Iron, vitamin E, and folate in the preterm infant

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Increasing numbers of small prematurely born infants are surviving as a result of intensified perinatal care. Soon after birth, they require a special approach to nutrition that takes into consideration the early cessation of transplacental supply of nutrients, certain underlying metabolic handicaps, and the potential for an unusually rapid rate of growth. The small premature infant differs from the term infant in that his nutritional requirements may not be completely met by breast milk (supplemented with vitamins A, C, and D) or by proprietary infant formulas. In order to meet some of these requirements, it has become the practice to give additional vitamins and/or minerals in the form of separate supplements, as new evidence of deficiency is discovered. Naturally, those deficiency states that are most familiar and easily diagnosed are the first to be corrected. For example, severe rickets was found to develop on a nutritional basis in small infants even though they were fed vitamin D-supplemented formula. There is increasing recognition of the need to detect and prevent not only such advanced malnutrition but also the milder and more subtle forms of malnutrition to be described here.

This discussion will focus primarily on iron, vitamin E, and folate requirements in premature infants with a birth weight below 2,000 gm. Each of the three nutrients must be supplied as a supplement, but optimal regimens in respect to dose and timing are still being developed. The deficiency states of iron, vitamin E, and folate also have in common the relative ease with which they are detected, since they produce changes that can be readily measured in the peripheral blood. Consequently, it is possible to evaluate various regimens of supplementation by laboratory means. Before discussing each of the nutrients individually, it is worth reviewing some general considerations that apply to all three.

GENERAL CONSIDERATIONS

Nutritional requirements and rate of growth. Nutritional recommendations for infants are designed to take into account their dietary requirements for growth. Thus, the recommended daily allowance for some nutrients is almost as high in the term infant as in the adult despite the enormous difference in body weight. The recommendations take into account the link between growth rate and caloric intake by also listing the allowances per 100 kcal in the diet. This is the basis for a supplementary table of RDA of the Nutrition Board of the National Research Council for 1968. Allotments for term infants are most logically made on the basis of caloric intake because most nutrients are adequately supplied in the form of breast milk. Thus the rapid changes in nutrient requirement during the first few months of life are met by changes in milk consumption. The values for term infants and the use of caloric intake as a reference may not apply to premature infants. Table I lists the requirements in premature infants for iron, vitamin E, and folate based on this review. These are compared with the recommendations of the Committee on Nutrition and the RDA for term infants. The amounts of these three nutrients provided from breast milk, cow's milk, and proprietary formulas are also listed. The requirements for vitamin E and folate are disproportionately large in preterm infants and are

Abbreviations used

RDA: recommended daily allowance
TPGS: a-tocopherol polyethylene glycol-1000-succinate

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Table I. Suggested iron, vitamin E, and folate intake per 100 kcal for infants compared with that in milk and proprietary formulas

<table>
<thead>
<tr>
<th>Intake recommended:</th>
<th>Iron (mg/100 kcal)</th>
<th>Vit. E (IU/100 kcal)</th>
<th>Folate (µg/100 kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Based on this review for a hypothetical 1,500 gm infant with a daily intake of 200 kcal</td>
<td>2.0*</td>
<td>2.5-12‡</td>
<td>25-35‡</td>
</tr>
<tr>
<td>By Committee on Nutrition—1967 (iron for premature infants; minimal requirement of vitamin E and folate for term infants)</td>
<td>2.0</td>
<td>0.3</td>
<td>4</td>
</tr>
<tr>
<td>Recommended Daily Allowances—1968 (for term infants 0-1 year)</td>
<td>1.2-1.7</td>
<td>1.0</td>
<td>10</td>
</tr>
<tr>
<td>Content in:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast milk</td>
<td>0.2</td>
<td>0.2</td>
<td>8</td>
</tr>
<tr>
<td>Cow's milk</td>
<td>0.2</td>
<td>0.1</td>
<td>8</td>
</tr>
<tr>
<td>Proprietary milk formulas Without added iron</td>
<td>0.2</td>
<td>0.7-1.3</td>
<td>7-15</td>
</tr>
<tr>
<td>With added iron</td>
<td>1.8-2.0</td>
<td>1.2-1.4</td>
<td>6-12</td>
</tr>
</tbody>
</table>

*aOptional before 3 months of age.
‡Vitamin E and folate requirements are disproportionately large/kcal in low-weight infants and are most conveniently prescribed at doses of 5-25 IU/day of vitamin E and 50-70 µg/day of folate, irrespective of weight or caloric intake.

