Vitamin E Supplementation and Periventricular Hemorrhage in Very Preterm Babies

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Plasma vitamin levels in newborn babies are low compared with older infants, children, and adults (1–3). In preterm babies the levels remain low for several months, whereas in term babies plasma vitamin E rises during the first few weeks of life. Red blood cells of newborn babies are susceptible to hemolysis in a dilute solution of hydrogen peroxide, and this is corrected by supplementation with vitamin E (4,5). A hemolytic anemia responsive to vitamin E supplementation was first described in artificially fed preterm babies at around 6 to 10 weeks of age by Hassan et al. (6) and Oski and Barness (7).

Hemolysis is the least controversial effect of vitamin E deficiency in the newborn, but overt hemolytic anemia rarely occurs nowadays partly because of improved supplementation of artificial milks with vitamin E in relation to the polyunsaturated fat content. The two disorders that provoke controversy about the role of vitamin E in their pathogenesis and prevention are retinopathy of prematurity (retrolental fibroplasia) and bronchopulmonary dysplasia.

Owens and Owens (1) drew attention to low serum vitamin E levels in preterm babies nearly 40 years ago and suggested that this was implicated in the causation of retrolental fibroplasia. Renewed interest in this subject in recent years has culminated in the proposal, based on electron microscopical observations, that vitamin E deficiency does indeed have an important role in the pathogenesis and prevention of what is now termed retinopathy of prematurity (8).

Bronchopulmonary dysplasia is a chronic lung disorder of preterm babies who have received mechanical ventilation in the neonatal period. It is likely that both pulmonary barotrauma and oxygen toxicity have a role in the
etiology. Initial optimism that this disorder might be ameliorated or prevented by vitamin E supplementation (9) has not been confirmed by subsequent investigations (10,11).

PERIVENTRICULAR HEMORRHAGE

In this chapter we explore the use of vitamin E supplementation in another important disorder of preterm babies, namely, periventricular hemorrhage (PVH). We define PVH as bleeding in and around the lateral ventricles of the brain. Before methods of imaging the brain in living newborn babies became available, the only way of confirming the presence of PVH was at necropsy. It was apparent that the lesion was essentially one of preterm babies and occurred especially in those who weighed less than 1,500 g (roughly equivalent to <32 weeks of gestation) in whom it was observed in about 75% of necropsies (12). The introduction of computerized tomography, and more important, ultrasound imaging that is carried out at cribside has led to a greater understanding not only of PVH, but other common neonatal brain conditions, such as ventriculomegaly and ischemic brain lesions.

Brain Ultrasonography and PVH

Modern ultrasound scanners provide high-quality images of the brain in the coronal and sagittal planes, an ultrasound probe (5.0 or 7.5 MHz) being positioned over the anterior fontanelle (13). The image is viewed in real time on a television screen, and a permanent record can be made on videotape or by polaroid photographs.

Serial ultrasound brain scanning has shown that about 40% of babies ≤32 weeks of gestation develop echo-dense lesions in and around the lateral ventricles, corresponding at necropsy to PVH (14–16). The hemorrhage originates in the subependymal region beneath the floor of the lateral ventricles, where it may be confined [subependymal hemorrhage (SEH); Fig. 1b] or may rupture into the ventricles [intraventricular hemorrhage (IVH); Fig. 1c] or into the brain parenchyma [parenchymal hemorrhage; Fig. 1d]. Most lesions occur during the first 3 days of life.

An important controversy surrounds parenchymal echodensities. Although undoubtedly some of them represent the worst stage in the progression of PVH, with extension of hemorrhage from the subependymal area or from the ventricles, certain of these lesions may have an ischemic basis and correspond to the precystic stage of periventricular leukomalacia. Also, PVH and ischemic lesions often coexist (17). It is therefore important that studies of brain lesions using ultrasonography be based on clear and consistent definitions of ultrasound findings.

An important complication of PVH that is not considered in the main study
FIG. 1. Ultrasound scans of the brain through the coronal plane. **A:** Normal appearance. The slit-like cavities of the lateral ventricles appear as echo-free areas indicated by arrows.

**B:** Subependymal hemorrhage. There is an echo-dense area at the inferolateral margin of the right lateral ventricle indicated by arrows. A less extensive echodensity is seen on the contralateral side. A sagittal view is necessary to distinguish this from normal choroid plexus.

