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Sialic Acid Content of Red Blood Cells from Protein-Calorie Malnourished Children and During Recovery, and from Normal Children and Adults[1]

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Sialic acids were measured in the red cells of two groups of subjects. One group consisted of 12 children with severe protein-calorie malnutrition (P.C.M.); six of them were followed longitudinally throughout the recovery period. The control group included 28 normal children and 11 normal adults. All subjects were studied hematologically and the sialic acid content of the red cells was determined in three layers of erythrocytes, separated according to their density by ultracentrifugation. The results indicate that there are no alterations in the content of sialic acids in the red cells of children with severe P.C.M. Furthermore, they show that the sialic acid content of the red cell is not influenced by various levels of red cell folates nor by differences in the concentration of serum proteins, serum iron, percentage saturation of transferrin, serum folates, or serum vitamin B_{12}.

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Chez deux groupes de sujets, nous avons mesuré le contenu en acides sialiques des globules rouges. Un groupe comprenait 12 enfants sujets à une malnutrition protéo-calorique (P.C.M.) grave; six d'entre eux ont été également observés durant le période de rétablissement. Le groupe témoin comprenait 28 enfants normaux et 11 adultes. Tous les sujets ont été soumis à des tests hématologiques. Nous avons déterminé le contenu en acides sialiques au niveau des trois couches de globules rouges telles qu'obtenues par ultracentrifugation. Les résultats indiquent que le contenu en acides sialiques des globules rouges n'est pas modifié chez l'enfant atteint de P.C.M. Ils indiquent également que le contenu des globules rouges en acides sialiques n'est pas modifié par le contenu globulaire en folates ni par le contenu sérique en proteines, fer folates et vitamine B_{12}, ni par le taux de saturation de la transférrine.

[Traduit par le journal]

Introduction

There is, to our knowledge, no information on the effect of nutrition on the sialic acid contents of the red blood cells, even though they are important components of membranes (Eylar *et al.* 1962; Cook *et al.* 1961) and of erythrocuprein (Kimmel *et al.* 1959), which partially determines the red cell life span (Markowitz *et al.* 1959).

In children suffering from kwashiorkor and marasmickwashiorkor, several authors (Shehata *et al.* 1965; Fayad *et al.* 1969; Patwardhan *et al.* 1971) have shown a marked increase in the total protein-bound hexose in serum. On the other hand, Patwardhan *et al.* (1971) observed altered concentrations of serum glycoproteins, a finding that the authors interpreted as the result of infection on protein-calorie deficiency.

In uncomplicated protein-calorie malnutrition (P.C.M.), changes such as low serum copper and ceruloplasmin (Lahey *et al.* 1958), a mild decrease in red cell survival (Lanzkowsky *et al.* 1967), and a reduced number of red cells (Viteri *et al.* 1968*a*, 1968*b*) are known to occur. Viteri and colleagues (1968*a*, 1968*b*) have suggested that this finding is the result of adaptation, which involves reduction of the total circulating hemoglobin as a consequence of diminution in active tissue mass. If hemolysis plays an important role in the hematological alterations observed in P.C.M., an increase in the sialic acid content of erythro-

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cytes in patients with severe P.C.M. would be expected since young red blood cells have a higher content of these acids, based on the results of Yachnin and Gardner (1961). On the other hand, if erythropoiesis were markedly impaired in severe P.C.M., the red blood cell content of these acids would be reduced, since older erythrocytes have a smaller content of sialic acids.

The concentration of sialic acids was measured in red blood cells of children with severe uncomplicated P.C.M. and during nutritional recovery in order to estimate the age composition of the circulating erythrocytes. Thereafter, relationships were investigated between the concentration of sialic acids in red blood cells and the serum and erythrocyte contents of important nutrients (proteins, iron, folates, and vitamin B_{12}) known to be deficient in cases of uncomplicated P.C.M., and during nutritional recovery on certain experimental diets (Viteri *et al.* 1964).

Materials and Methods

Two groups of subjects were studied: (1) Normal subjects, 11 well-nourished adults, and 28 children 43 to 96 months old; (2) malnourished subjects, 12 children with severe P.C.M. of the edematous type and without infectious complications, between 22 and 84 months of age, admitted to the pediatric section of the General Hospital of Guatemala and to the Clinical Center of the Institute of Nutrition of Central America and Panama (INCAP). Six of these patients were studied periodically during their nutritional recovery.