Not related to caloric intake. These are therefore also listed on the basis of total dose per day and are most conveniently administered in this manner.

Bioavailability. The absorption and utilization of essential nutrients is at least as important as the quantity ingested. This seemingly simple principle is only recently receiving proper emphasis. In the case of vitamin E, bioavailability is quite variable, and even the administration of more than ten times the recommended daily allowance of vitamin E, as α-tocopherol acetate, does not reliably overcome malabsorption in the small premature infant and result in a normal concentration of tocopherol in serum. In contrast, intestinal absorption of iron (ferrous sulfate) and folate in the preterm infant seems to be as effective as in the older child or adult.

Of particular importance after the perinatal period is the poor bioavailability of iron in the form of sodium iron pyrophosphate, ferric orthophosphate, and reduced iron of large particle size. Iron in these poorly assimilated forms has been used to supplement infant cereal and flour products, a situation that is in the process of being corrected by the food industry. Since this topic applies primarily to older infants, children, and adults, it will not be discussed in greater detail here.

Avoiding nutrient excess. Many nutrients, formerly thought to be innocuous in large amounts, are now recognized as being actually or theoretically harmful. The most familiar examples are those of vitamins A and D. Examples that relate to the nutrients which are the subject of this discussion are less well known. Perhaps the most important is iron excess as a cause of hemolytic anemia in vitamin E-deficient preterm infants. Vitamin E excess has not convincingly been proved to be harmful in man; however, evidence in animals of a decrease in the rate of wound healing suggests caution against large doses. Doses of folate larger than 100 µg/day are not warranted to meet any nutritional need and, in the rare case of occult vitamin B12 deficiency, can aggravate neurologic manifestations; this is a consideration particularly after 6 months of age when neonatal stores of vitamin B12 become depleted. Thus, iron, vitamin E, and folate all have a limited dosage range. It is rarely necessary, and it may be harmful to exceed substantially the doses recommended in Table I.

Prevention of asymptomatic deficiency. Mild anemia as a sign of iron or vitamin E deficiency or hypersegmentation of neutrophils as evidence of folate deficiency is not associated with recognizable symptoms or defects in growth and maturation. Nevertheless, good nutritional management requires that such hematologic manifestations not be ignored. Each of these deficiency states should be regarded as a systemic disease, although they happen to be recognized by their hematologic manifestations. In iron deficiency, anemia is a signal that an essential substrate, i.e., iron, is present in amounts that restrict not only the production of hemoglobin but also the synthesis of other vital iron-containing proteins. Similarly, hypersegmentation of neutrophils indicates that lack of folic acid or vitamin B12 is interfering with nuclear maturation not only in...
Table II. Hemoglobin* and hematocrit* in formula-fed premature infants: From two studies in which two or more regimens of supplementation were compared; the values listed are those of the least “anemic” group

