Cali-Harvard Nutrition Project

II. The Erythroid Atrophy of Kwashiorkor and Marasmus

JACOBO GHITIS, M.D.,* HERNAN VELEZ, M.D.,† FRANCISCO LINARES, M.D.,‡
LEONARDO SINISTERRA, M.D.§ AND JOSEPH J. VITALE, SC.D.||

PROTEIN DEFICIENCY is one of the most important public health problems in many areas of the world. A consistent clinical finding in severe malnutrition (kwashiorkor and marasmus) is the presence of anemia.[1-5] This anemia has usually been described as normocytic-normochromic and sometimes as macrocytic, hypochromic or dimorphic.[1,2,4,6-8]

In a review article on protein malnutrition in young children, Scrimshaw and Behar[9] recently wrote that "the exact role of protein deficiency per se in the production of some types of anemia is still not known." Investigators, with few exceptions,[10,11] have been reluctant to accept a direct cause and effect relationship between the lack of protein and the presence of anemia in human subjects. On the other hand, there are several studies which indicate that protein deficiency causes anemia in animals.[12-16] Presumably, protein deficiency resulted in a decrease in hemoglobin production or a decrease in red cell formation, but these terms have been used loosely, as Cartwright[17] pointed out, to refer to erythropoiesis.

Most studies on the role of protein deprivation and the resultant anemia have only included changes in the red cell counts, hemoglobin concentration, volume of packed red cells, total blood volume and radioiron uptake determinations. To our knowledge there have been few, if any, experimental studies with animals dealing with the effect of protein deprivation on the structure or metabolism of the red cell precursors (normoblasts). Smears of bone marrow aspirates from children with protein malnutrition have been examined,[18] but again, serial observations on the effect of malnutrition and subsequent protein feeding on this tissue have been neglected.

The present study deals with young children suffering from severe malnutrition (kwashiorkor and marasmus) in whom bone marrow aspirates were examined on admission and during the recovery period when increasing amounts of protein were fed.

MATERIALS AND METHODS

Fifteen children on whom it was possible to take serial bone marrow aspirates are included in this study. The patients chosen had clinical signs of severe protein malnutrition but not of severe infectious diseases (tuberculosis, meningitis, etc.). The patients, admitted from the emergency room of the University Hospital, Cali, Colombia, were fed a special metabolic diet. The diet consisted mainly of skim milk and brown sugar and supplied 10 μg. or less of total "folic acid activity" per day. The

From the Departments of Nutrition, Medicine and Pediatrics, Universidad del Valle, Cali, Colombia, South America, and the Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts.

* Department of Medicine, Universidad del Valle, Cali, Colombia; † Present address: Department of Nutrition, Universidad de Antioquia, Medellin, Colombia; ‡ Department of Nutrition, Universidad del Valle, Cali, Colombia; § Chairman, Department of Nutrition, Universidad del Valle, Cali, Colombia; || Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts.

This study was supported in part by Grants-in-Aid from The Rockefeller Foundation, The Kellogg Foundation and The Nutrition Foundation, New York City; the National Institutes of Health (No. C-1323 (C8) and H-3515 (C3)), Public Health Service, Bethesda, Maryland; and the Fund for Research and Teaching, Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts.