All children were treated following a standard protocol used at INCAP's Clinical Center (Alvarado *et al.* 1970): upon admission water and electrolyte imbalances were corrected while the children were maintained for approximately 4 days on an adaptation diet that provided 0.7 g of protein (casein + 0.2% methionine) and 70 cal/kg body weight per day. Twenty to thirty percent of the calories were given as vegetable fat. From then on, and depending on tolerance, the concentration of the diet was increased progressively to reach 3–4 g of protein and 120–150 cal/kg body weight per day. This level of intake was usually attained within 10 days of admission. Later on, casein was progressively substituted by milk. Throughout hospitalization a multivitamin and mineral supplement was administered. It included iron, folic acid, and vitamin B_{12}. Protein depletion and repletion were estimated by the creatinine–height index (Viteri and Alvarado 1970).

Red cells were obtained from heparinized venous blood, which was placed at 4 °C immediately after withdrawal. Packed red cell volume was measured by the method of McGovern *et al.* (1955). Blood samples were centrifuged at 2000 × *g* and 4 °C for 10 min. Plasma and the buffy coat containing leukocytes were removed by suction. The packed erythrocytes were transferred to polyallomer tubes and centrifuged at 67 000 × *g* and 4 °C for 2 h in a preparative ultracentrifuge (Spinco model L2-65), using a swinging bucket rotor (Type SW-39) and following the method of Rigas *et al.* (1961). The resulting packed erythrocyte column was arbitrarily divided into three equal layers by cutting the tubes to isolate erythrocytes of different densities. They were identified as fractions 1, 2, and 3 from top to bottom. Fraction 1 should contain most of the reticulocytes; fraction 2, mature red cells; and fraction 3, older red cells (Rigas *et al.* 1961; Borun *et al.* 1957). Red blood cell counts were routinely carried out on each layer using a Coulter counter model B (Coulter 1956). Reticulocyte counts were performed according to the method of Brecher and Schneiderman (1950) to check the success of blood cell fractionation. Chemical determination of sialic acids was performed on each of the three erythrocyte layers by the thiobarbituric acid method of Warren (1959), using a Beckman DB spectrophotometer. The absorption spectrum of the acid chromophore from each sample was always obtained in the wave length range of 480–600 (mμ) nm. A representative spectrum is illustrated in Fig. 1. The equation and constants proposed by Tishkoff (1966) were applied to calculate the sialic acid concentration. Sialic acid content was expressed as *N*-acetylneuraminic acid (NANA).

Two lots of crude neuraminidase-receptor-destroying enzyme from *Vibrio cholerae* filtrates were used.[2] In paired determinations ($n = 18$) enzyme lot No. 2 gave consistently lower values (81.6%) than enzyme lot No. 1. Therefore, values obtained with the enzyme lot No. 2 were corrected to express all results as if obtained with the enzyme lot No. 1. The purity of the *N*-glycolyl- and *N*-acetylneuraminic acids[3] used as reference standards was checked by descending paper chromatography, using as a solvent system a mixture of butanol, *n*-propanol, and 0.1 *N* HCl in the ratio of 1:2:1 (Mårtensson *et al.* 1958). All other chemicals used were reagent grade.

Plasma proteins were measured by refractometry, and hemoglobin content by the method of Crosby *et al.* (1954), while serum iron levels and total iron binding capacity were estimated by Ramsay's technique (Ramsay 1958). Serum folates were quantitatively determined using *Lactobacillus casei* as the assay microorganism (Herbert 1961). Estimation of serum B_{12} levels was made by the *Euglena gracillis* method of Anderson (1964).

Results

Data for P.C.M. children and for children fully recovered from P.C.M. are presented in

[2]Enzyme lot No. 1 was supplied by Sigma Chemical Company and enzyme lot No. 2 by Calbiochem.

[3]Supplied by Sigma Chemical Company.

