The early anaemia of the premature infant: is there a place for vitamin E supplementation?

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1. The efficacy of oral vitamin E supplementation in preventing the early anaemia of the premature infant was assessed in a 10-week double-blind trial. Forty-two babies received either a placebo or 5 or 15 mg supplementary vitamin E/d with oral feeding. No infant received less than the recommended vitamin E:polyunsaturated fatty acid (E:PUFA) value of 0.6. No iron supplement was given.

2. Weekly full blood counts were taken, and plasma vitamin E assay and in vitro haemolysis tests performed on blood sampled on day 1, and also at 6 and 10 weeks of age. All blood withdrawn and transfused and all feeds were documented.

3. Thirty-six (86%) of the babies had a plasma vitamin E level at birth below the accepted adult norm, i.e. < 5200 μg/l. At 6 weeks of age thirty-three (79%) and at 10 weeks thirty-five (83%) of the babies had levels within the normal adult range. No baby showed either clinical or haematological evidence of a vitamin E deficiency state during the trial.

4. It is concluded that in the absence of Fe supplementation and observing the minimum recommended E:PUFA value, contemporary feeding practices allow for the absorption of sufficient vitamin E by the premature baby to prevent the development of an early haemolytic anaemia.

5. No significant relation was found between plasma vitamin E levels and the degree of peroxide haemolysis.

The premature neonate has low blood levels of vitamin E at birth (Gyorgy et al. 1952; Goldbloom, 1963) probably because of relative placental impermeability (Wright et al. 1951; Cruz et al. 1983). Unless exogenous supplies of the vitamin are adequate and well absorbed a deficiency state is likely to develop. The clinical features of vitamin E deficiency were recognized by Hassan (1966). He described a syndrome with a characteristic skin lesion, pitting oedema, irritability, nasal catarrh and constipation, a raised platelet count and peripheral blood changes suggestive of deficient erythrocyte synthesis and increased destruction. Resolution of these signs and symptoms cleared with vitamin E therapy. Similar findings were reported by Ritchie (1968). Oski & Barness (1967) produced clear haematological evidence of a haemolytic anaemia in vitamin-E-deficient premature babies. They showed that supplementary vitamin E was able not only to correct the anaemia but, if adequately supplied, to prevent it.

The question now is not whether vitamin E deficiency is a clinical entity in neonatology, but rather whether it appears in present-day clinical practice and, if so, can it be prevented by dietary supplementation? Despite many studies the matter remains incompletely resolved. This is largely because of the failure to account adequately for complicating variables such as the importance of the ratio of vitamin E: polyunsaturated fatty acids (PUFA) in the diet (Harris & Embree, 1963; Williams et al. 1975), and the prescription of iron supplements (Melhorn & Gross, 1971a; Williams et al. 1975), which both influence the liability to erythrocyte haemolysis in the vitamin-E-deficient subject; and the influence of blood removed for tests, and blood transfused for anaemia, on the haemoglobin level, which is itself often used as an indicator of vitamin E deficiency. Additionally many studies have failed to use a randomized double-blind design (Goldbloom, 1963; Hassan et al. 1966;

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As a result, widely different recommendations on the need for supplementation have been made (Hassan et al. 1966; Dallman, 1974; Farrell, 1979; Phelps, 1979).

Bell & Filer (1981) after reviewing the literature, concluded that there is 'no proven need for supplemental vitamin E' to the diet of the premature infant, and question the existence of any protective effect that additional vitamin E might confer against the early anaemia of prematurity. The aim of the randomized double-blind study described in the present paper is to ascertain whether vitamin E supplementation has any role in the prevention of the early anaemia of prematurity in infants fed on a diet with no added Fe and with a vitamin E:PUFA value greater than 0.6.

EXPERIMENTAL

Patients and methods

Fifty-two consecutively admitted babies of birth weight less than 1760 g were entered into the study. Forty-two completed the full trial period. Median gestational age was 31 weeks (range 27–37 weeks), and median birth weight 1320 g (range 820–1760 g). No baby had problems of blood group incompatibility, erythrocyte enzyme defects or haemoglobinopathy. Gestational age was ascertained from ultrasound scan if this was performed at less than 18 weeks gestation, or by clinical assessment (Dubowitz et al. 1970). Fully-informed parental consent was obtained in all cases.