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>Average birth weight 1,840 gm (N = 69†)</th>
<th>Birth weight 1,000-1,500 gm (N = 12‡)</th>
<th>Birth weight 1,500-2,000 gm (N = 22§)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hemoglobin (gm/dl)</td>
<td>Hematocrit (%)</td>
<td>Hemoglobin (gm/dl)</td>
</tr>
<tr>
<td>1.5</td>
<td>10.6 (9.6-12.2)</td>
<td>32.0 (22.8-41.8)</td>
<td>9.7 (8.3-11.1)</td>
</tr>
<tr>
<td>2</td>
<td>9.7 (9.1-11.3)</td>
<td>8.5 (7.1-12.4)</td>
<td>9.7 (8.1-11.3)</td>
</tr>
<tr>
<td>2.5</td>
<td>9.3 (7.1-11.5)</td>
<td>28.4 (21.2-35.6)</td>
<td>9.8 (8.4-11.2)</td>
</tr>
<tr>
<td>4</td>
<td>10.9 (9.1-12.7)</td>
<td>33.7 (30.8-36.6)</td>
<td>10.6 (9.1-12.7)</td>
</tr>
<tr>
<td>6</td>
<td>11.3 (9.3-12.3)</td>
<td>35.6 (29.4-41.8)</td>
<td>9.8 (8.4-11.2)</td>
</tr>
<tr>
<td>9</td>
<td>11.5 (9.9-13.1)</td>
<td>35.8 (30.4-41.2)</td>
<td>10.6 (9.1-12.7)</td>
</tr>
<tr>
<td>12</td>
<td>11.9 (10.1-13.7)</td>
<td>36.9 (32.1-41.7)</td>
<td>10.6 (9.1-12.7)</td>
</tr>
</tbody>
</table>

*Mean and range (± 2 SD).
†Groton and Cross14: Formula with iron (Similac, Ross Laboratories, Columbus, Ohio), solid foods starting at 3 months of age.
‡Melhorn and Gross5: Formula without added iron (Enfamil, Mead Johnson Laboratories, Evansville, Ind.), supplementary vitamins A, C, and D, and α-tocopherol acetate, 25 IU/day.

white blood cells but also in cells of other tissues. Even if such deficiencies are mild, they should be suspected of contributing to suboptimal conditions for growth and development.

Laboratory monitoring. The hematocrit and the concentration of hemoglobin are commonly used screening tests in the newborn period. The definition of anemia in preterm infants, however, is complicated by the uncertainty of whether the available normal values represent optimal nutrition. Nutritional practices are changing rapidly, and it is also difficult to take into account the loss of large volumes of blood for laboratory determinations in infants who are critically ill. Hematocrit and hemoglobin values between birth and 6 weeks fall at a rapid rate, and normal limits are broad during this period.9-H The limits of normal values for hematocrit and hemoglobin in apparently healthy and well-nourished infants between 6 weeks and 1 year of age are listed in Table II.

The other generally available laboratory studies for evaluation of iron, vitamin E, and folate status are rarely requested for screening or for diagnostic purposes. The amount of blood needed by many laboratories, usually 5 ml each for determinations of serum iron and iron-binding capacity, folate, or α-tocopherol, is a major stumbling block to the nutritional assessment of individual patients and to comparing the merits of alternative dietary regimens. In our experience and in increasing numbers of other laboratories, these determinations have lent themselves readily to microadaptations of standard methods which require less than 1 ml of blood. More widespread use of micromethods should make laboratory assessment of nutritional status more practical in the small infant.

IRON

Unless the diet is supplemented with iron, small preterm infants fed proprietary formulas usually develop iron-deficiency anemia sometime after 6 months of age.12-16 There is evidence to suggest that breast-fed infants are less prone to iron deficiency,17 but this requires confirmation, particularly in premature infants. Although the need for additional iron is universally recognized, there is some disagreement concerning the timing of supplementation and the vehicles that should be used. These issues can be brought into focus by reviewing the perinatal changes in iron metabolism and balance.

Iron metabolism during development. Iron is required from early fetal development as a constituent not only of hemoglobin, but also of myoglobin, the cytochromes, and other iron-containing proteins which function primarily in the transport, storage, and utilization of oxygen. In utero, the fetus accumulates iron at a rate that is roughly in proportion to its increasing body weight, a total of about 75 mg/kg.18 primarily in hemoglobin (45 mg/kg) and iron stores. The concentration of iron in serum is higher in the fetus than in the mother, and the amount of iron delivered to the fetus, as estimated by hemoglobin concentration at birth, is almost independent of maternal iron nutrition.19,20

At birth, the concentration of storage iron per kilogram body weight is generous and of the same order as the 15 mg/kg of the average adult male. Any subse-
quent handicap of the premature infant lies simply in the fact that its stores of iron are small in absolute terms and are insufficient to bridge a prolonged period of rapid growth on an iron-poor diet.