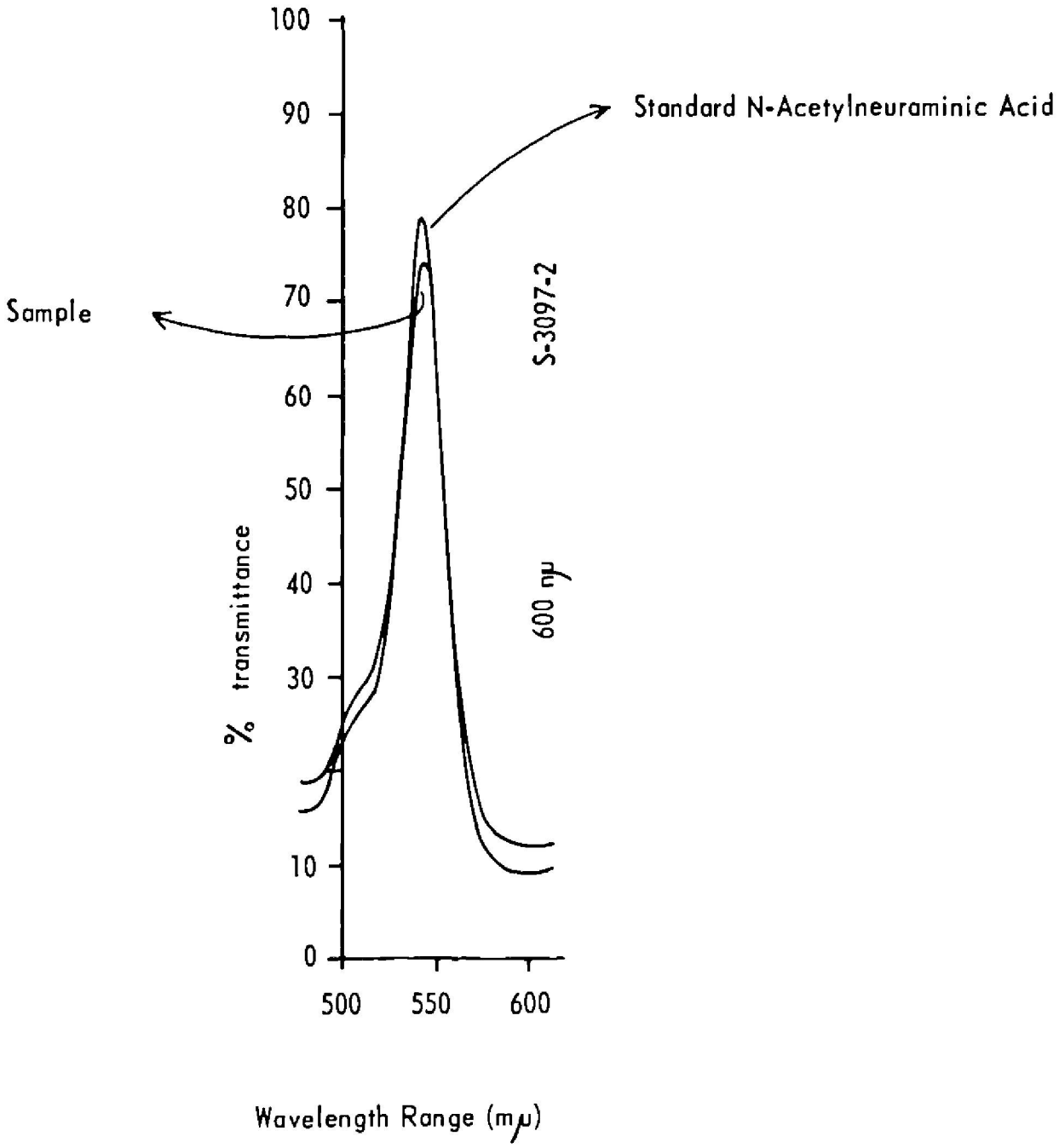

FIG. 1. Absorption spectrum of the thiobarbituric acid chromophore.