**C:** Intraventricular hemorrhage. Both lateral ventricles are dilated and contain echo densities consistent with blood clot. The clot in the left ventricle is indicated by an arrow.

**D:** Parenchymal hemorrhage. The left lateral ventricle is completely obscured by a large echo density that extends into the brain parenchyma. The slit-like cavity of the right ventricle is indicated by an arrow.
is ventriculomegaly (ventricular dilatation). It is discussed at this juncture, since ventricular size can be accurately assessed by ultrasonography. Slight, transient, and asymmetrical ventriculomegaly is very common in the acute stage of IVH and presumably results from distension of the ventricles with liquid blood. Obstruction to the outflow of cerebrospinal fluid (CSF) after the acute stage can lead to a more pronounced ventriculomegaly, which usually resolves spontaneously after several weeks or persists for months or even years but does not progress. Probably in only 5% to 10% of cases does ventriculomegaly become progressive and lead to the clinical features of hydrocephalus. Surgical treatment is necessary in these babies to divert CSF from the ventricles to the peritoneal cavity or right atrium of the heart.

Pathogenesis of PVH

Serial ultrasound imaging of the brain in populations of preterm babies has helped to clarify the clinical circumstances in which PVH is prone to occur (18–20). As well as confirming the vulnerability of babies <32 weeks gestation, various clinical factors have been associated with an increased risk of PVH. These include the occurrence of hyaline membrane disease (respiratory distress syndrome), pneumothorax, hypercapnia, acidemia, and the need for mechanical ventilation. Thus the incidence and severity of PVH is strongly influenced by the nature of the population under consideration. For example, in our own population of inborn babies during 1984 and 1985, about 50% of babies not supplemented with vitamin E suffered PVH, which had the following distribution: SEH, 26%, IVH, 67%, parenchymal, 7%. In contrast, in nonsupplemented babies transferred to us from maternity hospitals during the same period, the incidence of PVH was 67%, with the following distribution: SEH, 20%, IVH 60%, parenchymal 20%.

The subependymal layer (germinal matrix) from which PVH originates comprises an immature vascular bed of thin, poorly supported vessels. Bleeding originates from the arterial side of the circulation and is associated with varying degrees of destruction of the primitive vascular bed. Probably a key factor determining the occurrence of hemorrhage is loss of autoregulation of cerebral blood flow. Thus, surges in cerebral flow, as can occur during recovery from systemic hypotension, might expose the immature vascular bed to a pressure force sufficient to cause destruction of thin vessels and produce hemorrhage (21).

Perlman et al. (22) continuously recorded systemic blood pressure while simultaneously recording cerebral blood flow velocity using Doppler ultrasound with the probe positioned over the anterior fontanelle and directed toward one of the cerebral vessels. Those babies with an unstable blood pressure trace, with beat-to-beat fluctuations in systolic peaks and diastolic troughs, also had a fluctuating pattern of cerebral flow velocity. The inci-
 incidence of PVH was higher in these babies compared with others who had a stable pattern of systemic blood pressure and cerebral flow velocity. There would appear to be two dominant factors in the pathogenesis of PVH: first, immaturity of the vascular bed in the subependymal layer; and second, exposure of the vascular bed to excess perfusion pressure.

VITAMIN E AND PVH: INITIAL OBSERVATIONS

About 7 years ago, there was growing interest in our department in reports suggesting a role for vitamin E in the prevention of retinopathy of prematurity and bronchopulmonary dysplasia. We wished to clarify the effect of intramuscular supplementation on plasma levels of vitamin E and on the susceptibility of red blood cells (RBCs) to hydrogen peroxide hemolysis. We studied 35 newborn babies, whose gestational ages ranged from 25 to 36 weeks. Fourteen of them were randomly selected to receive vitamin E (α-tocopherol acetate), 20 mg/kg, within 24 hr of birth and thereafter at daily intervals for a total of four doses. This regimen was associated with a progressive rise in mean plasma vitamin E levels in supplemented babies from 0.6 mg/dl after birth to 4.5 mg/dl on day 3. The mean levels in the control group stayed in the range 0.5 to 0.6 mg/dl. Supplemented babies also had reduced susceptibility of their RBCs to hemolysis in hydrogen peroxide.