The postnatal changes in the iron status of the premature infant have been divided into three well-defined stages; they are diagrammed in Fig. 1. The first stage is that of decreased erythropoiesis which starts immediately after birth and lasts 1 to 3 months. A physiologic basis for this decrease has been related to the change in tissue oxygenation at birth. In utero, fetal tissues depend on the more circuitous transplacental circulation for their oxygen supply. A relative tissue hypoxia results and presumably leads to increased erythropoietin production and a stimulation of red cell production. Following birth, tissue oxygenation increases and hemoglobin synthesis declines. Consequently, most of the iron released from senescent red cells cannot be reutilized for production of new hemoglobin but is deposited in tissues, leading to a temporary increase in body stores of iron.

The second stage is characterized by the resumption of a rapid rate of hematopoiesis beginning between 1 and 3 months of age. This stage has its earliest onset in infants in the lowest range of birth weights. Total body hemoglobin rises as long as the supply of storage iron is available, usually for at least 1 month. The concentration of hemoglobin may increase slightly or remain stable through dilution in an expanding blood volume, which keeps pace with rapid body growth.

The third stage is that of exhausted iron stores and occurs only in infants who have not received sufficient supplemental iron. It is marked by an iron-limited decrease in reticulocyte count and rate of erythropoiesis. The total body iron remains relatively stable, but it becomes diluted by continuing body growth. Iron-deficient erythropoiesis ensues and eventually leads to iron-deficiency anemia. Administration of iron results in an increased concentration of hemoglobin only in this last stage, even though it can be assimilated earlier to augment iron stores. A dose of 12 mg iron/l of infant formula, in the form of ferrous sulfate, is sufficient to prevent the development of iron-deficiency anemia but does not influence the physiologic "anemia" of the first two stages, which is unrelated to iron lack. Possibly, supplementation with a lower dose of iron would also be sufficient. Indeed, there is evidence that merely lowering the concentration of the protein in the formula to that of breast milk decreases the incidence of iron deficiency in term infants.

**Progression of iron deficiency; diagnostic considerations.** The sequence of laboratory changes that characterizes the progression of iron deficiency in infants is essentially the same as in older children and adults. Evidence of early deficiency has always been the most difficult to obtain. **Depletion of iron stores** is the first consequence of iron lack. Until recently, this could be observed best by staining a marrow aspirate for iron. This method was used by Seip and Halvorsen who showed that infants of a birth weight below 2,000 gm who did not receive iron supplementation usually had exhausted the stainable iron in tibial marrow after 10 weeks of age. A new and apparently more practical method of estimating iron stores is the measurement of ferritin in serum. This employs a sensitive radioimmunoassay and requires less than 0.05 ml serum. The concentration of serum ferritin corresponds closely to the concentration of iron stores during normal development and appears to fall to subnormal concentrations before other laboratory evidence of iron deficiency develops. Thus, the ferritin concentration should prove useful in future studies to determine when iron supplementation should be started under current conditions of feeding and blood sampling.

**Preanemic (latent) iron deficiency** develops after mobilizable iron stores become exhausted. It is characterized by a serum iron saturation below 16%, the range...
where iron availability begins to limit the rate of hemoglobin production. The diagnosis of latent iron deficiency is hampered by the fact that most laboratories request 5 ml of blood for the assay, an amount which one is reluctant to remove from an otherwise healthy infant.

Iron-deficiency anemia develops gradually, as a substantial number of red cells become replaced by cells which are produced during the period of low serum iron saturation. A presumptive diagnosis of iron-deficiency anemia is readily established because the determination of hemoglobin, hematocrit, and red cell indices by electronic counter requires only minute amounts of blood and reveals the presence of a hypochromic microcytic anemia.

Prevention of iron deficiency. Iron deficiency is easy to prevent by either of the two following regimens which employ the same dose of iron but differ in timing: (1) Start iron shortly after birth in the form of ferrous sulfate-supplemented formula (12 mg elemental iron/l formula, about 2 mg/100 kcal, or 2.5 mg/kg). The same dose can be given to breast-fed babies in the form of ferrous sulfate drops. This is a dose that may be unfamiliarly low to many physicians who are accustomed to giving iron in larger therapeutic doses. (2) Initiate iron-supplemented formula, or the same dose of medicinal iron, only when iron stores are first depleted (2 or 3 months of age).