TABLE I
Hematologic Data on Fifteen Patients*

Patient, Sex and Age (yr.)	Hospital Day	Hemoglobin (gm. %)	Hematocrit (%)	Reticulocytes (%)	Nucleated Red Cells (/100 nucleated cells)	Myeloid: Erythroid Ratio	Normoblasts (/100 nucleated red cells)			Siderblasts (%)	Remarks
							Basophilic	Polychromatic	Orthochromatic		
M. S., F, 1½	2	10.9	33	0.8	0.3	180	...	...	...	...	Normocytic; normochromic; normoblastic; no pteroylglutamic acid or ferrous sulfate given; discharged on day 66 when hemoglobin 11 gm./100 ml.
	10	8.3	26	0.9	5	13	15	50	35	...	
	17	8.2	24	5.2	18	3.3	6	52	42	...	
	26	...	...	9.5	11	7	12	65	23	...	
	36	9.7	32	7.5	27	1.8	4	64	32	...	
S. R., M, 2½	1	7.6	24	3.0	10	7	18	52	30	60	Blood smear normocytic-normochromic; megaloblastic dysplasia grade 1 on day 20; normoblastosis on day 29; no pteroylglutamic acid given
	11	8.5	26	1.6	46	1	38	38	24	0	
	20	10.3	31	7.0	28	3	22	50	28	0	
	29	11.9	35	1.5	19	3	10	45	45	0	
M. E. L., F, 5	4	9.1	28	1.5	5	12	3	58	39	20	Initial bone marrow normoblastic; second bone marrow megaloblastic grade 2; third and fourth grade 1; became normoblastic on day 33; no pteroylglutamic acid given; macrocytosis not present on admission but seen on day 18 and gone on day 40
	11	7.8	25	1.6	21	3	27	27	46	78	
	18	7.9	23	6.8	26	2	6	49	45	64	
	25	9.8	31	2.3	23	2.8	3	67	30	2	
	33	9.7	29	1.4	29	2	6	48	46	0	
	40	9.9	31	1.6	18	3	0	45	55	0	
M. A., F, 7	2	11.4	34	0.8	9	6.4	0	36	64	...	Bone marrow megaloblastic grade 1; became normoblastic on day 41; no pteroylglutamic acid given; slight hypochromia on admission blood smear; marked on day 120; iron given and reticulocytosis up to 5 per cent with increase in hemoglobin; serum iron on admission 97 μg./100 ml.
	10	8.1	25	0.9	10	3	0	80	20	25	
	18	8.4	24	1.4	14	5	9	33	58	12	
	28	9.6	30	3.7	10	6	0	60	40	0	
	41	9.6	28	3.0	22	3	6	73	21	0	
	65	8.4	23	2.1	12	4	6	52	42	0	
A. B., M, 1	1	11.8	35	0.6	7	10	12	52	36	33	Initial bone marrow megaloblastosis grade 3; 10–20 μg. pteroylglutamic acid given per day from day 2; megaloblastosis grade 3 on day 28 and grade 1 on day 36; normoblastosis on day 42; slight macrocytosis on initial blood smear; hemoglobin on day 59 was 12.3 gm.
	7	10.7	29	1.2	8	8	48	44	8	60	
	14	9.5	25	1.4	16	2.5	3	15	72	56	
	21	8.8	23	3.5	37	1.3	33	51	16	36	
	28	...	26	8.2	37	1.2	24	33	43	27	
	36	12.0	31	9.1	14	4	27	36	37	0	
	42	11.4	31	4.9	20	2.3	0	33	67	0	
	49	...	...	3.2	26	1.2	10	45	45	0	
A. G., M, 1¼	3	12.9	40	...	2	29	...	...	...	...	Initial bone marrow showed 43 per cent promyelocytes; third bone marrow grade 3 megaloblastosis; 5–20 μg. pteroylglutamic acid given per day from day 15; normoblastosis on day 38; hypochromia from admission to day 47; 200 mg. iron (ferrous sulfate) given from day 47; reticulocyte peak 6.4 per cent and hemoglobin increase to 13.6 gm. on day 65
	7	10.8	32	0.2	1	58	...	...	...	...	
	13	8.1	25	1.5	33	1.6	14	36	50	31	
	24	8.9	29	4.3	26	2	2	28	70	0	
	38	9.1	30	2.4	20	3	14	54	33	0	