TABLE 1. Reticulocyte counts and sialic acids content of red blood cells from children with P.C.M. and fully recovered

	Red cell layers		
	1	2	3
Reticulocytes (% of total)	70.2 ± 3.3 ($n = 15$)	20.4 ± 2.2* ($n = 15$)	9.4 ± 1.7* ($n = 15$)
NANA (μg/10^{10} RBC)	146.0 ± 6.0 ($n = 16$)	124.0 ± 4.0* ($n = 16$)	117.0 ± 5.0* ($n = 14$)
NANA (μg/ml packed RBC)	161.0 ± 6.0 ($n = 16$)	145.0 ± 6.0 ($n = 16$)	145.0 ± 7.0 ($n = 14$)

*Significantly different ($p < 0.005$) from layer 1.
NOTE: The red cells are separated into three layers by centrifugation. Data are mean values ± S.E.

Table 1. This table shows the reticulocyte counts on each layer and confirms that, as expected, the reticulocyte content decreased from the top to the bottom layer. The levels of NANA also tended to diminish from top to bottom. When expressing the sialic acid content as concentration per 10^{10} red cells, a significant difference was observed between layer 1 and the other two layers. However, when NANA was expressed as micrograms per milliliter of packed red blood cells, no significant differences were observed between layers.

TABLE 2. Biochemical and hematological data and red blood cell sialic acid content in P.C.M. children, according to hospitalization period

Hospitalization period (days)	Serum proteins (g/100 ml)	Packed RBC volume (%)	Serum iron (μg/100 ml)	Saturation of TIBC* (%)	RBC folates (ng/ml)	Serum B_{12} (pg/ml)
0–3	4.4 ± 0.2 (n = 12)	32.0 ± 1.0 (n = 12)	51.0 ± 4.0 (n = 12)	52.0 ± 7.0 (n = 12)	169.0 ± 22.0 (n = 11)	442.0 ± 85.0 (n = 12)
4–20	6.6 ± 0.4 (n = 10)	29.0 ± 1.0 (n = 10)	186.0 ± 74.0 (n = 10)	46.0 ± 8.0 (n = 10)	136.0 ± 12.0 (n = 8)	395.0 ± 55.0 (n = 9)
21–40	7.5 ± 0.3 (n = 10)	30.0 ± 0.8 (n = 10)	87.0 ± 31.0 (n = 10)	25.0 ± 7.0 (n = 10)	150.0 ± 15.0 (n = 10)	334.0 ± 57.0 (n = 10)
41+	7.6 ± 0.1 (n = 16)	34.0 ± 0.6 (n = 16)	55.0 ± 7.5 (n = 10)	15.0 ± 2.0 (n = 10)	178.0 ± 26.0 (n = 10)	302.0 ± 34.0 (n = 7)

	RBC sialic acid content in red cell layer (NANA μg/ml packed RBC)			RBC sialic acid content in red cell layer (NANA μg/10^{10} RBC)		
	1	2	3	1	2	3
0–3	140.0 ± 7.0 (n = 11)	144.0 ± 8.0 (n = 12)	147.0 ± 10.0 (n = 12)	143.0 ± 10.0 (n = 5)	119.0 ± 8.0 (n = 6)	118.0 ± 10.0 (n = 6)
4–20	146.0 ± 10.0 (n = 10)	119.0 ± 12.0 (n = 9)	138.0 ± 9.0 (n = 9)	156.0 ± 20.0 (n = 4)	123.0 ± 16.0 (n = 3)	101.0 ± 10.0 (n = 2)
21–40	176.0 ± 30.0 (n = 10)	155.0 ± 9.0 (n = 10)	150.0 ± 8.0 (n = 9)	161.0 ± 0 (n = 1)	130.0 ± 0 (n = 1)	— —
41+	149.0 ± 8.0 (n = 16)	144.0 ± 9.0 (n = 16)	147.0 ± 8.0 (n = 16)	139.0 ± 5.0 (n = 6)	129.0 ± 5.0 (n = 6)	121.0 ± 6.0 (n = 6)

*TIBS is the total iron binding capacity.
NOTE: Data are mean values ± S.E.

