000/c.mm.; T.P., 8.3 g./100 ml.; A., 3.3 g./100 ml.; left hospital.

Case 3

A 2-year-old Kikuyu boy entered hospital on May 27, 1960, with typical kwashiorkor and a one-week history of cough and diarrhoea. Hb, 8.4 g./100 ml.; P.C.V., 31%; W.B.C., 5,500/c.mm.; T.P., 3.8 g./100 ml.; A., 1.1 g./100 ml.; marrow Fe, lumps grade 4; sids., 78%; R.C.P., 22%.

May 30: Moist sounds in lungs; temperature 100° F. (37.8° C.); penicillin and streptomycin.

June 10: Hb, 8.4 g./100 ml.; P.C.V., 31%; W.B.C., 14,000/c.mm.; T.P., 6.4 g./100 ml.; A., 1.72 g./100 ml.; marrow Fe, nil; sids., 10%; R.C.P., 37%.

June 23: Child much better clinically, but routine haematological examination showed: Hb, 8.4 g./100 ml.; P.C.V., 31%; W.B.C., 11,000/c.mm.; T.P., 7.3 g./100 ml.; A., 3.3 g./100 ml.; marrow Fe, nil; sids., 0%; R.C.P., 4%. Since the Hb had remained stable for three weeks and the marrow became hypoplastic, oral riboflavine 3 mg. daily was given for two weeks.

July 1: Seven days after the first dose of riboflavine marrow Fe nil; sids., 20%; R.C.P., 31%.

July 8: Hb and P.C.V. went up for the first time since the child entered hospital. Hb, 10.7 g./100 ml.; P.C.V., 40%; T.P., 7.7 g./100 ml.; A., 2.84 g./100 ml.; left hospital.

Case 4

A 1-year-old Kikuyu girl entered hospital on May 27, 1960, with typical marasmus/kwashiorkor and a two-weeks history of diarrhoea; temperature 100° F. (37.8° C.); Hb, 7.8 g./100 ml.; P.C.V., 27%; W.B.C., 6,300/c.mm.; T.P., 3.6 g./100 ml.; A., 1.1 g./100 ml.; marrow Fe, lumps grade 4; sids., 65%; R.C.P., 16%.

June 10: Hb, 4.5 g./100 ml.; P.C.V., 17%; W.B.C., 4,580/c.mm.; T.P., 6.3 g./100 ml.; A., 1.8 g./100 ml.; marrow Fe, grade 4; sids., 0%; R.C.P., 3%. As the patient's Hb, P.C.V., and R.C.P., went down so precipitously she was given a transfusion of 100 ml. of blood and 3 mg. of oral riboflavine for two weeks.

June 17: One week after the first dose of riboflavine marrow Fe, grade 3; sids., 0%; R.C.P., 78%; G.S.C. for which folic acid and vitamin B_{12} were given.

June 24: Hb rose to 8.4 g./100 ml.; P.C.V., 31%; W.B.C., 10,000/c.mm.; T.P., 6.9 g./100 ml.; A., 2.8 g./100 ml.

July 8: Hb, 9.7 g./100 ml.; P.C.V., 34%; W.B.C., 7,000/c.mm.; marrow Fe, grade 2; sids., 0%; R.C.P., 17%.

July 21: Hb, 9.7 g./100 ml.; P.C.V., 34%; W.B.C., 6,600/c.mm.; T.P., 6.8 g./100 ml.; A., 2.7 g./100 ml.; marrow Fe, grade 2; sids., 0%; R.C.P., 2%. As the Hb and P.C.V. stopped rising and the R.C.P. again fell riboflavine 5 mg. daily intramuscularly was given for two weeks.

August 5: Two weeks after the second course of riboflavine, Hb, 10.6 g./100 ml.; P.C.V., 37%; W.B.C., 8,400/c.mm.; T.P., 6.3 g./100 ml.; A., 2.96 g./100 ml.; marrow Fe, grade 2; sids., 0%; R.C.P., 26%. Left hospital.