J. L., M., 11/12	1	9.5	27	1.5	13	4.9	30	39	31	62	Bone marrow megaloblastosis on admission grade 1; grade 3 on day 16; grade 1 on day 23; normoblastic on day 24; 5–10 μg. pteroylglutamic acid given per day from day 2; slight hypochromia on admission increased on day 72; iron given from day 72; initial macrocytosis not present on discharge day 86 when hemoglobin was 11.8 gm.
	9	...	...	3.0	12	5.8	9	33	58	8	
	16	9.9	29	1.2	29	1.3	12	54	34	2	
	23	...	...	5.7	32	1.7	15	54	31	3	
	30	11.0	34	3.7	31	1.8	18	33	49	2	
	37	...	...	1.7	8	7.2	3	48	49	0	
	44	...	...	...	24	2.4	15	30	55	0	
	72	10.5	33	...	15	4.0	0	33	67	0	
	79	...	...	...	15	3.4	3	36	61	0	
N. J., F., 4	1	11.0	36	3.4	7	10	6	27	67	56	Bone marrow grade 1 megaloblastic from day 1–16; 5–10 μg. pteroylglutamic acid given per day from day 20; normoblastosis on day 24; initial blood smear normochromic-normocytic; hemoglobin 11.2 and hematocrit 32 on discharge day 56; iron given from day 11
	8	9.3	27	4.3	0.3	230	...	...	...	...	
	16	...	...	3.8	18	3.8	3	60	37	0	
	24	11.0	32	6.9	18	3.5	8	36	56	9	
	41	10.9	32	2.3	5	13	6	44	49	...	
L. G., M., 4	1	8.9	24	2.7	7	11	18	61	21	...	Megaloblastosis grade 1 on admission; iron given from day 3; normoblastic on day 44; macrocytosis and hypochromia on admission
	16	9.5	29	6.1	11	7	16	68	16	0	
	44	10.3	32	2.1	16	4.2	15	27	57	40	
F. C., F., 2	1	11.0	35	0.9	...	...	...	...	...	...	Megaloblastosis grade 1 on admission; normoblastic on day 21; no pteroylglutamic acid given; blood smear showed macrocytosis of slight degree on admission
	11	10.5	32	1.7	7	9	21	27	52	44	
	21	10.6	33	4.9	21	2.7	43	30	27	44	
	28	10.6	36	4.2	21	2.7	36	46	18	0	
D. I., M.,	1	9.5	31	0.6	6	11	4	40	56	0	First bone marrow grade 1 megaloblastic; became normoblastic on day 18; no pteroylglutamic acid given; slight hypochromia on admission; iron given from day 4; hemoglobin 11.5 gm. on day 44
	11	9.6	29	3.9	21	2.4	30	15	55	8	
	18	9.7	29	2.3	12	4.8	6	36	58	0	
C. M., M., 3 1/2	1	8.5	24	0.9	10	6.8	12	36	52	70	Hypochromia on admission; iron given from day 4; hemoglobin 12.1 gm. on discharge day 140
	13	7.1	22	3.4	34	1	14	42	44	38	
	20	8.4	26	2.4	6	10	35	50	15	3	
	55	10.5	33	0.5	6	8	0	25	75	6	
E. C., F., 2 1/2	1	8.1	27	1.9	9	5	18	55	27	14	Initial grade 1 megaloblastosis; increased to grade 2 on day 10; pteroylglutamic acid given from day 12; normoblastosis on day 39; 80 ml. blood transfusion on day 22; slight macrocytosis and moderate hypochromia on initial blood smear; iron given from day 40
	10	8.7	27	1.3	17	3	12	49	39	...	
	17	6.9	22	0.6	7	9	11	47	42	52	
	31	8.0	25	6.4	32	2	28	44	28	1	
	39	8.7	27	4.0	13	6	12	58	30	0	
	87	11.5	34	1.8	15	3.3	0	24	76	0	
R. R., M., 2	1	11.0	35	0.5	7	11	6	33	61	2	Bone marrow grade 1 megaloblastic from admission to day 44 when it became normoblastic; 5–10 μg. pteroylglutamic acid given per day from day 6; slight macrocytosis and hypochromia on admission; iron given from day 3
	12	9.5	29	0.8	22	3	12	33	55	5	
	19	10.6	34	2.7	16	4.4	12	45	43	54	
	35	11.8	35	1.2	27	2	3	33	64	...	
	44	11.9	37	3.4	22	2	2	53	45	0	
M, R., M., 2	4	9.5	29	0.3	5	15	4	56	40	...	Initial megaloblastosis grade 1 increased to grade 2 on day 12; normoblastic on day 29; no pteroylglutamic acid given; macrocytosis of moderate degree on admission; iron given from day 14 although hypochromia was not present
	12	10.0	33	0.5	17	4	0	34	66	55	
	19	10.4	34	1.1	22	2.5	4	42	54	6	
	29	11.5	35	2.2	15	4	9	63	28	0	
	37	11.7	36	2.1	20	2.5	24	30	46	0	

* All patients had kwashiorkor except A. G. and J. L. who had marasmus.

diet was adequate in all other respects. The methods used and the general management and care of the patients have been described in detail elsewhere.[19] Morphologic classification of the anemias was based on the observation of blood smears stained with Wright's stain.