TABLE 3. Total sialic acids of the three red blood cell layers in the four groups of subjects studied

Group	Packed cell volume (%)	Sialic acid content expressed as NANA μg/ml packed RBC in red cell layers		
		1	2	3
P.C.M. children	32.0 ± 1.0 (n = 12)	140.0 ± 7.0 (n = 11)	141.0 ± 8.0 (n = 12)	147.0 ± 10.0 (n = 12)
Children fully recovered from P.C.M.	34.0 ± 0.6 (n = 16)	149.0 ± 8.0 (n = 16)	144.0 ± 9.0 (n = 16)	147.0 ± 8.0 (n = 16)
Control children	37.0 ± 0.6 (n = 29)	148.3 ± 6.0 (n = 28)	146.0 ± 6.0 (n = 28)	143.0 ± 5.0 (n = 28)
Control adults	48.0 ± 1.0 (n = 16)	155.0 ± 3.0 (n = 15)	157.0 ± 4.0 (n = 15)	159.0 ± 4.0 (n = 16)

NOTE: Data are mean values ± S.E.

Table 2 shows the results obtained in the malnourished children, grouped by days of hospitalization in four categories: acute phase (0–3 days), early recovery stage (4–20 days), late recovery (21–40 days), and full recovery (41+ days). Differences in sialic acid content between layers generally persist when expressed by micrograms per 10^{10} red cells, although no differences were detected between the various stages of recovery. Furthermore, the NANA values obtained from severely malnourished children, fully recovered children, and normal children and adults (controls) are similar (Table 3). The values in the blood from normal adults tend to be higher in the three red cell layers.

An attempt was also made to correlate the sialic acid content of red blood cells from P.C.M. and recovered children with some of the biochemical and hematological parameters in-

vestigated. There exists no relationship between the sialic acid content and any other nutritional or hematological variable measured, either by groups of children or individually. These included packed cell volume, hemoglobin concentration, serum iron, percentage saturation of transferrin, red cell folates, and serum vitamin B_{12}. Tables 4 and 5 are presented as illustrations of this fact. Red cell folate level was chosen, in this instance, as the independent variable; based on the distribution of these levels the children were grouped in quartiles.

Discussion

The results obtained provide evidence that in contrast to alterations in serum glycoproteins, red cell sialic acid content remains normal in P.C.M. Findings in the three red blood cell (RBC) layers also indicate that old cells contain less sialic acid than young cells. This suggests that even though during the process of malnutrition bone marrow production may have decreased or even stopped (Viteri *et al.* 1968*a*; Kho and Tumbelaka 1960; Ghitis *et al.* 1963; Adams *et al.* 1967), when the malnourished children were studied their red cell production was probably keeping up with its demands, mostly determined by the children's active tissue mass. This interpretation is in agreement with the blood reticulocyte content of P.C.M. children (Viteri *et al.* 1968*a*).

The fact that the red cell content of sialic acid does not appear altered in severe uncomplicated P.C.M. also agrees with our unpublished observations (Viteri, F. E., and Alvarado, J.: unpublished observations) that the red cell half-life is essentially normal in children with severe protein-calorie malnutrition and without infectious complications. Also it may be concluded that protein, iron, folate, and vitamin B_{12} deficiencies diagnosed on the bases of serum and/or red cell contents of these nutrients do not influence the total sialic acid content of red cells.

TABLE 4. Hematological and nutritional biochemical data of children with P.C.M. and during recovery. Results are grouped based on red cell folates

		Group No.			
		1	2	3	4
RBC folates (ng/ml)	$\bar{X}$	74.0	127.0	168.0	250.0
	S.E.	9.0	4.0	3.0	15.0
	(*n*)	6	12	12	9
Packed RBC volume (%)	$\bar{X}$	30.0	31.0	31.0	33.0
	S.E.	2.0	1.0	1.0	0.8
	(*n*)	6	12	12	18
MCV (μm^3)	$\bar{X}$	86.0	82.0	84.0	84.0
	S.E.	2.0	2.0	3.0	2.0
	(*n*)	5	11	10	13
MCHC (g/100 ml RBC)	$\bar{X}$	31.0	32.0	32.0	32.0
	S.E.	0.8	0.6	0.7	0.4
	(*n*)	5	11	10	13
Serum iron (µg/100 ml)	$\bar{X}$	77.0	92.0	123.0	72.0
	S.E.	27.0	26.0	65.0	11.0
	(*n*)	6	12	12	12
Saturation of TIBC (%)	$\bar{X}$	36.0	33.0	30.0	43.0
	S.E.	13.0	6.0	8.0	8.0
	(*n*)	6	12	12	12
Serum vitamin B_{12} (pg/ml)	$\bar{X}$	251.0	488.0	305.0	420.0
	S.E.	82.0	70.0	35.0	76.0
	(*n*)	6	11	12	9

NOTE: MCV is the mean corpuscular volume and MCHC the mean corpuscular hemoglobin concentration.

