

Some Studies of Tocopherol in Infants and Children

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IT IS our purpose to summarize data on the hemolysis of erythrocytes in hydrogen peroxide and on plasma tocopherol levels of infants and children. Studies were begun at the Colorado General Hospital during the last months of 1951 and have been continued at the Sinai and Johns Hopkins Hospitals in Baltimore for the past four years.

Our interest dates from the suggestion by the Owens, in 1949,¹ that defective absorption of fat by premature infants and the use of partially skimmed cow's milk mixtures²⁻⁴ might lead to a deficiency of vitamin E. The reports by György and Rose^{5,6} that hemolysis of erythrocytes in dialuric acid could be used as a measure of tocopherol deficiency in rats led to studies of dialuric acid hemolysis of erythrocytes of premature infants, with negative results.⁷ Because low plasma tocopherol levels were being reported^{1,8-10} for newborn full-term and premature infants, because the Owens' original suggestion still seemed reasonable, and because the variations in manifestations of tocopherol deficiency in different species had been stressed by Mason,¹¹ we did not believe the negative results with dialuric acid ruled out tocopherol deficiency. After György and his co-workers¹² reported that hemolysis in hydrogen peroxide could also be used as a test of tocopherol deficiency,

we began our studies before publication of the method. With only slight modification, the test has proved technically satisfactory in our laboratories during the past four years.

Our first studies, made in a small group of premature infants fed partially skimmed or evaporated whole milk mixtures and in several adults, indicated that the red cells of the adults showed less than 10 per cent hemolysis, while for the majority of the premature infants, hemolysis was over 50 per cent.¹³ The administration of tocopherol acetate was uniformly effective in reversing susceptibility to peroxide. Because it was not clear whether this evidence of tocopherol deficiency was a reflection of prematurity or of diet, studies were extended to other subjects: (1) newborn infants, full-term or premature, who had received no feedings or only glucose-water; (2) thriving young full-term infants who were studied in the well-baby clinic at an average age of seven weeks; (3) thriving young premature infants, less than three months of age, while still in the nurseries; (4) infants and children with steatorrhea, e.g. cystic fibrosis of the pancreas, biliary atresia, etc.

In addition, studies have been made in a group of mothers during the first post-partum day, and in healthy adult hospital personnel. Most of the data have been presented in detail elsewhere.^{14,15}

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HEMOLYSIS OF ERYTHROCYTES IN HYDROGEN PEROXIDE

Healthy Adults, Post-partum Mothers, and Unfed Newborn Infants

A summary of the results of hemolysis tests in 60 normal adults, 71 post-partum mothers, and 342 unfed newborn infants are presented in Table I. Virtually all adults showed hemolysis of less than 10 per cent, whereas only

TABLE I

Hemolysis in Hydrogen Peroxide—Summary of Results for Adults and Unfed Newborn Infants

Subjects	No.	Hemolysis	
		Mean and stand. error	Observations less than 10% hemolysis
		% ^a	%
Healthy hospital personnel ^b	60	2 ± 0.3	100
Post-partum mothers ^b			
Private white	23	2 ± 0.4	100
Service white	21	3 ± 0.6	100
Service Negro	27	7 ± 3.0	89
Newborn infants—unfed			
Full-term			
Private white	81	34 ± 3.2	26
Service white	133	43 ± 2.4	14
Service Negro	68	49 ± 4.0	16
Premature			
White ^c	42	35 ± 4.3	21
Negro	25	53 ± 6.1	8

^a Per cent hemolysis is ratio of hemoglobin liberated from aliquots of red blood cells incubated in hydrogen peroxide and diluted with a buffer to that liberated by dilution in distilled water.

^b No dietary surveys were made for any of these subjects.

^c Lack of precise information prevented division of these infants according to economic status.

from 8 to 26 per cent of unfed newborn infants showed this resistance to incubation in hydrogen peroxide. No differences in mean hemolysis were found between full-term and premature infants in either the white or Negro groups. Significant differences were found, however, between the means for private white and service Negro full-term infants (difference ± S. E. diff. 15 per cent ± 5.16; $P < 0.01$) for private white and service white full-term infants (9 per cent ± 4.04; $0.01 < P < 0.05$) and for white and Negro premature infants (18 per cent ± 7.61; $P < 0.02$).