At this time we were providing ventilatory support for preterm babies with severe respiratory distress; it was clear that an important factor limiting survival was not lung disease itself but massive IVH, especially among very preterm babies. We were aware of Minkowski’s (23) earlier and somewhat neglected observations, which suggested that vitamin E reduced capillary permeability. In our preliminary investigation, 8 of the 35 (22.9%) babies died, and they were all <30 weeks gestation. We observed that among these babies, the incidence of IVH at necropsy was 69.2% in the controls and 22.2% in the supplemented group (24). Subsequently, brain ultrasound imaging was introduced in our department, and we initiated a small randomized controlled pilot study of vitamin E supplementation for the possible prevention of PVH (25). We studied 44 babies <37 weeks gestation and used a similar vitamin E supplementation regime to that outlined above. We observed that among babies <32 weeks gestation, the incidence of IVH in the nonsupplemented group was 56.3% (nine of 16), whereas the corresponding incidence in the supplemented babies was 18.8% (three of 16). There was no apparent influence of vitamin E on mortality.

No trials have subsequently been conducted to verify these preliminary findings. Phelps (26) warned against the use of vitamin E in preterm babies and suggested that intravenous vitamin E was associated with an increased risk of severe grades of PVH. In contrast, during an investigation into the possible protective effect of vitamin E in retinopathy of prematurity, Speer
et al. (27) incidentally observed a protective effect against PVH, but their study population included only babies requiring supplemental oxygen for respiratory distress and excluded outborn referred babies, an ill population at special risk of PVH.

We now report the results of a randomized controlled trial of vitamin E in the prevention of PVH in babies of ≤32 weeks gestation who underwent care at the Neonatal Medical Unit of this hospital, which is the Regional Perinatal Referral Centre for the North Western Region of England. The study was approved by the Hospital Ethical Committee.

PATIENTS AND METHODS

Between January 1984 and September 1985 we enrolled in the trial 231 babies ≤32 weeks gestation. One hundred and fifty were born at this hospital, and 81 were referred from other maternity hospitals in the North Western Region. Each baby was randomly allocated to a vitamin E-supplemented or control group, without stratification for place of birth, according to directions contained on a card drawn from a sealed envelope. For inborn babies the card was drawn just before or immediately after birth. Referred babies had an ultrasound scan of the brain carried out on admission, and those with parenchymal hemorrhage (n = 2) were not enrolled in the trial. Randomization of the referred babies occurred immediately after the initial brain scan.

Babies allocated to the supplemented group received three intramuscular doses of vitamin E (Ephynal, DL-α-tocopherol acetate), 20 mg/kg, commencing within 2 hr of randomization into the trial (day 0), and 24 hr and 48 hr later (days 1 and 2). The vitamin E preparation was manufactured by Hoffmann-La Roche, Basel. Samples of heparinized venous or arterial blood (1.5 ml) were drawn from babies immediately before the first dose of vitamin E was given and at comparable times in the control babies (day 0). The following measurements were made: susceptibility of RBCs to hydrogen peroxide hemolysis, plasma vitamin E concentration, and plasma total lipids. These measurements were repeated at 24, 48, and 72 hr (days 1, 2, and 3).

The susceptibility of RBCs to peroxidation was measured by the hydrogen peroxide (H₂O₂) hemolysis test using a modification of the method originally described by Rose and Gyorgy (28) and later by Gordon et al. (29). The result was judged to be abnormal if more than 10% of the RBCs hemolyzed in the H₂O₂ solution. Plasma vitamin E was measured as total tocopherols by a colorimetric method in which ferrous iron produced by reduction of ferric iron by vitamin E was used as an index of vitamin E concentration (30). The results were expressed as milligrams per deciliter (× 23.2 = µmol/liter) and as a ratio to plasma lipids (mg/g). A colorimetric method was used to measure plasma total lipid (31).
Ultrasound Scans of the Brain

Each baby was scanned through the anterior fontanelle using a Technicare Mechanical Sector Scanner (Model 8100) with a 7.5-MHz probe. Inborn babies had their first scan within 2 hr of birth, and outborn babies were scanned on admission to the unit (range, 4–26 hr; median, 10 hr). Thereafter, each baby was scanned daily during the first week of life and at least twice weekly until discharge from the unit. Scans were made in coronal and sagittal planes, with several sequential sections in each plane. The scans from each baby were recorded and stored on videotape and reviewed in detail at the end of the study. Except where indicated, the final grading on serial scanning is reported.