TABLE 5. Sialic acid content of the three red blood cell layers in blood obtained from P.C.M. children and during recovery. Results are grouped based on red blood cell folates

Group No.		RBC folates ng/ml	Sialic acid (NANA µg/ml RBC) in red cell layers		
			1	2	3
1	$\bar{X}$	74.0	142.0	147.0	143.0
	S.E.	9.0	10.0	9.0	7.0
	(*n*)	6	6	6	6
2	$\bar{X}$	127.0	159.0	136.0	150.0
	S.E.	4.0	22.0	15.0	12.0
	(*n*)	12	12	11	11
3	$\bar{X}$	168.0	150.0	137.0	145.0
	S.E.	3.0	16.0	9.0	8.0
	(*n*)	12	11	12	11
4	$\bar{X}$	250.0	151.0	144.0	145.0
	S.E.	15.0	7.0	6.0	7.0
	(*n*)	9	18	18	18
			Sialic acid (NANA µg/10^{10} RBC) in red cell layers		
			1	2	3
1	$\bar{X}$	74.0	127.0	128.0	112.0
	S.E.	9.0	0	9.0	4.0
	(*n*)	6	2	2	2
2	$\bar{X}$	127.0	172.0	123.0	128.0
	S.E.	4.0	0	0	0
	(*n*)	12	1	1	1
3	$\bar{X}$	168.0	143.0	121.0	103.0
	S.E.	3.0	7.0	6.0	17.0
	(*n*)	12	4	4	3
4	$\bar{X}$	250.0	149.0	125.0	122.0
	S.E.	15.0	10.0	7.0	6.0
	(*n*)	9	9	9	8

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ADAMS, E. B., SCRAGG, J. N., NAIDOO, B. T., LILJESTRAND, S. K., and COCKRAM, V. I. 1967. Observations on the aetiology and treatment of anaemia in kwashiorkor. Br. Med. J. **3**, 451–454.

ALVARADO, J., VITERI, F., and BEHAR, M. 1970. Tratamiento hospitalario de la desnutrición proteínico-calórica severa. Rev. Col. Med. (Guatemala), **21**, 231–245.

ANDERSON, B. B. 1964. Investigations into the Euglena method for the assay of the vitamin B_{12} in serum. J. Clin. Pathol. **17**, 14–26.

BORUN, E. R., FIGUEROA, W. G., and PERRY, S. M. 1957. The distribution of Fe^{59} tagged human erythrocytes in centrifuged specimens as a function of cell age. J. Clin. Invest. **36**, 676–679.

BRECHER, G., and SCHNEIDERMAN, M. 1950. A time-saving device for the counting of reticulocytes. Am. J. Clin. Pathol. **20**, 1079–1083.

COOK, G. M. W., HEARD, D. H., and SEAMAN, G. V. F. 1961. Sialic acids and the electrokinetic charge of the human erythrocyte. Nature, **191**, 44–47.

COULTER, W. 1956. Proc. Natl. Electron. Conf. Hialeah, Florida.

CROSBY, W. H., MUNN, J. I., and FURTH, F. W. 1954. U.S. Armed Forces Med. J. **5**, 693.

EYLAR, E. H., MADOFF, M. A., BRODY, O. V., and ONCLEY, J. L. 1962. The contribution of sialic acid to the surface charge of the erythrocyte. J. Biol. Chem. **237**, 1992–2000.

FAYAD, I. M., METWALLI, O. M., SHUKRY, A. S., and ISMAIL, S. M. 1969. Serum mucoproteins in protein-calorie deficiency. Gaz. Egypt. Pediatr. Assoc. **17**, 199.

GHITIS, J., VELEZ, H., LINARES, F., SINISTERRA, L., and VITALE, J. J. 1963. Cali-Harvard nutrition project. II. The erythroid atrophy of kwashiorkor and marasmus. Am. J. Clin. Nutr. **12**, 445–451.