Starting iron shortly after birth is the most expedient of these alternatives and is in accord with the recommendation of the Committee on Nutrition of the Academy of Pediatrics. It has the advantage of augmenting iron stores during hospitalization to tide the infant over a period at home when dietary insufficiency of iron is more likely to develop because of an early shift from formula to regular cow’s milk or through erratic administration of drops.

There are, however, reasons for delaying iron supplementation for a few months. Preterm infants under 3 months of age may develop hemolytic anemia in association with a supplement of 8 mg iron/kg. The risk is greater in those infants not supplemented with adequate vitamin E (discussed under vitamin E). Whether smaller doses of iron produce a similar effect remains to be determined. Iron supplements may be safe over a broader dosage range if vitamin E deficiency can be prevented by α-tocopherol polyethylene glycol-1000-succinate, as current studies suggest.

When iron supplementation is delayed until 3 months of age, the dose of 2-3 mg iron/kg is still adequate. Gorten and Cross have shown that this dose, in the form of iron-supplemented formula, is even adequate to treat the established iron-deficiency anemia that had developed after 6 months of age in previously unsupplemented premature infants.

The iron may be given in formula or in medicinal form. The addition of iron and other trace nutrients to formula is the more reliable and convenient of the two methods, but medicinal iron is also effective. Some physicians oppose using milk products as a vehicle for iron because they feel it sanctions the later use of milk to the exclusion of other sources of calories. Such large intakes of regular cow’s milk increase the risk of intestinal blood loss, a contributing factor in some cases of iron deficiency.

Another more speculative reason for not using milk as a vehicle for supplemental iron comes from recent evidence that two iron-binding proteins in milk, lactoferrin and transferrin, lose their bacteriostatic properties when they are saturated with iron. Normally, both iron-binding proteins are about one-third saturated in milk. Theoretically, the amount of iron added to supplemented formula would be sufficient to saturate the two iron-binding proteins and reduce the bacteriostatic activity of milk. It would be teleologically satisfying if the unusually low iron content of milk were finally found to be advantageous to the infant. It is possible that milk has bacteriostatic properties of importance during a period characterized by physiologically low concentrations of immunoglobulins. Until there is more experimental evidence, the theoretic objections to the use of formula as a vehicle for iron seem outweighed by the convenience and efficacy of these products in preventing iron-deficiency anemia. The use of iron-supplemented formula is also strongly endorsed by the Committee on Nutrition. The alternative of medicinal iron has the disadvantages of inconvenient and unreliable home administration and the risk of acute poisoning if accidentally ingested by older siblings.

VITAMIN E

The evidence for a vitamin E-deficiency syndrome in preterm infants was at first regarded with skepticism by some but is becoming increasingly convincing. Criteria for diagnosis and the regimens for prevention and treatment of deficiency, however, are still evolving.

Alpha-tocopherol is the most abundant and most biologically active compound in the vitamin E group and is also the compound that is used therapeutically. One milligram of synthetic d, l-α-tocopherol acetate is equivalent to 1 IU of vitamin E.

Pathophysiology. The best documented metabolic role of the vitamin E compounds is the protection of biologic membranes against oxidative breakdown of lipids. In addition, vitamin E appears to play a meta-
The requirement for vitamin E depends on the degree of saturation of the fats in the diet. Diets particularly high in polyunsaturated fatty acids gradually produce a corresponding change in the composition of the fatty acid in cellular and intracellular membranes. The membranes then become more susceptible to damage as a result of lipid peroxidation, which increases the requirement of the antioxidant effect of vitamin E. In the adult, whose requirements have been most carefully studied, 6 mg/day of α-tocopherol is the recommended intake if the diet is normal (10% linoleic acid), but 20-30 mg/day is suggested if the diet is rich in corn oil, safflower oil, and other unsaturated fats (35-50% linoleic acid).Breast milk contains a low proportion of unsaturated fats, including only 8% linoleic acid. In contrast, proprietary formulas, in which milk fat is replaced by vegetable oil, are rich in unsaturated fatty acids in which linoleic acid accounts for as much as 50% of the total. Consequently, formulas have been fortified with vitamin E to a concentration of at least 5 IU/l (about 0.7 IU/100 kcal); these are higher values than those for breast milk.