The babies were randomly allocated to one of three treatment groups. Each received orally in double-blind fashion either a placebo (twelve babies), 5 mg (fifteen babies) or 15 mg (fifteen babies) of α-tocopherol acetate in an aqueous base per day as soon as enteral feeding was commenced. This was continued until 10 weeks of age. If at any time oral feeding was discontinued due to intercurrent illness, the placebo or supplement was discontinued and recommenced simultaneously with the oral feeding. No Fe supplementation was given until 10 weeks of age. All babies received 0.6 ml Abidec (Parke-Davis, Eastleigh, Hampshire) twice daily and 0.5 mg folate daily at commencement of oral feeding or from 6 days of age if orally fed from birth. During the period of parenteral feeding the babies received Vamin glucose (KabiVitrum, Uxbridge, Middx), dextrose solution with added electrolytes, and intralipid (200 g/l; KabiVitrum). The parenteral feed was supplemented with vitamins in the form of 1 ml Multibionta (Merck, Alton, Hampshire) /500 ml dextrose, and 13 ml Vitlipid (KabiVitrum)/100 ml intralipid. This provided a vitamin E intake of 0.1 mg/kg per day, assuming an average parenteral feeding regimen of 2.5 g Vamin glucose/kg per day, 1.5 g lipid/kg per day and a total fluid intake of 150 ml/kg per day. The babies received as oral feed either breast-milk (their mothers' own or banked), Nenatal (Cow and Gate, Trowbridge, Wiltshire), SMA Goldcap (Wyeth, Taplow, Berks) or Osterfeed (Farley Health Products, Plymouth, Devon), as shown in Table 1. The vitamin E content (mg/l) of these feeds is respectively 5.4, 40, 9.5, 4.8. Table 2 shows the percentage fatty acid composition of the four oral feeds used. No infant received less than the recommended E:PUFA value 0.6. The mean vitamin E intake from these oral feeds was 3 mg/day.

Plasma vitamin E assay was performed for all forty-two babies on blood sampled within the 1st day of life, before transfusion of any blood or blood products, and also at 6 and 10 weeks of age. Full in vitro haemolysis test results were recorded at birth, 6 and 10 weeks of age in all except seven babies. A colorimetric method modified from Martinek (1964) was used for the former. Spontaneous haemolysis was determined according to the method of F. C. Jager (personal communication) and peroxide haemolysis, with and without the addition of glucose to the incubation buffer, using a modification of the method of Rose & Gyorgy (1952).

Estimations of haemoglobin reticulocyte and platelet counts were performed at birth on all babies and at weekly intervals until 10 weeks of age. On each occasion the blood film
was examined for signs of haemolytic anaemia. The number of blood counts and films obtained from week 6 to week 10 varied between thirty-three and forty-two. All blood withdrawn and transfused was documented. Transfusions were given to ill, ventilator-dependent babies in order to maintain a blood haemoglobin level of greater than 14 g/l, and to those babies showing clinical evidence of anaemia.
Table 2. The percentage fatty acid composition of the four oral feeds used

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Breast-milk</th>
<th>Nenatal</th>
<th>Osterfeed</th>
<th>SMA Goldcap</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td>3.24</td>
<td>1.37</td>
<td>8.63</td>
<td>12.96</td>
</tr>
<tr>
<td>14:0</td>
<td>6.69</td>
<td>0.73</td>
<td>4.88</td>
<td>6.13</td>
</tr>
<tr>
<td>14:1ω5</td>
<td>0.08</td>
<td>nd</td>
<td>0.18</td>
<td>0.22</td>
</tr>
<tr>
<td>15:0</td>
<td>0.21</td>
<td>nd</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>16:0</td>
<td>26.11</td>
<td>10.57</td>
<td>14.32</td>
<td>13.15</td>
</tr>
<tr>
<td>16:1ω7</td>
<td>3.27</td>
<td>0.15</td>
<td>0.38</td>
<td>1.13</td>
</tr>
<tr>
<td>18:0</td>
<td>10.62</td>
<td>2.69</td>
<td>8.43</td>
<td>9.51</td>
</tr>
<tr>
<td>18:1ω9</td>
<td>30.55</td>
<td>24.95</td>
<td>47.11</td>
<td>36.73</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>12.37</td>
<td>55.21</td>
<td>13.14</td>
<td>16.96</td>
</tr>
<tr>
<td>18:3ω3</td>
<td>1.07</td>
<td>0.59</td>
<td>0.66</td>
<td>2.17</td>
</tr>
<tr>
<td>20:0</td>
<td>0.29</td>
<td>0.43</td>
<td>0.95</td>
<td>0.27</td>
</tr>
<tr>
<td>20:1ω9</td>
<td>1.60</td>
<td>0.42</td>
<td>0.81</td>
<td>0.39</td>
</tr>
<tr>
<td>20:2ω6</td>
<td>0.61</td>
<td>0.05</td>
<td>0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>0.53</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>0.91</td>
<td>0.03</td>
<td>0.04</td>
<td>nd</td>
</tr>
<tr>
<td>20:2ω9</td>
<td>0.12</td>
<td>0.05</td>
<td>0.28</td>
<td>nd</td>
</tr>
<tr>
<td>24:0</td>
<td>1.09</td>
<td>nd</td>
<td>nd</td>
<td>0.12</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>0.65</td>
<td>2.74</td>
<td>0.04</td>
<td>0.05</td>
</tr>
</tbody>
</table>