HERBERT, V., FISHER, R., and KOONTZ, B. J. 1961. The assay and nature of folic acid activity in human serum. J. Clin. Invest. **40**, 81–91.

KHO, L. K., and TUMBELAKA, W. A. 1960. The pathogenesis of anemia in kwashiorkor. Ann. Paediatr. **194**, 257–272.

KIMMEL, J. R., MARKOWITZ, H., and BROWN, D. M. 1959. Some chemical and physical properties of erythrocuprein. J. Biol. Chem. **234**, 46–50.

LAHEY, M. E., BEHAR, M., VITERI, F., and SCRIMSHAW, N. S. 1958. Values for copper, iron and iron-binding capacity in the serum in kwashiorkor. Pediatrics, **22**, 72–79.

LANZKOWSKY, P., MCKENZIE, D., KATZ, S., HOFFENBERG, R., FRIEDMAN, R., and BLACK, E. 1967. Erythrocyte abnormality induced by protein malnutrition. II. 51-Chromium labeled erythrocyte studies. Br. J. Haematol. **13**, 639–649.

MARKOWITZ, H., CARTWRIGHT, G. E., and WINTROBE, M. M. 1959. Studies on copper metabolism. XXVII. The isolation and properties of an erythrocyte cuproprotein (erythrocuprein). J. Biol. Chem. **234**, 40–45.

MÅRTENSSON, E., RAAL, A., and SVENNERHOLM, L. 1958. Sialic acids in blood serum. Biochim. Biophys. Acta, **30**, 124–129.

MCGOVERN, J. J., RICHARDSON JONES, A., and STEINBERG, A. G. 1955. The hematocrit of capillary blood. New Engl. J. Med. **253**, 308–312.

PATWARDHAN, V. N., MAGHRABI, R. H., MOUSA, W., GABR, M. K., and EL MARAGHY, S. 1971. Serum glycoproteins in protein-calorie deficiency disease. Am. J. Clin. Nutr. **24**, 906–912.

RAMSAY, W. N. M. 1958. Plasma iron. *In* Advances in clinical chemistry. Vol. 1. *Edited by* H. Sobotka and C. P. Stewart. Academic Press Inc., New York. pp. 1–39.

RIGAS, D. A., KOLER, R. D., and SWISHER, K. 1961. Ultracentrifugal fractionation of human erythrocytes on the basis of cell age. J. Lab. Clin. Med. **58**, 242–246.

SHEHATA, A. H., ABDEL HAY, A., KAMEL, G., FAYAD, I., and TALAAT, M. 1965. Biochemical studies in kwashiorkor. IV. Protein-bound polysaccharides. J. Endocrinol. Metab. (Egypt), **11**, 33.

TISHKOFF, G. H. 1966. Erythrocyte mucoids in acquired autoimmune hemolytic anemia. Blood, **28**, 229–240.

VITERI, F. E., and ALVARADO, J. 1970. The creatinine–height index: its use in the estimation of the degree of protein depletion and repletion in protein calorie malnourished children. Pediatrics, **46**, 696–706.

VITERI, F. E., ALVARADO, J., LUTHRINGER, D. G., and WOOD, R. P., II. 1968*a*. Hematological changes in protein-calorie malnutrition. Vitam. Horm. **26**, 573–615.

——— 1968*b*. Adaptation of the erythropoietic system to protein calorie deficiency. Fed. Proc. **27**, 485 (Abstr. 1509).

VITERI, F., BEHAR, M., ARROYAVE, G., and SCRIMSHAW, N. S. 1964. Clinical aspects of protein malnutrition. *In* Mammalian protein metabolism. Vol. II. *Edited by* H. N. Munro and J. B. Allison. Academic Press Inc., New York and London. p. 523.

WARREN, L. 1959. The thiobarbituric acid assay of sialic acids. J. Biol. Chem. **234**, 1971–1975.

YACHNIN, S., and GARDNER, F. H. 1961. Measurement of human erythrocyte neuraminic acid: relationship to haemolysis and red blood cell virus interaction. Br. J. Haematol. **7**, 464–475.