In the preterm infant at birth, the body stores of α-tocopherol are disproportionately low: 3 mg at a birth weight of 1,000 gm in contrast to 20 mg in 3,500 gm term infants. Consequently, there is greater dependence on exogenous sources of vitamin E shortly after birth. The concentration of serum α-tocopherol in the preterm infant usually remains low until about 2 to 3 months of age, particularly in the formula-fed infant. It then increases spontaneously and remains at values that are characteristic of the older child and adult.

The interaction between iron and vitamin E is particularly important during the first few months. Melhorn and associates placed preterm infants on one of four supplementation regimens, in addition to feedings of proprietary formula: iron and vitamin E, iron only, vitamin E only, or no additional supplement. The infants who received solely 8 mg/kg of iron as ferrous sulfate were significantly more anemic and had higher reticulocyte counts than infants in any of the other groups, suggesting the possibility of hemolysis. Since iron is a co-factor that catalyzes the oxidative breakdown of red cell lipids in vitro, it is postulated that large doses of iron could have the same effect in vivo. The oxidative damage would be most pronounced if vitamin E were not exerting its antioxidant effect. In addition, there is evidence that concurrent administration of 8 mg/kg of iron and 25 mg/day of α-tocopherol results in slightly impaired absorption of α-tocopherol. These observations raise the question whether iron supplementation may predispose to vitamin E deficiency in the first 2 or 3 months of life. Since iron stores are rarely depleted this early, it may be better to withhold iron supplementation in the preterm infant until 3 months of age. (See above under Iron.)

Clinical manifestations. Physical manifestations of vitamin E deficiency consist primarily of edema of the legs, labia, scrotum, and eyelids. There may also be a watery nasal discharge, tachypnea, and restlessness. Other characteristics are restricted to laboratory abnormalities. Hematologic manifestations include a mild normochromic normocytic anemia and an elevation of the reticulocyte count, though usually not above 10%; thrombocytosis is frequently present. The primary mechanism of the anemia is hemolysis, presumably owing to oxidative damage to the red cell membr-
Fig. 3. Serum concentration of vitamin E (α-tocopherol) after an oral dose of 100 IU α-tocopherol. The curves represent the mean values at 3, 6, and 10 weeks of age of 6 infants with birth weights of 1,000-1,500 gm. (Figure redrawn from Melhorn DK, Gross S, and Childers G: J. PEDIATR 79:581, 1971.)

brane. Red cell morphology is characterized by the presence of acanthocytes, or cells with up to eight irregular, pointed, thorny projections from an irregular central mass. Acanthocytes (pyknoocytes or spur cells), however, are also a relatively common and self-limited finding in presumably normal infants and are, therefore, of help only in supporting a diagnosis.

The concentration of serum α-tocopherol is normally greater than 0.5 mg/dl. It is commonly below this value in small premature infants. In one study of infants receiving a proprietary formula without added iron, 75% of those with a birth weight between 1,000 and 1,500 gm had a concentration below 0.5 mg/dl at 6 weeks of age. The corresponding figure was 15% in those with a birth weight between 2,000 and 2,500 gm. Anemia and reticulocytosis were correlated with a depressed concentration of serum α-tocopherol. The physical manifestations of edema appear to be far less common than the laboratory abnormalities.

An abnormal concentration of serum lipid can be a source of error in evaluating vitamin E status because the values tend to be proportional to one another. The total serum lipid of preterm infants is normally low during the first week of life but then remains stable, only slightly below adult values. Consequently, variations in concentration of serum lipids are unlikely to be a major source of error in evaluating the vitamin E status of otherwise healthy infants.