Case 5

A 4-year-old Mkamba boy entered hospital on July 7, 1960, with typical kwashiorkor and a two-months history of diarrhoea and giardiasis, for which mepacrine was given. Hb, 5.2 g./100 ml.; P.C.V., 17%; W.B.C., 8,500/c.mm.; T.P., 6 g./100 ml.; A., 2.9 g./100 ml.; marrow malaria pigment, no Fe; sids., 60%; R.C.P., 47%.

July 21: Developed measles. Hb, 8.3 g./100 ml.; P.C.V., 34%; W.B.C., 4,800/c.mm.; T.P., 6.8 g./100 ml.; A., 2.64 g./100 ml.; marrow malaria pigment, no Fe; sids., 0%; R.C.P., 16%.

August 8: Recovered from measles. Hb, 9.7 g./100 ml.; P.C.V., 39%; W.B.C., 10,500/c.mm.; T.P., 7.3 g./100 ml.; A., 3.1 g./100 ml.; marrow no malaria pigment, no Fe; sids., 0%; R.C.P., 2%.

August 17: Child much better clinically; but Hb, 8.4 g./100 ml.; P.C.V., 32%; W.B.C., 14,000/c.mm.; T.P., 7.8 g./100 ml.; A., 4 g./100 ml.; marrow Fe, nil; sids., 0%; R.C.P., 1%. As the marrow was found to be aplastic on two occasions, riboflavine 5 mg. daily intramuscularly was given for five weeks.

August 24: One week after the first dose of riboflavine. Marrow Fe, nil; sids., 0%; R.C.P., 28%.

August 30: Hb, 9.3 g./100 ml.; P.C.V., 31%.

September 9: Hb, 9.9 g./100 ml.; P.C.V., 37%.

September 15: Hb, 9.9 g./100 ml.; P.C.V., 37%; marrow, no malaria pigment, no Fe; sids., 0%; R.C.P., 19%; heavy giardia infection, mepacrine given.

September 26: Hb, 11 g./100 ml.; P.C.V., 41%. The slow haematological response of this child was probably

due to repeated occurrences of giardia infection, and once these were effectively treated the haemoglobin rose to normal.

September 28: Left hospital.

Discussion

Our attention was first drawn to this red-cell aplasia of the marrow in a patient from whom two successive marrow specimens taken from the spinous processes yielded what appeared to be blood; a third puncture, from the tibia, produced a similar fluid, which, however, on examination was found to contain marrow particles, proving upon scrutiny to be completely devoid of all R.C.P.s.

The aetiological factors concerned in the present cases of selective red-cell aplasia are obscure. Gross malnutrition and diarrhoea were present in all the 23 cases of marasmus and kwashiorkor, and most had respiratory as well as other infectious processes, but only nine of them developed aplasia or hypoplasia, for which it was impossible to decide what particular factors were responsible.

In none of the present cases was there obvious clinical or radiological thymic enlargement, or history of exposure to toxic substances or ionizing radiations. Indirect bilirubin was never raised and there was no sign of haemolysis. Some of the patients had been given mepacrine (an antagonist of riboflavine) for giardia infection, but others had not. One had miliary tuberculosis, and one had malaria pigment in the marrow, but we know of no evidence to incriminate these as causes of pure red-cell aplasias. That tetracycline or penicillin and streptomycin, which are currently used in the treatment of marasmus and kwashiorkor, were in any way involved in the causation of these aplasias seems unlikely. So far as we are aware, there is little evidence that these antibiotics produce depression of the red-cell series in the bone-marrow. In fact, there was no common denominator that could be specifically incriminated as a cause of the aplasias.

On admission the bone-marrow was always erythronormoblastic and sometimes had considerable numbers of R.C.P.s (Table I); two patients had G.S.C.s. In all cases hypoplasia or aplasia developed during treatment at a time when the serum proteins were returning to normal (Table I). At the stage when the marrow was aplastic or hypoplastic in the red-cell series, the myeloid series and platelets were normal in all the patients and there was no sign of panmyelopathy, the only series affected being the erythroid. In Table II are shown the myeloid series at a time when the marrow was hypoplastic or aplastic in the red-cell line.