nd, not detected

A Kruskall Wallis non-parametric analysis of variance was used to assess differences between groups. This test is the equivalent of the Wilcoxon unpaired test for greater than two groups and was used in preference to Student’s unpaired t test because of the non-normality of the data. The Friedman analysis of variance was used to analyse the profile of change of the haemoglobin level within each group for those babies with haemoglobin values recorded for each of 0, 6 and 10 weeks (ten in the placebo group, thirteen in the low-dose and ten in the high-dose vitamin E groups). Spearman correlation coefficients were calculated to assess other data.

RESULTS

Mean gestational age and age-ranges (weeks) for the three treatment groups at the start of the trial were respectively 31.2 (28–37), 30.3 (27–36), 31.2 (28–36). Birth weight means and ranges (g) were respectively 1327 (900–1660), 1285 (820–1700), 1380 (900–1760). There was no significant difference between the three groups during the trial for weeks of parenteral feed, days of treatment with the trial medicine, or net blood changes.

Median plasma vitamin E (µg/l) at birth was 3360 (range 1200–9520). Thirty-six (86%) of the babies had a level below the accepted adult norm (5200–11400 µg/l).

The Spearman correlation coefficients for the initial plasma vitamin E level with birth weight and with gestational age were 0.12 and 0.05 respectively.

Plasma vitamin E levels at 0, 6 and 10 weeks of age in the three treatment groups are shown in Table 3 and a comparison of the plasma vitamin E levels in the placebo and high-dose groups at 6 and 10 weeks in Table 4. At 6 weeks, eleven of the twelve babies in the placebo group, ten of the fifteen babies in the low-dose vitamin E group, and twelve of the fifteen babies in the high-dose vitamin E group had plasma vitamin E levels within the normal adult range. At 10 weeks the corresponding numbers were eleven, eleven and thirteen respectively. Analysis of all forty-two babies showed no significant difference in the vitamin E levels at 10 weeks in babies of 30 weeks gestation and less compared with those of 31 weeks gestation and above (P > 0.05).
Table 3. Plasma vitamin E levels in premature infants receiving a placebo or low- or high-dose vitamin E supplements (5 and 15 mg/d respectively)

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Plasma vitamin E level (μg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment group</td>
<td>Median</td>
</tr>
<tr>
<td>Placebo</td>
<td>3320</td>
</tr>
<tr>
<td>Low-dose vitamin E</td>
<td>3000</td>
</tr>
<tr>
<td>High-dose vitamin E</td>
<td>3420</td>
</tr>
</tbody>
</table>

Statistical significance of difference between groups: \( P = 0.53 \) for 0 weeks, \( P = 0.62 \) for 6 weeks, and \( P = 0.09 \) for 10 weeks.

Table 4. Comparison of placebo and high-dose vitamin E (15 mg/d) at 6 and 10 weeks for plasma vitamin E level and fall in initial haemoglobin

<table>
<thead>
<tr>
<th>Age (Weeks)</th>
<th>Plasma vitamin E (μg/l)</th>
<th>Change in initial haemoglobin (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P value</td>
<td>95% confidence limits</td>
<td>P value</td>
</tr>
<tr>
<td>6</td>
<td>0.34</td>
<td>–6320 and +2520</td>
</tr>
<tr>
<td>10</td>
<td>0.07</td>
<td>–8830 and +880</td>
</tr>
<tr>
<td>Treatment group</td>
<td>Age (weeks)…</td>
<td>Hb (g/l)</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------</td>
<td>----------</td>
</tr>
<tr>
<td>Placebo</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Low-dose vitamin E</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>High-dose vitamin E</td>
<td>10</td>
<td>15</td>
</tr>
</tbody>
</table>

Statistical significance of difference between groups: \( P = 0.98 \) for 0 weeks, \( P = 0.07 \) for 6 weeks, \( P = 0.08 \) for 10 weeks.
Table 5 shows the haemoglobin values obtained at 0, 6 and 10 weeks of age in the three treatment groups. No differences approaching statistical significance were found in platelet and reticulocyte counts, or percentage of irregular erythrocytes on routine films, between the three groups. Similarly there was no significant difference in haemoglobin values or reticulocyte counts at 6 or 10 weeks when, irrespective of group, those babies with a plasma vitamin E level > 5200 µg/l were compared with those babies with a level < 5200 µg/l. (No baby showed evidence of an Fe-deficient anaemia in the absence of Fe supplementation.)