Thriving Full-term and Premature Infants Less Than Three Months of Age

In Table II is presented a summary of the results in thriving young full-term and premature infants. It is seen that at average age, seven weeks, both white and Negro full-term infants, who had been fed ordinary evaporated milk mixtures, showed significant decreases in

TABLE II

Hemolysis in Hydrogen Peroxide of Erythrocytes of Thriving Infants Less Than 3 Months of Age

Subjects	No.	Hemolysis	
		Mean and stand. error	Observations Less than 10% hemolysis
		%	%
Full-term			
White			
Newborn—unfed	133	43 ± 2.4	14
7 weeks—cow's milk ^a	29	21 ± 4.1	55
7 weeks—breast milk ^b	7	4	100
Negro			
Newborn—unfed	68	49 ± 4.0	16
7 weeks—cow's milk ^a	25	31 ± 4.3	32
7 weeks—breast milk ^b	11	11	73
Premature			
White			
Newborn—unfed	42	35 ± 4.3	21
<30 days—cow's milk ^c	43	59 ± 4.9	9
30 days and over ^c	41	69 ± 4.5	5
Negro			
Newborn—unfed	25	53 ± 6.1	8
30 days—cow's milk ^c	31	62 ± 5.2	0
30 days and over ^c	22	53 ± 7.4	14

^a The cow's milk mixtures consisted of ordinary dilutions of evaporated milk with added carbohydrate, estimated to contain 0.10 mg per 100 ml.⁸

^b Human milk has been reported to contain 0.24 mg per 100 ml.¹⁷

^c Although some of the infants were receiving unskimmed cow's milk mixtures at the time of testing, partially skimmed cow's milk mixtures, estimated to contain 0.04 mg per 100 ml,⁸ had been fed during most of the time before the test.

mean hemolysis from 43 to 21 per cent and from 49 to 31 per cent, respectively (differences: 22 per cent ± 4.76; $P < 0.001$, 18 per cent ± 6.23; $P < 0.01$). The erythrocytes of breast-fed infants showed even less hemolysis; in all seven white and eight of eleven Negro infants hemolysis was less than 10 per cent, the level ordinarily found in adults. The spontaneous decrease in hemolysis on these diets which were supplemented only with vitamins A, D, and C is analogous to the spontaneous repair

H₂O₂ HEMOLYSIS OF RBC OF HOSPITALIZED FULL TERM INFANTS ON LOW FAT DIETS

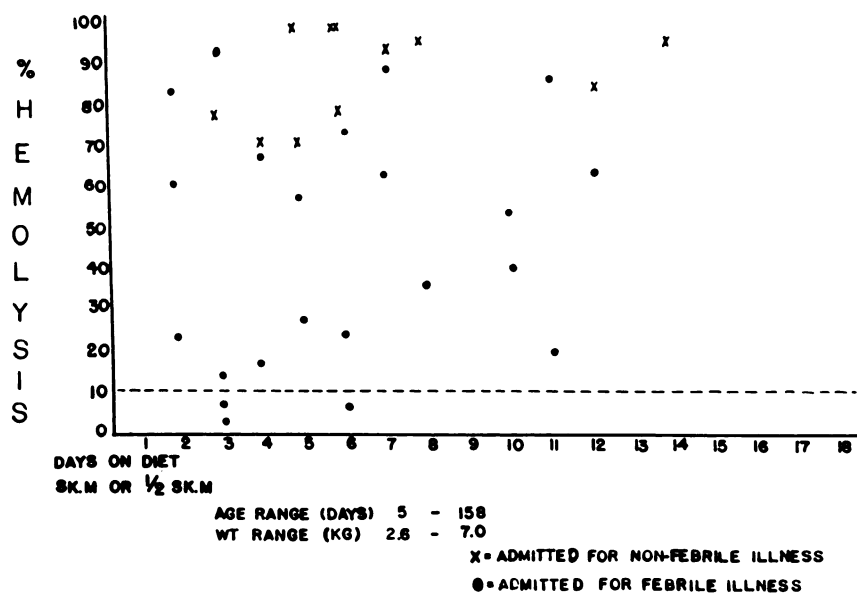


Figure 1

of neonatal hypoprothrombinemia without administration of vitamin K.