Since the sensitivity of the red cell membrane to oxidative damage is probably as much a function of its lipid composition as it is of vitamin E status, the hydrogen peroxide fragility test has a diagnostic role by providing a crude estimate of the resistance of the red cell membrane. The results of serum α-tocopherol and the hydrogen peroxide fragility test tend to be correlated. The hydrogen peroxide fragility test, however, may also be abnormal in hemolytic conditions other than in vitamin E deficiency.

The reticulocytosis that characterizes vitamin E deficiency is difficult to distinguish from that which normally accompanies the acceleration of erythropoiesis in the second or third month of life. In the latter case, the increased rate of hematopoiesis appropriately raises the total body hemoglobin content. In contrast, the vitamin E-deficient infant is likely to have an anemia which, because it is due to increased red cell destruction, may progress or remain stable in spite of a markedly elevated reticulocyte count. The new technique of detecting hemolysis by determination of carbon monoxide in exhaled air has been used to diagnose hemolysis in infants and should prove useful in distinguishing these two conditions in the future. Carbon monoxide is derived almost exclusively from the degradation of heme, and the amount exhaled is proportional to the rate of heme breakdown. At present, this remains a research procedure and is not generally available.

**Prevention and treatment.** The management of vitamin E nutrition in the first 12 weeks of life is complicated by the poor assimilation of α-tocopherol acetate, the form of the vitamin that is commercially available. A dose of 25 IU α-tocopherol acetate per day, which is a generous amount even for an adult, does not consistently raise the serum tocopherol concentration to the normal range during the first 2 to 3 months of life. The physical manifestations of edema appear to be far less common than the laboratory abnormalities.

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would provide a large excess of vitamin E to the individual infant with better than average absorption of the vitamin.

In experimental animals, large doses of vitamin E inhibit wound healing, probably by the same mechanism attributed to corticosteroids, through excessive stabilization of the lysosomal membrane. Lysosomal digestion of dead cells and other debris from wounds is presumably decreased, and repair is delayed. Such evidence of toxicity in the experimental animal warrants caution against the use of very large doses of vitamin E in man.

A recent report of Gross and Melhorn indicated that intestinal absorption of vitamin E can be facilitated by a new oral preparation. Water-soluble $\alpha$-tocopherol polyethylene glycol-1000-succinate was found to be significantly better assimilated than $\alpha$-tocopherol acetate by premature infants. If these data are confirmed, a dose of 5-10 IU/day is likely to be adequate during the first 3 months of life. Intramuscular administration of $\alpha$-tocopherol acetate is also effective, particularly in the treatment of vitamin E deficiency, but it is unlikely to have wide application in its prevention.

Until the problem of intestinal malabsorption is conclusively resolved and TPGS becomes generally available, it seems prudent to provide $\alpha$-tocopherol acetate in an oral dose of at least 5 IU and not exceeding 25 IU/day to preterm infants during the first 3 months of life. This dose will raise the serum tocopherol concentrations and decrease peroxide hemolysis in most infants.

Clinical manifestations of deficiency. Premature infants who are fed boiled, pooled breast milk or evaporated milk formulas often have a decreased serum concentration of folic acid (below 5 μg/ml) within 2 to 6 weeks after birth (Fig. 4). In one study of infants fed an evaporated milk formula, nine out of 15 of those who weighed less than 1,700 gm at birth had low concentrations of folate at 1 or 2 months of age whereas only one out of 15 infants with birth weights between 1,700 and 2,500 gm had a concentration of folate less than 5 μg/ml. The concentration of erythrocyte folate does not become markedly decreased until after 6 weeks because the complement of folic acid in the red blood cell is laid down, like hemoglobin, primarily during early maturation and is then retained. For laboratory monitoring, the concentration of erythrocyte folate is a good indicator of chronic folate deficiency, whereas concentration of serum folate is more sensitive in reflecting early or
Table III. Folate status of premature infants fed evaporated milk formula (folate therapy: 100 µg IM every other day for 14 doses after birth)

<table>
<thead>
<tr>
<th>Age</th>
<th>Serum folate (ng/ml)</th>
<th>RBC folate (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>Treated</td>
</tr>
<tr>
<td>17 days</td>
<td>4.8</td>
<td>26.4</td>
</tr>
<tr>
<td>28 days</td>
<td>4.3</td>
<td>19.7</td>
</tr>
<tr>
<td>3 mo*</td>
<td>5.0</td>
<td>13.5</td>
</tr>
</tbody>
</table>

*Hypersegmented neutrophils are increased in number in the untreated infants (p < 0.05).