At 6 weeks the initial median haemoglobin value (g/l) had fallen significantly by 79 (P < 0.01), 56 (P < 0.05) and 60 (P < 0.05) in the placebo, low- and high-dose vitamin E groups respectively. This difference remained significant at 10 weeks. There was, however, no significant fall in haemoglobin in any group from week 6 to week 10. Comparison of the change in the haemoglobin value in the placebo and high-dose groups at 6 and 10 weeks is shown in Table 4.

At 6 and 10 weeks no clinically useful association was shown between the plasma vitamin E level and the haemoglobin value, platelet and reticulocyte counts, or percentage of irregular erythrocytes.

No significant relation was found between plasma vitamin E levels and the degree of peroxide haemolysis (P > 0.05). Of 116 standard peroxide haemolysis test samples, sixty-four (92%) of sixty-nine with plasma vitamin E levels > 5200 µg/l had normal (< 5%) haemolysis test results. However, in twenty-nine (62%) of the remaining forty-seven with plasma vitamin E levels < 5200 µg/l, normal haemolysis results were also obtained. No improvement in the relation occurred when glucose was added to the haemolysis medium, nor when spontaneous haemolysis was observed.

**DISCUSSION**

The benefit of adequate plasma vitamin E derives from its potent antioxidant capacity. Vitamin-E-deficient erythrocytes are susceptible to haemolysis due to an unopposed collection of lipid peroxides (Younkin et al. 1971), resulting in cleavage of unsaturated fatty acids at their double bonds and a marked decrease in the phosphatidyl ethanolamine zone in the erythrocyte membrane (Jacob & Lux, 1968). Vitamin E opposes this process by stabilizing PUFA and minimizing lipid peroxidation.

The finding of low initial plasma vitamin E levels, not associated with birth weight or gestational age, in the large majority of the study population is in agreement with other authors (Wright et al. 1951; Gyorgy et al. 1952; Goldbloom, 1963) and confirms the poor transplacental passage of the vitamin to the baby. The small and gestationally immature baby is further compromised by his erythrocyte membrane having a higher phospholipid and cholesterol content than his full-term counterpart (Gross & Melhorn, 1972) and, therefore, being particularly susceptible to vitamin E deficiency. Previously such a propensity to develop clinical evidence of inadequate plasma levels of the vitamin was compounded by the high content of PUFA in proprietary baby milks (Hassan et al. 1966; Ritchie et al. 1968; Dallman, 1974).

In our study at 6 and 10 weeks nine and seven babies respectively had plasma vitamin E levels below the accepted adult norm. Further consideration of the latter group did not reveal any clear reasons for this. The seven babies did not differ from the rest of the study population in terms of gestational age (weeks; mean 30, range 27–32), or birth weight (g; mean 1244, range 900–1280).

Gross (1983) demonstrated that premature infants fed on breast-milk, pooled separately according to postpartum week from mothers 1–6 weeks following preterm deliveries, maintained a higher vitamin E level than those fed on mature human milk. None of the
seven babies had received their mother’s own milk. However, only eight of thirty-one babies with plasma vitamin E levels $>5200 \mu g/l$ who received breast-milk received their mother’s own. The remaining twenty-three received mostly mature human milk from the ‘bank’.

Three of the seven babies had prolonged periods of parenteral nutrition (5, 6 and 7 weeks respectively). Two of the seven, however, had been orally fed from birth (Table 1).

Horwitt et al. (1972) maintain that plasma vitamin E levels cannot be interpreted when isolated from concurrent blood lipid levels, demonstrating the tendency for plasma tocopherol values to rise and fall in proportion to the lipid concentration. However, it has recently been argued (Gutcher et al. 1984) that the total tocopherol:total lipid value, although showing a close correlation in healthy adults and children, may not be an appropriate index of vitamin E sufficiency in the rapidly fluctuating nutritional state of the low-birth-weight premature infant. No assessment of blood lipids was made in the present study and thus we cannot support either argument with our findings. The possibility that the low levels in seven babies at 10 weeks were a reflection of concurrent low blood lipid concentrations should nonetheless be considered.