RESULTS

On admission, M. S. (Table 1) had a hemoglobin of 10.9 gm. per 100 ml. of blood, hematocrit of 33 per cent and reticulocyte count of 0.8 per cent. The blood smear showed normocytic and normochromic red cells. Normoblasts were almost completely absent in the bone marrow smear, and no megaloblastic changes were evident in the granulocytes (Fig. 1). The amount of protein in the diet was raised progressively from 0.5 gm. per kg. per day to a level of approximately 3 gm. per kg. per day by the end of the second week. During this time much of the edema present in the patient on admission disappeared.

On the tenth day the hemoglobin had decreased to 8.3 gm. per cent, probably due to the effect of hydration, mobilization of body fluids and increased plasma volume. This has been observed by other investigators.[20,21] There was a progressive increase in the percentage of normoblasts resulting in erythroid hyperplasia on the thirty-sixth day; the reticulocyte peak of 9.5 per cent was seen on the twenty-sixth day. Neither hypochromia nor macrocytosis was noted, and the bone marrow remained normoblastic (Fig. 2). At the time of discharge, on the sixty-sixth day, the hemoglobin had risen to 11 gm. per 100 ml.

S. R. responded rapidly to protein feeding in a similar manner to M. S. The hemoglobin increased from 7.6 to 11.9 gm. per 100 ml. of blood in twenty-nine days. There was an initial erythroid hypoplasia followed by marked hyperplasia, reticulocytosis, and finally normal erythroid activity (Table 1).

M. E. L. showed a decrease in hemoglobin concentration during the initial period. With protein feeding there was a rapid increase in the number of erythroblasts and a change in the bone marrow from normoblastic soon after admission (Fig. 3) to megaloblastic seven days later (Fig. 4). A proliferation of pronormoblasts and basophilic normoblasts was seen at this time, whereas a reticulocyte peak of 6.8 per cent occurred on the eighteenth day. The changes in the sideroblast counts (Table 1) also reflected the transition of the erythropoietic system from inactive to active.

With the increased production of red cell precursors a latent folic acid deficiency became apparent as manifested by the morphologic changes in the erythroblasts (megaloblastic dysplasia) and the appearance of macrocytes in the blood. With continued feeding of a diet containing 10 to 13 μg. of total folic acid activity the bone marrow became normoblastic.

Folic acid requirements are extremely low and thus, are measured in micrograms.[19,22,23] It was suggested by Sheehy et al.[22] that the complex substances associated with folic acid activity are not as well absorbed as pteroylglutamic acid in patients with tropical sprue. It is reasonable to assume that the gastrointestinal disturbances that occur in protein deficiency produce a similar malabsorption of folic acid complexes. Some of our patients recovered from the megaloblastic dysplasia with the administration of protein but no pteroylglutamic acid therapy. Whereas this might indicate that improvement in the intestinal structure and metabolism facilitated the absorption of the small amounts of folic acid complexes in the hospital diet, it fails to explain why other patients showed a persistent megaloblastosis, and remission was effected only by the administration of pteroylglutamic acid.[19]

In M. A. anemia became apparent only several days after admission. The slight degree of bone marrow megaloblastosis present on admission was slow in disappearing on diet alone. The hemoglobin concentration, which had increased by the twenty eighth day, showed a secondary decrease on the sixty-fifth day. By this time the patient had gained a considerable amount of weight and had, presumably, a concomitant increase in blood volume. A slight hypochromia was now apparent, and it became marked on the hundred and twentieth day. With the administration of ferrous sulfate, reticulocytosis and a rapid increase in hemoglobin occurred. In this patient serum iron was normal on admission, and there were a good