That young full-term infants fed cow's milk may have what Mason¹⁶ has called a "precarious tocopherol status" is indicated by the data in Figure 1. Thirty-four observations were made on 17 young infants (age 5 to 15 days) admitted to the hospital because of febrile or non-febrile illnesses. The infants had all received unskimmed cow's milk before admission and completely or partially skimmed cow's milk mixtures after admission. Initial observations made from 2 to 14 days after beginning the low fat diets showed over 50 per cent hemolysis in 12 of 17 infants. One infant showed a rise in hemolysis from 1 per cent on the third day of the diet to 59 per cent on the seventh day; another showed virtually the same lack of hemolysis (2 and 12 per cent) on the eleventh as on the third day.

Erythrocytes of prematurely born infants fed partially skimmed cow's milk, which contains less tocopherol than either the evaporated cow's milk mixtures or breast milk^{8,17} continued to show significant degrees of hemolysis. In view of their known defect in fat

absorption, and the lower content of tocopherol in stored than in fresh human milk,¹⁷ supplementation of their diets may be indicated even if one uses human milk in their feeding. We have been reluctant to recommend supplementation while we continue to search for some clinical, physiologic, or biochemical correlations of the deficiency.

Because the presence of positive hemolysis tests was related to low tocopherol intakes, infants and children with steatorrhea were studied. As has been previously reported,¹⁸ these subjects also showed markedly positive tests. Thirty-eight observations have now been made in malnourished subjects with steatorrhea and 21 in subjects of comparable age, i.e., up to 15 years, with malnutrition due to other causes. In some of the children with steatorrhea a decreased fat intake had been prescribed, but this prescription was not being followed. Thirty observations, 79 per cent, showed over 50 per cent hemolysis, and four of the eight tests below 50 per cent were in patients receiving pancreatin. In the patients with malnutrition not associated with steatorrhea, only two observations showed hemolysis

TABLE III
Plasma Tocopherol Levels—Summary of Results

Subjects	Diet	No.	Plasma Tocopherol	
			Mean	Stand-Error
<i>mg/100 ml</i>				
Adults				
Healthy hospital personnel	^a	15	0.75	± 0.040
Post-partum mothers				
Private white	^a	23	1.56	± 0.080
Service white	^a	22	1.33	± 0.052
Service Negro	^a	28	1.07	± 0.060
Infants				
Newborn				
Term				
Unfed				
Private white	"	35	0.22	± 0.003
Service white	"	59	0.23	± 0.015
Service Negro	"	28	0.26	± 0.023
Total		122	0.23	± 0.010
Premature	"	14 ^b		0.26 ± 0.042
Young Infants				
Full-term				
Ave. age 46 days	Cow's milk	55	0.33	± 0.022
Ave. age 46 days	Breast milk	14	0.72	± 0.071
Premature				
11-30 days	Partially	17		0.20 ± 0.018
31 days and over	skimmed cow's milk	11		0.13 ± 0.024
Infants and children with steatorrhea ^c		31		0.18 ± 0.028

^a No dietary surveys were made for any of these subjects.

^b Nine were white and five were Negro.

^c Subjects had proved cystic fibrosis of pancreas or biliary atresia.

over 50 per cent; these were in an eczematous infant whose mother had eliminated everything but a cereal-water mixture from his diet.