In some cases there was an increase in the reticulum cells. Every case showed an increase in the lymphocytes such as was described by Chalmers and Boheimer (1954). Perhaps some of these lymphocytes were the "erythrogones" of Israëls (1948) and may be precursors of the red cells. Alternatively, the factors responsible for the aplasia may also be causing the lymphocytosis, in which case there is no need to suppose that these lymphocytes are taking any part in red-cell production.

From admission till the development of aplasia there were great fluctuations in the cellularity of the marrow, but ultimately the R.C.P.s fell to less than 5% in all patients but one (Case 2).

The development of this aplasia during the course of treatment is interesting in view of the findings of Gómez *et al.* (1952, 1955), who described a "recovery

TABLE I

Date	Hb g./100 ml.	P.C.V. %	M.C.H.C. %	R.C.P. %	E.E. %	L.E. %	N. %	Sids. %	Marrow Iron Grade	Retics. %	T.P. g./100 ml.	A. g./100 ml.
Case 1												
30/4/'60	9·5	33	29	17	3	5	9	70	4	—	3·0	1·04
20/5/60	8·5	31	28	38	2	15	21	0	2	—	5·5	2·04
11/6/60	9·4	35	27	11	1	3	7	0	1	—	6·5	2·2
24/6/'60	9·4	35	27	32	9	4	19	0	1	—	6·8	3·5
11/7/60	9·4	35	27	0	0	0	0	0	1	0	6·7	3·1
18/7/60	8·0	—	—	0	0	0	0	0	1	0	—	—
Riboflavine												
25/7/60	—	—	—	—	—	—	—	—	—	1		
26/7/60	—	—	—	—	—	—	—	—	—	2		
27/7/60	—	—	—	—	—	—	—	—	—	4		
28/7/60	—	—	—	—	—	—	—	—	—	9		
29/7/60	—	—	—	—	—	—	—	—	—	7		
30/7/60	—	—	—	—	—	—	—	—	—	6		
2/8/60	8·8	33	27	25	3	5	17	4	0	3	6·8	3·1
9/8/60	9·6	36	27	—	—	—	—	—	—	—	—	—
16/8/60	10·5	38	28	17	5	6	6	29	0	3	7·8	3·5
29/8/60	11·7	43	27	—	—	—	—	—	—	—	—	—
Case 2												
30/3/60	9·4	33	28	10	2	2	6	0	0	—	5·6	1·7
20/5/60	10·4	35	30	12	3	1	8	0	0	—	6·3	2·4
4/6/60	10·2	35	29	17	0	2	15	40	0	—	7·3	2·4
21/6/60	10·0	35	29	9	3	2	4	0	0	—	8·3	2·6
Riboflavine												
30/6/60	—	—	—	32	3	12	17	8	0	—	—	—
7/760	11·6	40	29	—	—	—	—	—	—	—	8·3	3·3
Case 3												
27/5/60	8·4	31	27	22	6	7	9	78	4	—	3·8	1·1
10/6/60	8·4	31	27	37	2	17	18	10	0	—	6·4	1·7
23/6/60	8·4	31	27	4	3	1	0	0	0	—	7·3	3·3
Riboflavine												
1/7/60	—	—	—	31	3	10	18	20	0	7	—	—
8/7/60	10·7	40	27	—	—	—	—	—	—	—	7·7	2·8
Case 4												
27/5/60	7·8	27	29	16	1	5	10	65	4	—	3·6	1·1
10/6/60	4·5	17	26	3	2	0	1	0	4	—	6·3	1·8
Riboflavine												
17/6/60	—	—	—	78	10	49	19	0	3	—	—	—
24/6/60	8·4	31	27	—	—	—	—	—	—	—	6·9	2·8
8/7/60	9·7	34	28	17	1	3	13	0	2	—	—	—
21/7/60	9·7	34	28	2	0	1	1	0	2	—	6·8	2·7
Riboflavine												
5/8/60	10·6	37	29	26	3	6	17	0	2	—	6·3	3·0
Case 5												
7/7/60	5·2	17	30	47	3	20	24	60	M.P.	—	6·0	2·9
21/7/60	8·3	34	24	16	4	1	11	0	M.P.	—	6·8	2·6
8/8/60	9·7	39	25	2	1	1	0	0	No M.P.	0	7·3	3·1
17/8/'60	8·4	32	26	1	1	0	0	0		0	7·8	4·0
Riboflavine												
24/8/60	—	—	—	28	6	10	12	0	0	3		
25/8/60	—	—	—	—	—	—	—	—	—	8		
26/8/60	—	—	—	—	—	—	—	—	—	6		
27/8/60	—	—	—	—	—	—	—	—	—	4		
30/8/60	9·3	36	25	—	—	—	—	—	—	2		
9/9/60	9·9	37	27	—	—	—	—	—	—	—		
15/9/60	9·9	37	27	19	1	8	10	0	0	—		
26/9/60	11·0	41	27	—	—	—	—	—	—	—		