Subependymal hemorrhage (SEH) was defined as echodensity confined to the subependymal region with the floor of the ventricles apparently intact. Intraventricular hemorrhage (IVH) was represented by echodensity occupying the cavity of the ventricle. Parenchymal hemorrhage was indicated as echodensity within the brain parenchyma clearly contiguous with intraventricular or subependymal echodensity. Parenchymal echodensities that were not contiguous with intraventricular echodensity, presumed ischemic lesions, were not considered in this report. The expression periventricular hemorrhage (PVH) includes all types of observed hemorrhages.

Clinical Management

Medical and nursing care was according to our routine ward protocol. The babies were nursed in incubators set within the thermoneutral range. All babies with respiratory distress, and other ill babies in whom it was judged imprudent to feed orally, received intravenous dextrose (10%) or dextrose-saline (30 mmol/liter) commencing at 60 ml/kg bodyweight and increasing to 90, 120, and 150 ml/kg on days 1, 2, and 3, unless there were clinical indications to modify this protocol. After 10 days, amino acids, lipids, and vitamins A, D₂, and K₁ were added to the intravenous fluid regimen.

Those requiring supplemental oxygen for respiratory distress had their arterial \( P_{O_2} \) monitored, either continuously using an indwelling umbilical arterial catheter with an oxygen electrode at its tip, or by 4-hourly arterial sampling from a conventional umbilical arterial catheter, or by radial artery puncture. Blood pressure was continuously monitored with a transducer connected to the umbilical artery catheter, and the waveform was visually displayed. Mechanical ventilation was provided by a “pressure-preset” ventilator interfaced with a nasotracheal tube. The ventilator settings and adjustments were similar to those suggested by Reynolds (32). Babies judged to be breathing asynchronously with the ventilator such that their blood gases were difficult to control were paralyzed with pancuronium. Intravenous
phenobarbital was used for the control of seizures, and in a few babies it was used to correct an unstable or fluctuating blood pressure trace. We used a loading dose of 20 mg/kg, followed by 5 mg/kg daily, adjusted to keep the serum level within the range of 15 to 30 μg/ml.

Clinical information relevant to the occurrence of PVH was collected prospectively in each baby. Mothers of certain inborn babies received either vitamin E or placebo capsules during preterm labor as part of a different ongoing investigation into the influence of maternal supplementation on the incidence of PVH. This “blinded” study is not complete; however, the treatment code was temporarily broken to verify that maternal supplementation occurred in a similar proportion of neonatally supplemented babies (22.5%) and their controls (19.2%).

Statistical Analysis

The significance of comparison between mean values was evaluated by the unpaired or paired Student’s test. Incidences in various groups were compared by chi-square test with Yates’s correction, and the 95% confidence intervals (CI) of differences were calculated where appropriate. The independent effect of vitamin E supplementation on the incidence of IVH or parenchymal hemorrhage was evaluated by multivariate analysis using a binomial logic model (33).

RESULTS

Three inborn babies for whom randomization cards were drawn before birth had lethal major malformations, and they were immediately excluded from the trial for ethical reasons.

Plasma Vitamin E and Total Lipid Concentration

On day 0, the mean ± SD plasma vitamin E concentration (mg/dl) was identical in supplemented (0.44 ± 0.20) and control groups (0.44 ± 0.21). Subsequently, there was a significant rise in vitamin E levels in the supplemented group, and on each day the supplemented group had a significantly higher mean plasma vitamin E level compared with the controls (Fig. 2). On day 0, the mean ± SD vitamin E concentration:plasma lipid ratio (mg/g) was similar in supplemented (1.40 ± 0.89) and control groups (1.39 ± 0.68). Thereafter, the trend of the mean values in the two groups was identical to that observed with plasma vitamin E concentration, reaching a value on day
3 of 7.38 ± 2.97 in supplemented babies and 1.11 ± 0.46 in controls (p < 0.001).

Trends in plasma lipid concentration and the relationship between plasma vitamin E and total lipids were analyzed in control babies only (Table 1). The mean (± SD) plasma lipid concentration (g/dl) on day 0 was 0.325 ± 0.092. There was a significant rise in the mean level from day 0 to day 1 and from day 1 to day 2. There was a significant positive correlation between plasma vitamin E and total lipids on each day of the study; however, the coefficients of correlation (r) were very low, ranging from 0.25 to 0.43.