PLASMA TOCOPHEROL LEVELS

A summary of the results of 15 determinations in healthy hospital personnel, 73 in post-partum mothers, 136 in unfed newborn infants, 69 in thriving full-term infants, 28 in thriving premature infants, and 31 in infants and children with steatorrhea, are presented in Table III. The mean values for the healthy adults and for the post-partum mothers are in accord with previous observations¹⁹⁻²¹. Significant differences were found between mean values for private white and service white mothers (differences ± S. E. diff. = 0.23 mg ± 0.096; $P < 0.01$) and for service white and service Negro women (0.26 mg ± 0.086; $P < 0.01$). No differences were found in the groups of unfed newborn infants, although such

differences might have been expected from the hemolysis tests. No differences were found between full-term and premature infants, the levels for both groups being much lower than in mothers, in accord with previous findings^{20,22,23} which suggest a relative impermeability of the placenta to tocopherol.

In the thriving full-term infants at average age of 46 days, the mean tocopherol levels had risen to 0.33 mg per 100 ml for infants fed whole cow's milk mixtures (difference = 0.10 ± 0.024; $P < 0.01$) and to 0.72 mg per 100 ml for infants fed at the breast. On the other hand, the premature infants fed partially skimmed cow's milk showed significant ($P < 0.01$) decreases in plasma tocopherol concentration with continuation of low tocopherol intakes. These results were to be expected from the findings on the hemolysis tests, from the reports of tocopherol concentrations in various milks, and from previous reports on tocopherol

values in both full-term and premature infants.

Thirty-one observations of plasma tocopherol were made in infants and children with steatorrhea. The mean level was only 0.18 mg per 100 ml, and all but two determinations were below 0.4 mg per 100 ml. These results confirm those of Filer²⁴ and Darby²⁵ and their co-workers.

CORRELATION OF HEMOLYSIS AND TOCOPHEROL CONCENTRATION

Since the hemolysis test has proved to be a simply performed index of deficiency of either tocopherol intake or absorption, an analysis has been made of observations in which simultaneous measurements of plasma tocopherol and erythrocyte hemolysis had been performed.

In 125 measurements in which plasma tocopherol was above 0.5 mg per 100 ml, hemolysis was below 10 per cent in 119, or 95 per cent. This relatively uniform lack of hemolysis of erythrocytes in hydrogen peroxide when the plasma tocopherol level is above 0.5 mg per 100 ml is in accord with *in vitro* studies,^{26,27} as well as with the findings of MacKenzie in premature infants fed supplements of alpha-tocopherol acetate.²⁸ A summary of the correlations found when plasma tocopherol was below 0.5 mg per 100 ml is presented in Table IV.

It is seen that significant inverse correlations of from 0.5 to 0.7 were obtained for all but the unfed newborn infants. In these the plasma tocopherol concentrations were almost always low, but hemolysis occurred less frequently than in older infants and children with similar levels. This finding is a little ironic, since the first demonstration of positive hemolysis tests was in newborn infants;¹² its explanation is not clear. The data suggest that other factors than plasma tocopherol concentration may play a role in the hemolysis of erythrocytes in hydrogen peroxide, and these will be discussed in the next paper.²⁷

COMMENT

The studies of hemolysis and plasma tocopherol indicate, however, that newly born full-term and premature infants have a

TABLE IV

Correlation of Simultaneous Measurements of Hydrogen Peroxide Hemolysis and Plasma Tocopherol*

Subjects	Number of Observations	r	p
Patients with steatorrhea	63	-0.67	<0.001
Thriving full-term infants	69	-0.49	<0.001
Thriving premature infants	14	-0.71	<0.01
Unfed newborn infants	122	-0.03	>0.1

* Below 0.5 mg per 100 ml.

r = coefficient of correlation.

deficiency of tocopherol. In the former, it is remedied quickly by breast feeding and less consistently by feeding whole cow's milk mixtures, but in the latter it persists on customary feeding mixtures of partially skimmed cow's milk. Because of their defect in fat absorption and the limited tocopherol content of both whole cow's milk mixtures and stored human milk, supplementation of the diets of premature infants may be necessary to repair the deficiency. Using the average concentration of 0.24 mg per 100 ml of human milk reported by Harris, Quaife, and O'Grady¹⁷ as a guide, one might estimate daily supplementation at 0.5 mg/kg. We are, however, reluctant to make such a recommendation while we search for correlates of the tocopherol deficiency both in these infants, and in the infants and children with steatorrhea who have prolonged deficiency.

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