Despite these babies having low absolute plasma vitamin E levels, with contemporary artificial feeds observing at least the minimum recommended $E:PUFA$, and with no supplemental Fe, there was neither clinical nor haematological evidence of a vitamin E deficiency state in any of the study population.

Smith & Dunkley (1962) showed ferrous- and ferric-Fe catalysis of linoleate peroxidation. Melhorn & Gross (1971a; Gross & Melhorn, 1972) demonstrated the deleterious effect of Fe on vitamin E absorption and the increased susceptibility to erythrocyte haemolysis in potentially vitamin-E-deficient babies given added Fe. These findings were confirmed by further studies (Chadd & Fraser, 1970; Williams et al. 1975; Gross, 1983) and the problem is now well recognized (American Academy of Pediatrics, 1977; Phelps, 1979).

Other papers recommending that supplemental vitamin E be given to the premature baby have included added oral Fe in their study protocol (Oski & Barness, 1967; Chadd & Fraser, 1970; Lo et al. 1973). We suggest that the overall efficient absorption of vitamin E and the failure to demonstrate any haematological evidence of a deficiency state in our study is in part due to none of the babies receiving additional Fe supplementation.

Although there was a trend in median values towards a higher plasma vitamin E level with increasing vitamin E supplementation, the differences between the groups were not statistically significant. The wide range of the 95% confidence limits suggests that a true significance is not being hidden by the sample size.

There was no statistical difference between any of the three groups in the haemoglobin values obtained at 0, 6 or 10 weeks, nor in the number of babies with a haemoglobin $<105 \text{ g/l}$. This value has been shown by Wardrop et al. (1978) to be that below which symptoms of anaemia may occur. There was no significant difference in the fall from the initial haemoglobin level at 6 weeks when this was compared in the placebo and high-dose vitamin E groups (Table 4). Comparison of the change from week 0 to week 10, however, in the same two groups reached significance ($P = 0.05$) but with 95% confidence limits of $-21$ and 0, and analysis showing no significant fall in haemoglobin from week 6 to week 10 within any of the three groups, the question of a real haematological benefit from the vitamin supplementation remains uncertain. Furthermore, at the plasma levels achieved, vitamin E had no practically useful correlation with any of the blood indices examined.

Previous studies have suggested that 15 mg oral vitamin E supplementation/d is at least adequate (Oski & Barness, 1967; Chadd & Fraser, 1970; Lo et al. 1973). Moreover, Gross & Melhorn (1974) demonstrated significantly higher serum tocopherol levels and maintenance of higher haemoglobin values in premature infants given water-soluble as opposed to fat-soluble preparations of the vitamin, leading Dallman (1974) to suggest that
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A dose of 5–10 mg/d of such a preparation is likely to be adequate in the first 3 months of life. In our study an oral supplement of 15 mg water-soluble α-tocopherol acetate/d did not result in significantly higher plasma vitamin E levels compared with the placebo group, suggesting that enough vitamin E can be absorbed by the baby from that provided in the commercial feeds.

Melhorn & Gross (1971b) and Farrell (1979) found less-efficient intestinal absorption of vitamin E in the more premature baby. Our results, however, tend to support the recent work of Bell et al. (1979) who, in a 6-week trial, achieved normal adult tocopherol levels in infants of less than 1.5 kg given vitamin E daily by orogastric tube, and Jansson et al. (1984) who showed good intestinal vitamin E absorption in premature babies of less than 1000 g birth weight.

The increased haemolytic susceptibility of vitamin E-deficient erythrocytes was noted by Rose & Gyorgy (1952) and further studies have claimed the hydrogen peroxide haemolysis test to be a reliable indicator of low plasma vitamin E levels with good reproducibility (Binder et al. 1965; Lo et al. 1973). However, discrepancies between vitamin E levels and the percentage haemolysis have been recognized and wide fluctuations noted (Mackenzie, 1954; Hassan et al. 1966). Our results show the haemolysis test to be a poor predictor of plasma vitamin E levels. In an attempt to achieve more meaningful results glucose was added to the medium. This results in a regeneration of glutathione peroxidase (EC 1.11.1.9), a faster detoxification of the hydrogen peroxide, and a greater reproducibility of the test. There remained, however, no significant relation between the degree of haemolysis and the plasma vitamin E level. As discussed, other factors apart from the absolute plasma vitamin E levels are important in determining the susceptibility of the erythrocytes to haemolysis and it may be that the percentage haemolysis in hydrogen peroxide will be more useful as an effective indicator of a relative vitamin E-deficient haemolysis, if not of the absolute vitamin E level.

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REFERENCES

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