**TABLE 1.** Plasma total lipids (g/dl) and correlation of plasma vitamin E with total lipids on different days in control babies

<table>
<thead>
<tr>
<th>Day</th>
<th>Plasma total lipids (g/dl) (mean ± SD)</th>
<th>Correlation of plasma vitamin E with lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>0.325 ± 0.092</td>
<td>r = 0.279; p &lt; 0.005</td>
</tr>
<tr>
<td>Day 1</td>
<td>0.367 ± 0.114</td>
<td>r = 0.430; p &lt; 0.001</td>
</tr>
<tr>
<td>Day 2</td>
<td>0.397 ± 0.122</td>
<td>r = 0.251; p &lt; 0.02</td>
</tr>
<tr>
<td>Day 3</td>
<td>0.411 ± 0.125</td>
<td>r = 0.410; p &lt; 0.001</td>
</tr>
</tbody>
</table>

* Paired comparisons of plasma lipids:
  - Day 0 to 1 (n = 101 pairs; t = 4.371; p < 0.001)
  - Day 1 to 2 (n = 98 pairs; t = 2.927; p < 0.005)
  - Day 2 to 3 (n = 93 pairs; t = 1.473; not significant)
TABLE 2. Percentage of supplemented and control babies with an abnormal hydrogen peroxide (H$_2$O$_2$) hemolysis test on different days

<table>
<thead>
<tr>
<th></th>
<th>Supplemented</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>52.7</td>
<td>52.7</td>
</tr>
<tr>
<td>Day 1</td>
<td>4.7</td>
<td>46.6</td>
</tr>
<tr>
<td>Day 2</td>
<td>1.0</td>
<td>51.5</td>
</tr>
<tr>
<td>Day 3</td>
<td>1.0</td>
<td>37.2</td>
</tr>
</tbody>
</table>

* An abnormal H$_2$O$_2$ hemolysis test is one where >10% of the RBCs hemolyze in a dilute solution of H$_2$O$_2$.

H$_2$O$_2$ Hemolysis

The results of the H$_2$O$_2$ hemolysis tests are shown in Table 2. On day 0, the same proportion of supplemented and control babies (52.7%) had an abnormal H$_2$O$_2$ hemolysis test. Thereafter, in supplemented babies the proportion with an abnormal result fell to 4.7% on day 1 and to 1.0% on days 2 and 3. In contrast, among control babies the proportion with an abnormal result remained high from days 0 to 3.

Brain Scan Findings

Among the 228 babies entered into the trial, 18 already had PVH on the initial scan carried out within 2 hr of birth (inborn: SEH, $n = 1$; IVH, $n = 1$), or on admission to the unit (referred: SEH, $n = 13$; IVH, $n = 3$). These 18 babies were excluded from the analysis to determine the effect of vitamin E on the incidence of PVH, but were included in the analysis to determine the effect of vitamin E on the progression of PVH.

The 210 babies without PVH on the initial scan comprised 145 inborn and 65 referred. The referred babies were a selected very sick population with an incidence of hyaline membrane disease of 85%, mechanical ventilation 92%, and pneumothoraces 31%; however, the incidence of various clinical factors that might influence the occurrence of PVH in inborn and referred babies was not significantly different between the supplemented and control groups (Table 3).

The incidence and distribution of the individual types of hemorrhage in inborn and referred babies is shown in Table 4. Among the inborn population, the proportion of supplemented babies without any hemorrhage (74.2%) was higher compared with the control group (48.2%; $p < 0.005$). The combined incidence of IVH and parenchymal hemorrhage in supplemented babies (8.1%) was lower compared with the corresponding incidence in controls (38.6%; $p < 0.001$) (95% CI for difference, 18.0%-43.0%). This was largely
accounted for by the markedly lower incidence of IVH in the supplemented group (6.5%) compared with the controls (34.9%; 95% CI for difference 16.5%-40.4%).

Among the referred population the proportion of supplemented babies without any hemorrhage (62.5%) was also higher compared with the control group (40.0%), although this difference was not statistically significant. There was a trend toward a higher incidence of SEH in supplemented babies compared with controls, but the combined incidence of IVH and parenchymal hemorrhage was lower in the supplemented group (15.0% vs. 48.0%) (p < 0.005).

Among all supplemented babies (inborn and referred), the proportion without any hemorrhage (69.6%) was higher compared with the controls (46.3%; p < 0.001). The incidence of IVH, and the combined incidence of IVH and parenchymal hemorrhages (10.8%) was lower in the supplemented group compared with the corresponding incidence in the control group (40.7%; p < 0.001) (95% CI for difference, 18.0%-41.0%).