Acute changes in folate nutrition. Hypersegmented neutrophils appear in the peripheral blood concurrently with the decrease in concentration of erythrocyte folate. This is the first easily recognizable manifestation of folate deficiency (Table III). In infants of low birth weight the mean number of nuclear lobes averages between 1.8 and 2.2 during the first year of life when folate supplementation is sufficient to keep the serum concentration of the vitamin in the normal range. Folate deficiency should be suspected in more than 8% of the neutrophils have four or more lobes or any cells have five lobes or more. Ordinarily, the subjective impression of hypersegmented neutrophils leads to confirmation of the diagnosis by the determination of serum and/or erythrocyte concentrations of folate. It is not surprising that anemia should be a late manifestation of folate deficiency because the early period of deficiency is characteristically superimposed on the period of physiologic erythroid hypoplasia.

After 3 months of age there is a gradual return to normal folate status; this is coincident with the addition of solid food to the infant’s diet. Although folic acid deficiency occurs principally in preterm infants who are otherwise well, it also occurs in term infants who are sick, especially with diarrhea, or who are fed goat’s milk, which is a particularly poor source of folate.

Prevention and treatment. In healthy infants, malabsorption of folate does not appear to be a problem as it is in the case of vitamin E. Most of the folate in milk, as well as that in vitamin preparations, is in the monoglutamate form, which is absorbed without further digestion. Folic acid in almost all other foods exists in the form of polyglutamates which are broken down to the absorbable monoglutamate by intestinal hydrolases. Samuel and associates showed that absorption of both orally administered polyglutamates and monoglutamates is adequate in low weight infants.

An important consideration in utilization of folate is not only how much is in food but also how the food is treated. Folic acid is heat labile in milk or milk products that are poor in ascorbic acid. This could explain the high incidence of folate deficiency in infants fed boiled breast milk or evaporated milk formulas that are sterilized.

The standard recommendation for folate in infants is shown in Table I: 10 µg/100 kcal/day (RDA) or a minimum of 4 µg/100 kcal/day (Committee on Nutrition). Proprietary formulas supply 8-13 µg/100 kcal. Thus, small infants who take 200 kcal/day receive only about 20 µg/day. In infants of low birth weight this dose does not prevent the development of subnormal serum concentrations of folate after 2 weeks of age, whereas 50 µg/day is effective. Additional supplementation therefore is warranted to supply 50 µg/day for the well infant weighing less than 2,000 gm and 100 µg/day for those who are sick.

Vitamin B₁₂ deficiency. In contrast to folate, nutritional vitamin B₁₂ deficiency is rare in infants. An unusual example is that of an infant nursed by a vitamin B₁₂-deficient mother. Since neonatal stores of vitamin B₁₂ are normally large and adequate amounts are present in breast milk or formula, no vitamin B₁₂ supplement is necessary. Cases of congenital pernicious anemia are unlikely to become apparent during the first 6 months of life.
tocopherol or reverse an abnormal hydrogen peroxide fragility test in all premature infants. Until dosage regimens for other preparations and routes of administration are established, \( \alpha \)-tocopherol acetate (Aquasol E) should be given orally in a dose of 5-25 IU/day to reduce the risk of deficiency in infants with a birth weight below 2,000 gm.

**Folate deficiency** is likely to develop in mild form from 2 weeks to 2 to 4 months after birth, before folate-rich solid foods are introduced into the diet. A supplement of 50 \( \mu \)g/day to formula or breast milk keeps the concentrations of serum and erythrocyte folate within the normal range and prevents hypersegmentation of neutrophils.

Folate and vitamin E can be conveniently combined in a single preparation by a hospital pharmacy since they are needed during nearly the same age period and each is prescribed in a dose unrelated to caloric intake or body weight. Iron can be provided by proprietary formula or in a separate medicinal preparation.

**REFERENCES**