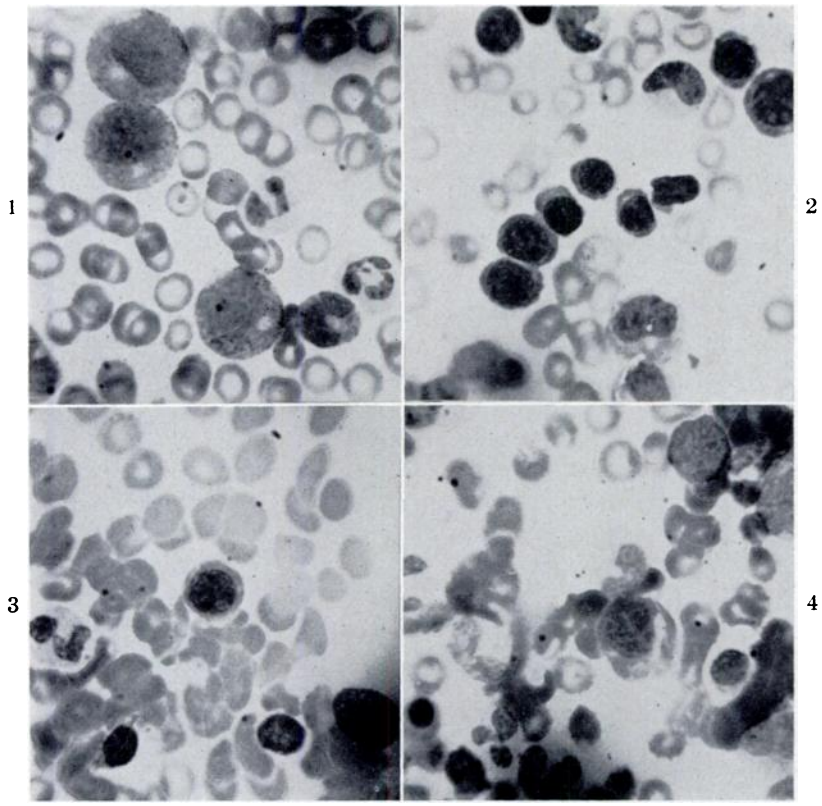

FIG. 1. Photomicrograph of bone marrow smear (first aspirate) of Patient M.S. No megaloblastic changes are seen in the granulocytes.

FIG. 2. Photomicrograph of bone marrow smear (fifth aspirate) of Patient M. S. There is erythroid hyperplasia without megaloblastic changes.

FIG. 3. Photomicrograph of bone marrow smear (first aspirate) of Patient M. E. L. Megaloblastic changes are not seen.

FIG. 4. Photomicrograph of bone marrow smear (second aspirate) of Patient M. E. L. showing an orthochromic megaloblast.

number of sideroblasts in the bone marrow (Table 1). With the increase in red cell production, the latent iron deficiency became apparent as it did in other patients (A. G., J. L. and E. C.) and as was reported in a previous publication.[19] All patients receive a diet which provides the recommended daily allowance of 12 to 15 mg. of iron.

It is generally agreed that protein deficiency in animals results in a hypoproteinemia and that this condition may lead to anemia of moderate degree.[12–16] In children with kwashiorkor, not only is there hypoproteinemia, a protein deficiency state, but also there may exist deficiencies of other essential nutrients. In addition, the child's condition is usually complicated further by infection, electrolyte imbalance, diarrhea, dehydration and marked edema. Thus, it is understandable that there has been some reluctancy to attribute the anemia seen in such children solely to protein deficiency.

However, electrolyte deficiency, presumably of potassium and magnesium, is probably a direct consequence of protein deficiency and secondary to the diarrhea and vomiting. Whereas all the various lipid fractions have been shown to be low in children with kwashiorkor, the levels of these substances usually rise rapidly in the course of treatment when little,

if any, lipid is given. This is similarly true of the fat soluble vitamins.[9] Scurvy is rarely, if ever, seen, and calcium and phosphorous levels are usually normal as are vitamin B_{12} levels. If hypochromia is present, it is usually indicative of an iron deficiency and will persist for a relatively long period of time even with the administration of large doses of ferrous sulfate.

Megaloblastic dysplasia due to folic acid deficiency may be present on admission but usually becomes apparent only after treatment with food begins.

Thiamine and pyridoxine deficiencies are not usually seen in kwashiorkor, and riboflavin deficiency may or may not be present.[9]

Recently, Shahidi et al. reported two cases of protein deficiency anemia in children found to have cystic fibrosis.[11] These infants, aged two and three months, had a normochromic-normocytic anemia and an erythroid hypoplasia which responded to "an adequate intake of high quality protein" and the administration of a pancreatic enzyme (viokase). The high quality protein fed was evaporated milk. These two cases are similar to those cases of kwashiorkor which are uncomplicated by such things as infection, severe dehydration or marked electrolyte imbalance. They resemble the "sugar baby" cases, those uncomplicated by the effects of prolonged and sustained protein deficiency.