A multivariate analysis using a binomial logic model adjusting for factors other than vitamin E that might influence the occurrence of PVH showed that vitamin E had an independent protective effect against PVH (all grades
TABLE 4. Incidence and distribution of the final grade of hemorrhage in supplemented babies and controls.

<table>
<thead>
<tr>
<th></th>
<th>No hemorrhage [No. (%)]</th>
<th>Subependymal [No. (%)]</th>
<th>Intraventricular [No. (%)]</th>
<th>Parenchymal [No. (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inborn (n = 145)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 83)</td>
<td>40 [48.2]</td>
<td>11 [13.3]</td>
<td>29 [34.9]</td>
<td>3 [3.6]</td>
</tr>
<tr>
<td>Referred (n = 65)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 25)</td>
<td>10 [40.0]</td>
<td>3 [12.0]</td>
<td>8 [32.0]</td>
<td>4 [16.0]</td>
</tr>
<tr>
<td>All babies (n = 210)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 108)</td>
<td>50 [46.3]</td>
<td>14 [13.0]</td>
<td>37 [34.3]</td>
<td>7 [6.5]</td>
</tr>
</tbody>
</table>

*a* *p* < 0.005.

*b* *p* < 0.001.

*c* *p* < 0.01.

of hemorrhage combined; (*p* < 0.001). The factors adjusted for included gestational age, birthweight, gender, inborn or referred, method of delivery, intubation at birth, Apgar score at 5 min, cardiorespiratory problem, mechanical ventilation, pneumothorax, use of muscle relaxant, and anticonvulsant treatment.

Within both supplemented and control groups (analyzed separately), mean plasma vitamin E levels were similar in those without hemorrhage and in those with all types of hemorrhage combined.

**Progression of Hemorrhage**

The overall incidence of hemorrhagic lesions among the 228 babies who entered the trial was 46.9% (107 babies) and the initial grade of hemorrhage was SEH or IVH in 101 babies. The six babies whose first abnormal scan showed parenchymal hemorrhage all belonged to the control group, and all had normal scans on entry to the trial. To assess the possible influence of vitamin E supplementation on the progression of SEH and IVH, the final grade of hemorrhage was compared with the initial lesion in the 101 babies. In only 16 babies (15.8%) was the final grade of hemorrhage worse compared with the initial grade; i.e., the hemorrhage was observed to progress. The incidence of progression was similar in supplemented (16.3%) and control groups (15.5%). Similar results were obtained when inborn and referred babies were analyzed separately.
SUPPLEMENTATION AND HEMORRHAGE IN BABIES

TABLE 5. Incidence and distribution of hemorrhages on the first abnormal brain scan in babies whose initial scan was normal

<table>
<thead>
<tr>
<th></th>
<th>Subependymal [No. (%)]</th>
<th>Intraventricular [No. (%)]</th>
<th>Parenchymal [No. (%)]</th>
<th>Intraventricular or parenchymal [No. (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplemented</td>
<td>23 (74.2)*</td>
<td>8 (25.8)</td>
<td>0</td>
<td>8 (25.8)</td>
</tr>
<tr>
<td>(n = 31)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>18 (31.0)</td>
<td>34 (58.6)</td>
<td>6 (10.3)</td>
<td>40 (69.0)</td>
</tr>
<tr>
<td>(n = 58)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.0001 (2 x 2 chi-square analysis; SEH vs. IVH, or parenchymal combined).

When only those babies whose initial scans were normal (n = 210) but who subsequently developed hemorrhage were considered (n = 89), a marked difference in the distribution of hemorrhage between supplemented and control babies was already apparent on the first abnormal scan. In the supplemented group SEH predominated, whereas in the control group a significantly greater proportion of babies had already established IVH or parenchymal hemorrhage (Table 5).

Mortality

Of the 228 babies in the trial, 184 (80.7%) survived to be discharged from the unit, and the survival rate was similar in supplemented and control groups. Although the survival rate of inborn babies (85.7%) was somewhat higher than that of the referred babies (71.6%), no significant effect of vitamin E treatment on survival was observed in either group; however, among supplemented babies who died, the combined incidence of IVH and parenchymal hemorrhage was 14.3%, whereas the corresponding incidence in the controls who died was 73.9% (p < 0.0001).

Among supplemented babies who survived, 14 (15.1%) had IVH or parenchymal hemorrhage, whereas among control survivors 31 (34.1%) had these lesions (p < 0.005). When the data are reanalyzed to include only the 210 babies whose scan on entry to the trial did not show any hemorrhage, 10.7% of the supplemented survivors had IVH or parenchymal hemorrhage compared with 32.6% of the control survivors (p < 0.001) (95% CI for difference, 9.9%-33.8%).