Hb = Haemoglobin. P.C.V. = Packed cell volume. M.C.H.C. = Mean corpuscular haemoglobin concentration. R.C.P. = Red-cell precursors. E.E. = Early erythroblasts. L.E. = Late erythroblasts. N. = Normoblasts. Sids. = Sideroblasts. Retics. = Reticulocytes. T.P. = Total protein. A. = Albumin.

TABLE II.—*Differential Marrow Counts at Time of Red-cell Aplasia*

	Case 1		Case 2	Case 3	Case 4		Case 5	
	11/7	18/7	21/6	23/6	10/6	21/7	8/8	17/8
Myeloblasts, %	3	0	0	0	0	3	2	0
Promyelocytes, %	1	2	1	2	3	1	0	1
Myelocytes, %	7	8	13	5	12	3	4	3
Metamyelocytes, %	11	10	9	2	3	2	3	2
Polymorphs, %	45	34	40	32	25	18	46	32
Lymphocytes, %	26	41	23	48	37	45	37	53
Eosinophils, %	3	4	2	7	1	4	2	3
Basophils, %	0	0	0	0	2	0	1	0
Reticulum cells, %	2	0	2	0	6	14	2	5
Megakaryocytes, %	1	0	0	0	0	0	0	0
Monocytes, %	1	0	0	0	4	6	1	0
Plasma cells, %	0	1	1	0	4	2	0	0
W.B.C.	6,600	—	10,000	11,000	4,580	6,600	10,500	14,000

syndrome" in malnutrition developing two to three weeks after the beginning of treatment and involving hepatomegaly, ascites, eosinophilia, and perhaps associated with "dysadrenocorticism" (Iversen, 1955), in which riboflavine deficiency plays a part (Forker and Morgan, 1954, 1955; Slater, 1959; *Nutrition Reviews*, 1960). Perhaps this red-cell aplasia is another manifestation of the "recovery syndrome." The aplasia and the other symptoms of the "recovery syndrome" may be due to deficiency of cortisone which is itself linked to riboflavine deficiency.

We do not know what proportion of cases of red-cell aplasia occurring in marasmus and kwashiorkor recover spontaneously without riboflavine, or how many deaths are due to bone-marrow failure. Spontaneous recovery, although not unknown, is exceedingly rare (Heilmeyer and Begemann, 1951). It is possible that the aplasia may be symptomatic and self-limiting; in two cases, however, an aplastic marrow was found in patients who had left hospital and who later died. Whatever the ultimate prognosis of these aplasias, they appear to be a common accompaniment of marasmus and kwashiorkor in Kenya, and must be regarded as a serious complication.