The definitive study can hardly be carried out in children suffering from protein malnutrition and all of its sequelae. Kwashiorkor patients would not survive for long if given all of the essential nutrients, save protein. However, preliminary studies in which a soy protein isolate (94 per cent protein dry weight) was substituted for milk produced similar hematologic responses.[24]

The observations made in this study and in others coupled with animal studies,[25] in which erythroid atrophy occurred quickly, strongly support the belief that protein deficiency *per se* is primarily the cause of the anemia seen in kwashiorkor and that such a deficiency has a marked effect on the production of red cell precursors, the lack of which would certainly lead to anemia.

Figure 5 is intended to illustrate the hematologic events that may occur with protein deficiency and subsequent protein refeeding. The figure is self-explanatory and illustrates what is usually seen in children with kwashiorkor and marasmus with whom we have worked. In a total of forty-seven such patients in whom bone marrow aspirates were examined on admission, erythroid hypoplasia of a variable degree was present in all. Sanchez[18] also reported an erythroid hypoplasia on admission in severely malnourished children. Table 1 shows that recovery started within one to two weeks after the initiation of protein feeding. In several patients, there was "overshooting"

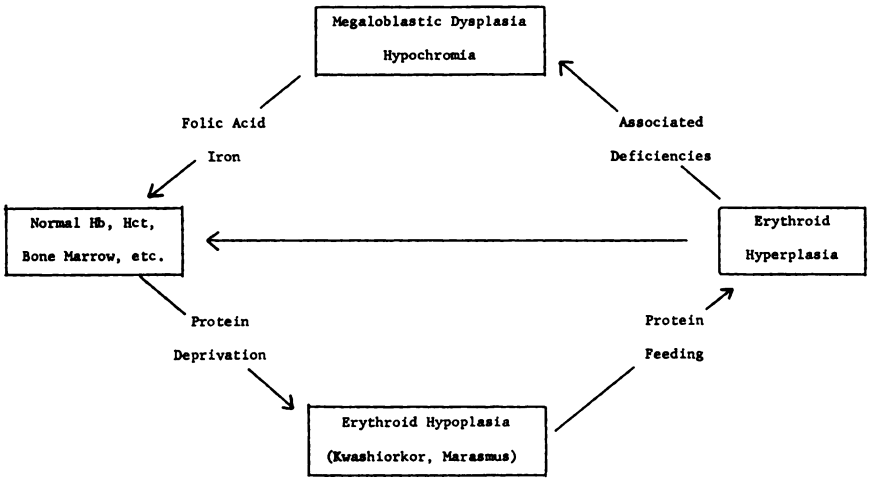

FIG. 5. Hematologic events that may occur with protein deficiency and subsequent protein refeeding.

(erythroid hyperplasia), including those whose myeloid:erythroid ratios and normoblast counts on admission did not depart much from normal values. Such observations in the latter type of patients suggest that a functional impairment of the red cell precursors precedes their decrease in number.

SUMMARY

Erythroid hypoplasia was a consistent finding in children with malnutrition (kwashiorkor and marasmus). With subsequent protein feeding, there was an increased production of normoblasts, and in most cases, erythroid hyperplasia occurred. With increased erythropoiesis, the associated deficiencies became apparent or more marked. It is postulated that the anemia of kwashiorkor is primarily due to the protein deficiency which results in a decreased production of red cell precursors.

ACKNOWLEDGMENT

We wish to thank the Misses Doris Diez, Virginia Bonelli, Enoe Norena, Dolly Quintero and Mary Gonzalez for their able technical assistance at various stages in the work.