Side Effects and Possible Toxicity of Vitamin E

Only one baby developed induration and inflammation at the injection site resulting from superficial placing of the injection. Among the 228 babies who
entered the trial, 27 (11.8%) developed septicemia confirmed by a positive blood culture, and in 22 of them this occurred ≥72 hr after birth ("late sepsis"). Sixteen (7.0%) of the babies in the trial had clinical evidence of a bleeding tendency, but in only two did laboratory data suggest disseminated intravascular coagulation. The incidence of early or late sepsis and bleeding tendency were similar in supplemented and control babies. Three (1.3%) babies developed necrotizing enterocolitis confirmed by abdominal X-ray. Each had been supplemented with vitamin E. Their maximum plasma vitamin E levels were 1.21, 2.93, and 4.48 mg/dl.

DISCUSSION

The normal range of plasma vitamin E (total tocopherols) in adults is 0.5 to 1.6 mg/dl, based on studies cited by Farrell (34). Our observations confirm that newborn babies have low circulating levels of this vitamin; the mean plasma concentration observed in supplemented and control babies on day 0 was similar to that observed by other investigators (2,3). Vitamin E supplementation caused a significant rise in plasma concentration 24 hr after the first dose and further rises thereafter. There is no satisfactory definition of vitamin E deficiency in adults, let alone in babies who have been born very prematurely. We will not address this problem except to say that it is too simplistic to imagine that measurements of the plasma concentration of vitamin E are a reliable guide to deficiency states.

The transport of vitamin E in the plasma is associated with lipoproteins. In children and adults there is a positive correlation between plasma vitamin E concentration and plasma total lipids or cholesterol. Thus it has been argued that plasma vitamin E levels should be expressed as a ratio to plasma lipid or cholesterol (35). We observed a weak but nonetheless highly significant positive correlation between plasma vitamin E and plasma lipid in unsupplemented babies during the first 3 days of life.

The mean plasma lipid concentrations in these very premature babies ranged from 0.325 g/dl on day 0 to 0.410 g/dl on day 3, which are certainly low compared with children and adults. It is interesting that the mean ratio of vitamin E to plasma lipid during the first 3 days in unsupplemented babies was well above 0.8 mg/g. It has been suggested that this level indicates vitamin E sufficiency (36).

It is well known that the RBCs of newborn babies have an increased susceptibility to hemolysis in a dilute solution of \( \text{H}_2\text{O}_2 \), and our findings confirmed the original observations that vitamin E supplementation is associated with increased resistance to \( \text{H}_2\text{O}_2 \) hemolysis (37,38).

Among the 210 babies who had normal brain scans on entry to the trial, those receiving vitamin E had, on final grading, a higher incidence of hemorrhage-free scans, a lower incidence of IVH and parenchymal hemorrhage,
but a similar incidence of SEH compared with the untreated group. This protective effect of vitamin E was observed among inborn and referred babies but was more pronounced in the former group, in whom the most significant benefit was against IVH.

Although it is not possible to define accurately progression of hemorrhage without continuous brain scanning, our results, based on scanning at 24-hr intervals, suggest that progression of hemorrhagic lesions in terms of worsening of grades occurs only in a minority of cases (15.8%). This is almost identical to the observations of Levene and de Vries (39), whereas Partridge et al. (40) report extension of hemorrhages occurring in up to 43% cases. In our study no protective effect of vitamin E was observed against SEH, and progression of hemorrhage was observed in a similar proportion of supplemented and control babies. It is important to note that on the first abnormal scan, supplemented babies had an excess of SEH and no parenchymal hemorrhage, whereas controls had an excess of IVH and a 10.3% incidence of parenchymal hemorrhage. These findings suggest that vitamin E protects by rapidly limiting the extent of SEH within 24 hr of its occurrence and imply the importance of early supplementation.