As in the previous case of pure red-cell aplasia reported in an African (Foy and Kondi, 1953), the response to riboflavine in the present patients was clear-cut. So far as was consistent with the clinical and haematological condition, riboflavine administration was begun only when the R.C.P.s had fallen to 5% or less, and was continued for from two to six weeks either orally or intramuscularly. One week after the first dose of riboflavine the bone-marrow passed from a state of hypoplasia or complete red-cell aplasia to a condition in which 25%–78% of the cells were R.C.P.s, consisting of early and late erythroblasts and normoblasts, and followed by peripheral reticulocytosis. One week after this increase in marrow activity there was a rise in the Hb and P.C.V. The time-relation between the administration of riboflavine and the subsequent haematological response makes it appear that there was an association between the two. Further, in Case 4 an increase in marrow activity followed the administration of riboflavine, but when this was stopped the cellularity of the marrow decreased, becoming reactivated when riboflavine was resumed; this second response must, we think, be regarded as significant. As was to be expected the increase in R.C.P.s after riboflavine was more pronounced in the most anaemic cases.

Altman and Miller (1953) reported an increased excretion of anthranilic acid in the urine of a patient with pure red-cell aplasia; after treatment with riboflavine the urinary anthranilic acid disappeared but the aplasia was unaffected. Altman and Miller attributed the presence of anthranilic acid to upsets in tryptophan metabolism due to a deficiency of riboflavine. Mason (1953) has shown that when tryptophan is administered to riboflavine-deficient rats there is a considerable increase in the output of anthranilic acid. These reports indicate that riboflavine is in some way linked with tryptophan metabolism. We had no facilities at the time for chromatographic studies, and we cannot at present say whether the patients had abnormal outputs of urinary anthranilic acid; serum riboflavine was not estimated in the present cases.

Whatever the cause of the pure red-cell aplasia in the patients under consideration, it seems that it is favourably influenced by riboflavine. According to Scrimshaw *et al.* (1956), serum riboflavine is often very low in kwashiorkor, and rises during treatment because of the milk diet. In the present patients, in spite of the milk diet, the serum riboflavine may have failed to increase enough to stimulate and sustain marrow activity, or their demands for riboflavine were greater than in those who did not become aplastic. The low level of serum riboflavine that occurs in these conditions may be due to the malnutrition, the diarrhoea, or the infections, and may apply to other vitamins as well (Scrimshaw *et al.*, 1956).

It is possible also that the high-protein diet which is an essential part of the treatment of these conditions increases the demand for riboflavine, leaving insufficient for sustaining increased marrow activity, just as excessive protein in the diet has been found to increase demands for vitamin B_{12} in both megaloblastic and iron-deficient anaemias (Foy and Kondi, 1958; Foy, Kondi, and Sarma, 1958).

Haemosiderin was present in the marrow as lumps in three cases, and this is usually regarded as an indication of some infectious process (Rath and Finch, 1948; Heilmeyer and Wöhler, 1958; Wallerstein and Pollycove, 1958; Heilmeyer and Keiderling, 1959). It may, however, have been associated with the very low serum proteins and a consequent reduction in transferrin leading to precipitation of the absorbed iron as haemosiderin (Shoden and Sturgeon, 1959). This shift of the iron from the red cells to the storage depots, resulting in an increase in marrow haemosiderin, is common in many anaemias, and the marrow iron in such cases decreases with treatment (Sheehy *et al.*, 1960). One of the patients, although miliary tuberculosis was present, had no haemosiderin in the marrow; this may have been because the iron stores were excessively low. Another had masses of malaria pigment in the marrow, and it is our experience that when iron is present in the marrow in the form of malaria pigment iron in other forms cannot be found.

With the exception of the tuberculosis patient, all the others had high sideroblast levels on admission to hospital, and these fluctuated widely throughout their illness.

In spite of the presence of haemosiderin and sideroblasts in the marrow on admission, the anaemia was always hypochromic, as it is in chronic infection (Heilmeyer and Begemann, 1951). During the course of the illness marrow iron and sideroblasts fell or disappeared, but iron therapy was necessary to meet the demands of increased marrow activity and to sustain haemopoiesis.

The serum iron was low on admission and fluctuated in relation to variations in haemopoiesis. The unsaturated iron-binding capacities and iron-saturation indices paralleled the fluctuations in serum iron and protein variations. The changes in these variables form a very striking picture in marasmus and kwashiorkor, and will be dealt with in a later paper.