REFERENCES

1. Protein malnutrition. In: Proceedings of a Conference in Jamaica. Cambridge, 1953. Cambridge University Press.
2. TROWELL, H. C., DAVID, J. N. P. and DEAN, R. F. A. Kwashiorkor. London, 1954. Edward Arnold, Ltd.
3. SENECAL, J. The treatment and prevention of kwashiorkor in French West Africa. *Ann. New York Acad. Sc.*, 69: 916, 1958.
4. BEHAR, M., VITERI, F., BRESSANI, R., ARROYAVE, G., SQUIBB, R. L. and SCRIMSHAW, N. S. Principles of treatment and prevention of severe protein malnutrition in children (kwashiorkor). *Ann. New York Acad. Sc.*, 69: 954, 1958.
5. GOMEZ, F., RAMOS-GALVAN, R., CRAVIOTO, J. and FRENK, S. Prevention and treatment of chronic severe infantile malnutrition (kwashiorkor). *Ann. New York Acad. Sc.*, 69: 969, 1958.
6. TROWELL, H. C. Tropical macrocytic anemia and nutritional macrocytic anemia. *South African J. M. Sc.*, 12: 21, 1947.
7. SCRIMSHAW, N. S., BEHAR, M., VITERI, F., ARROYAVE, G. and TEJADA, C. Epidemiology and prevention of severe protein malnutrition (kwashiorkor) in Central America. *Am. J. Pub. Health*, 47: 53, 1957.
8. MACDOUGALL, L. C. The hematological picture in kwashiorkor and marasmus. *Tr. Roy. Soc. Trop. Med. & Hyg.*, 54: 37, 1960.
9. SCRIMSHAW, N. S. and BEHAR, M. Protein malnutrition in young children. *Science*, 133: 2039, 1961.
10. ALTMAN, A. and MURRAY, J. F. The anemia of malignant malnutrition (infantile pellagra, kwashiorkor): protein deficiency as a possible etiological factor. *South African J. M. Sc.*, 13: 91, 1948.
11. SHAHIDI, N. T., DIAMOND, L. H. and SHWACHMAN, H. Anemia associated with protein deficiency. *J. Pediat.*, 59: 533, 1961.
12. PEARSON, P. B., ELVEHJEM, C. A. and HART, E. B. The relation of protein to hemoglobin building. *J. Biol. Chem.*, 119: 749, 1937.
13. WHIPPLE, G. H. Hemoglobin and plasma proteins: their production, utilization, and interrelation. *Am. J. M. Sc.*, 203: 477, 1942.
14. METCOFF, J., FAVOUR, C. B. and STARE, F. J. Plasma protein and hemoglobin in the protein deficient rat. *J. Clin. Invest.*, 24: 82, 1945.
15. CARTWRIGHT, G. E. and WINTROBE, M. M. Studies of free erythrocyte protoporphyrin, plasma copper and plasma iron in protein deficient and iron deficient swine. *J. Biol. Chem.*, 176: 571, 1948.
16. BETHARD, W. F., WISSLER, R. W., THOMPSON, J. S., SHROEDER, M. A. and ROBSON, M. J. The effect of acute protein deprivation upon erythropoiesis in rats. *Blood*, 13: 216, 1958.
17. CARTWRIGHT, G. E. Dietary factors concerned in erythropoiesis. *Blood*, 2: 111, 1947.
18. SANCHEZ, L. Las alteraciones de la medula osea en el desnutrido de tercer grado. In: La Pediatria, p. 659. Mexico City, 1956. Hospital Infantil de la Ciudad de Mexico.
19. VELEZ, H., GHITIS, J., PRADILLA, A. and VITALE, J. J. Megaloblastic anemia in kwashiorkor. *Am. J. Clin. Nutrition*, 12: 54, 1962.
20. ADAMS, E. B. Anemia in kwashiorkor. *Brit. M. J.*, 1: 537, 1954.
21. TROWELL, H. C. and JELLIFFE, D. B. Diseases of children in the subtropics and tropics. London, 1958. Edward Arnold Ltd.
22. SHEEHY, T. W., RUBINI, M. E., PEREZ-SANTIAGO, E., HADDOCK, J. and DAPENA, R. B. The effect of "minute" amounts of folic acid on the megaloblastic anemia of tropical sprue. *Bol. Asoc. méd. Puerto Rico*, 53: 197, 1961.
23. ZALUSKY, R. and HERBERT, V. Megaloblastic anemia in scurvy with response to 50 micrograms of folic acid daily. *New England J. Med.*, 265: 1033, 1961.
24. GHITIS, J., LINARES, F., CANOSA, C., SINISTERRA, L. and VITALE, J. J. Hematological responses in kwashiorkor cases fed soy protein isolate. *Fed. Proc.*, 22: 550, 1963.
25. GHITIS, J., PIAZUELO, E. and VITALE, J. J. The erythroid atrophy of severe protein deficiency in monkeys. *Am. J. Clin. Nutrition*, 12: 452, 1963.