An antipurpuric action of vitamin E has been reported in animals and humans, including newborn babies. Indeed, over 30 years ago Minkowski, on the basis of maternal supplementation experiments, suggested that vitamin E might protect against "hémorragies cérébro-méningées du prématuré" (23). Even earlier, Pappenheimer and Goettsch (41) observed that brain hemorrhage was a feature of "nutritional encephalopathy" in growing chicks fed diets deficient in vitamin E and enriched with linoleic acid. Progressive endothelial cell changes occurred, including increased lysosomal activity. This is interesting because vitamin E, in combination with selenium, stabilizes lysosomes and prevents lipid peroxidation of subcellular organelles. Another example of a vitamin E deficiency disorder from the veterinary sciences is spontaneous hemorrhagic necrosis of the central nervous system, which occurs in fetal hamsters. The initial lesion involves the subependymal vasculature in 12- or 13-day-old fetuses, and progression to IVH and necrosis of the central nervous system are moderated by treatment of the dams with vitamin E (42).

One factor thought to be important in the pathogenesis of PVH is impaired autoregulation of cerebral blood flow (21), and in many preterm babies it is likely that cerebral blood flow is pressure passive. The subependymal region (germinal matrix) of the newborn beagle pup is relatively poorly perfused and vulnerable to ischemic injury (43); experimentally induced hypovolemic hypotension followed by volume reexpansion provokes PVH (44). We speculate that vitamin E, which is a free-radical scavenger, protects against PVH by trapping free radicals generated during ischemic injury of the subependymal layer. This would limit progression of tissue damage (45) and limit the magnitude of hemorrhage on reperfusion, which is consistent with our
findings that vitamin E did not prevent SEH but presumably rapidly limited its extent. The strongest evidence supporting this theory is derived from experiments in newborn beagles, which showed that the free-radical scavenger, superoxide dismutase, protected against PVH in pups exposed to hemorrhagic hypotension and volume reexpansion (46).

Another source of potentially damaging free radicals occurs during the production of prostaglandins by the cerebral microvasculature when arachidonic acid is converted to endoperoxide compounds [cyclo-oxygenase pathway (47)]. It is relevant that the prostaglandin synthesis inhibitors indomethacin (48) and ethamsylate (49) also protect against PVH in newborn beagles despite the disturbance in cerebral blood flow in this experimental model. Both indomethacin (50) and ethamsylate (51) have been reported to reduce the incidence of PVH in preterm humans.

Overall mortality was uninfluenced by vitamin E supplementation, but among babies who died the incidence of IVH or parenchymal hemorrhage was significantly lower in the supplemented group. This observation should be interpreted cautiously because in ill preterm babies, the precise contribution of hemorrhage to the cause of death is often difficult to determine. What is certain is that surviving supplemented babies had a lower incidence of IVH and parenchymal hemorrhage. The potential importance of this observation must wait until sufficient time has elapsed for the neurodevelopmental assessment of survivors; however, we have estimated the magnitude of any potential benefit assuming that our vitamin E supplementation regimen was widely adopted throughout England and Wales. A reasonable assumption is that 1% of all live births are \( \leq 32 \) weeks gestation and that 70% survive the neonatal period. Based on an annual number of live births in England and Wales of 650,000, about 4,550 would be neonatal survivors of 32 weeks gestation or less. The 95% CI for the reduction in intraventricular or parenchymal hemorrhage among survivors in our study was 9.9% to 33.8%. Thus one might anticipate that if our results were applied to England and Wales, then among the neonatal survivors there would be about 450 to 1,540 fewer babies with these forms of hemorrhage. Aside from the potential reduction in the occurrence of neurodevelopmental handicap, including that generated by posthemorrhagic obstructive hydrocephalus, there are other subtle potential benefits. For example, many pediatricians advise against whooping cough immunization for preterm babies whose brain scans have shown PVH.

The optimal vitamin E supplementation regimen for protection against PVH needs to be determined. Our data show that the plasma vitamin E level is not a reliable indicator of protection, inasmuch as mean levels at different times in supplemented or control babies did not distinguish those with and without hemorrhage. The choice of a suitable supplementation regimen is further complicated by the fact that there are different vitamin E preparations available that may be given orally, intramuscularly, or intravenously, and
there are conflicting reports about their efficacy in raising plasma vitamin E levels as well as about their toxicity. The three babies with radiologically confirmed necrotizing enterocolitis in the present study were all supplemented, but their highest plasma vitamin E levels were disparate. Finer et al. (52) observed an association between orally administered α-tocopherol acetate (200 mg/kg/day) and necrotizing enterocolitis; plasma levels were <3.5 mg/dl in the few babies in whom measurements were available. Although the intravenous route produces the most rapid increase in plasma vitamin E, this cannot be recommended until concerns about serious toxicity have been resolved (53,54).

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REFERENCES