Whatever factors are involved in the high incidence of pure red-cell aplasia in marasmus and kwashiorkor, it seems that riboflavine is playing an important part in haemopoiesis, and is one of the substances that take part in some enzyme system that potentiates the maturation of a primitive stem cell into erythroblasts acting either directly or through the steroid hormones.

Summary

Selective red-cell aplasia or hypoplasia occurred in 9 out of 23 cases of marasmus and kwashiorkor in Kenya.

Serial bone-marrow punctures showed that the red-cell precursors were normal on admission, and the marrow became hypoplastic or completely aplastic during recovery, at a time when the serum proteins were returning to normal.

There was no single factor that could be regarded as a cause of these red-cell aplasias.

Treatment with oral or intramuscular riboflavine reactivated the marrow and was followed by reticulocytosis and a rise in haemoglobin and packed cell volume.

The implications of the marrow aplasia in marasmus and kwashiorkor are discussed.

Both the adrenal dysfunction of the "recovery syndrome" and the red-cell aplasia may be associated with riboflavine deficiency, particularly as the serum riboflavine is low in marasmus and kwashiorkor and the aplasia responds to riboflavine.

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A Government White Paper, *Disabled Persons in Government Employment*, shows that the Government employ 41,744 registered disabled persons. In the non-industrial Civil Service there are 627,127 people employed, of whom 26,554 (4.2%) are disabled. In the industrial Civil Service there are 349,711 people employed and 14,768 of them are disabled, again 4.2%. There are 452 passenger-lift attendants and 422 of them are disabled, or 93.4%. The standard percentage for the purpose of the Disabled Persons (Standard Percentage) Order, 1946, is 3%.

PRIMAQUINE-SENSITIVITY OF RED CELLS IN VARIOUS RACES IN SOUTHERN AFRICA

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Some cases of drug-induced haemolytic anaemia have been shown to be due to the action of the drug on congenitally defective red blood cells (Beutler, 1959). Phenacetin, acetanilide, sulphanilamide, sulfoxone, thiazosulphone (Dern *et al.*, 1955), nitrofurantoin (Kimbro *et al.*, 1957), pamaquin, and primaquine (Dern *et al.*, 1955) belong to this group. In addition, favus beans (Sansone and Segni, 1957) and naphthalene (Zinkham and Childs, 1957) also cause haemolysis in sensitive individuals. The congenitally defective red cell has a normal life span unless exposed to one of the precipitating substances, and the trait has not been shown to lead to any other disability. Since the biochemical lesion was first characterized in subjects sensitive to the antimalarial primaquine the condition is commonly referred to as "primaquine sensitivity."

Red-cell Abnormalities in Susceptible Subjects

The pattern of haemolysis in susceptible subjects exposed to one of the precipitating agents has certain characteristic features (Dern *et al.*, 1954; Flanagan *et al.*, 1958). It begins on about the third or fourth day of administration and lasts until about the twelfth day. About half the red-cell population is destroyed, with the result that the haemoglobin and haematocrit fall to roughly half their original values. Haemolysis then ceases, and the blood picture rapidly returns to normal. This is true even when the patient continues to take the drug. The clinical picture is thus one of an acute self-limited haemolytic anaemia.

The defective red cells can be identified in several ways. The first distinguishing characteristic to be discovered was abnormal Heinz-body formation *in vitro*. Following on the clinical observation that Heinz bodies appeared transitorily in the red cells of sensitive volunteers taking primaquine (Beutler *et al.*, 1954), it was demonstrated *in vitro* that the Heinz bodies produced in sensitive cells differed from those in normal cells both in size and in number (Beutler *et al.*, 1955), and the Heinz-body test has been used as a method of predicting primaquine sensitivity.

Subsequent investigations have revealed several biochemical abnormalities in the red cells of susceptible subjects. The concentration of reduced glutathione tends to be lower than normal, though the difference is not great enough to be used as a test of sensitivity. Of more significance is the fact that the glutathione level drops sharply on incubation *in vitro* with acetylphenylhydrazine, while the level in normal blood does not change. This glutathione stability test has proved an accurate means of predicting primaquine sensitivity (Beutler, 1957).

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