0031-3998/87/2201-0016$02.00/0
PEDIATRIC RESEARCH
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Vol. 22, No. 1, 1987
Printed in U.S.A.

Disposition of Vitamin E in the Eye[1]

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ABSTRACT. To study the effect of exogenous vitamin E on its plasma and eye tissue levels, we administered 100 mg/kg of dl-α-tocopherol either by intravenous, intramuscular, or oral routes in 3-day-old newborn kittens. Controls did not receive the drug. α-Tocopherol levels in the plasma, retina, choroid, and vitreous were analyzed by high-performance liquid chromatography at selected intervals following drug administration. Our results showed that after intravenous administration of vitamin E, the retinal α-tocopherol levels increased 300 and 500% the baseline value at 2 and 4 h, respectively. By contrast, comparable retinal levels were achieved at 24 h following intramuscular administration and 72 h following oral administration. In the intravenous group high retinal levels were maintained up to 168 h, while in the intramuscular and oral groups retinal levels continued to increase up to 192 h. Irrespective of route of administration, the retinal concentration of α-tocopherol remained high even when the plasma levels were declining; plasma levels did not reflect retinal levels at any time. Following exogenous vitamin E administration, α-tocopherol levels increased both in the choroid (4 to 6 times the control) and in the vitreous (1.5 to 4 times the control). Significant differences in tocopherol levels were also seen between the eyes in the same animal in all groups. Our studies suggest 1) a single dose of vitamin E by intravenous, intramusuclar, or oral routes increases retinal levels from 3 to 5 times the control levels. However, peak retinal levels are achieved earlier by parenteral than by the oral route. 2) In addition to the retina, a significant amount of vitamin E is transferred to the choroidal and vitreal tissues. 3) Plasma level does not reflect retinal level. 4) Following vitamin E supplementation by any route, the retinal α-tocopherol levels could vary significantly between the eyes in the same animal. (*Pediatr Res* 22: 16–20, 1987)

Abbreviations

ROP, retinopathy of prematurity
IV, intravenous
IM, intramuscular
HPLC, high-performance liquid chromatography
ANOVA, analysis of variance

Studies using vitamin E to reduce the severity of ROP have reported contradictory results (1–5), while those attempting to alleviate bronchopulmonary dysplasia have failed to demonstrate any significant benefit from this drug (6, 7). Vitamin E was administered in these studies for its antioxidant effect in the retina and the lungs, respectively (8, 9). Although the local concentration of α-tocopherol is considered crucial for its antioxidant effect, little is known about the disposition of vitamin E in the eye tissue following exogenous administration. Nishida and Tagari (10) reported that in preterm neonates, retinal levels increased 30 h after intramuscular administration of vitamin E; however, plasma levels were not presented herein. We measured α-tocopherol levels in the retina, choroid, and vitreous obtained at autopsy from 12 preterm infants (11). In these cases high serum levels were associated with high retinal levels, but because of wide variation in the time of oral vitamin E administration and the age at death, no correlation could be established between the serum and retinal values. Other than these two studies, to our knowledge, there are no reports examining retinal levels of vitamin E in human neonates. Although both oral and parenteral preparations of vitamin E have been used in clinical studies, the influence of the route of administration on its target tissue disposition is not known.

To investigate the plasma kinetics and eye tissue disposition of vitamin E when administered by different routes, we carried out the following studies in a kitten model. Because their retinal development matches that of the preterm infants' retina (12, 13), this model has been used in many ROP studies. The study aims were: 1) quantitate α-tocopherol levels in the retina, choroid, and vitreous following administration of vitamin E by IV, IM, and oral routes. 2) Study the rate of change in these levels and in plasma. 3) Examine the relation between the plasma and eye tissue levels.

MATERIALS AND METHODS

Pregnant queens bred and housed in the Biological Resources Laboratory of the University of Illinois were used in our studies. The animals were fed Purina Cat Chow (Purina, St. Louis, MO), given water *ad libitum*, and were observed twice daily. After delivery the newborn kittens were housed with the mothers and were let to suckle freely.

On the 3rd day of age kittens from each litter were randomly assigned to different treatment groups or control. The injectable form of vitamin E, (Hoffman-La Roche Laboratories, Nutly, NJ), containing 50 mg/ml α-tocopherol dissolved in an aqueous mixture, ethyl alcohol (10%), propylene glycol (10%), and Emulphor EL-620 (10%), were used in the treatment. A single dose of 100 mg/kg vitamin E was administered either by IV using a 25-gauge scalp vein needle via the femoral vein (IV group), by IM injection at a site in the anterior aspect of the thigh (IM group), or orally using an orogastric tube, (oral group). The scalp vein needle and the orogastric tubes were flushed with 0.5 and 1.5 ml saline, respectively. At least one kitten from each litter did not receive the drug (control group). Kittens were sacrificed using ether anesthesia at 4, 8, 24, 72, 120, 168, and 192 h following drug administration. In addition in the IV group, some kittens were sacrificed at 1 and 2 h to determine the early drug effect. Immediately after sacrifice, blood was collected and plasma was

Received November 14, 1986; accepted January 28, 1987.
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Supported in part by the National Institutes of Health Grant NIH-AM 39855 and PHS-BRSG 82526.
[1] Presented in part at the Society for Pediatric Research Meeting, San Francisco, May 1984.

separated, and the eyes and other organs were removed. Plasma and the organs were frozen and stored at −80° C in the dark for later analysis (14). Our preliminary studies showed that with this method of collection and storage of specimen, no significant loss of vitamin E occurs.

The frozen eyes were dissected to separate various parts. The anterior chamber was cut and the lens was removed followed by the removal of vitreous *en bloc*. The retina and the choroid were then separated under microscopic dissection. The retina, choroid, and vitreous from each eye were stored separately.

Vitamin E assays. The plasma α-tocopherol level was measured using an HPLC method (15). One hundred μl of plasma was mixed with 10 μl of 25% ascorbic acid (pH 4.5); the latter acidifies the sample and acts as an antioxidant (16). Four μg of vitamin E acetate was then added as an internal standard. The vitamin E was extracted with 1.5 ml heptane and the mixture was centrifuged, following which the heptane was evaporated using a vacuum centrifuge (Savant Instrument Co., Farmingdale, NY) and the resulting residue was dissolved in 100 μl of methanol. Recovery studies have shown that the extraction efficiency for vitamin E is greater than 90%. Quantitation of vitamin E was performed by HPLC using a Waters Radial-Pak C8 cartridge column eluted with 97% methanol, 3% H_2O at 1 ml/min. A Hitachi Spectrophotometer was used to measure the UV absorbance at 292 nm. The retention times for α-tocopherol and vitamin E acetate were 3.3 and 3.9 min, respectively. The concentration of α-tocopherol (per volume of plasma) was determined from a standard curve using the tocopherol/vitamin E acetate peak height ratio. The lower limit of sensitivity for this assay based on analysis of standards was 50 ng vitamin E.

Vitamin E was extracted from each of the eye tissues using 2.5 ml Fisher HPLC acetone. After vortex mixing and sonication to solubilize the vitamin E, the sample was centrifuged and the acetone supernatant was retained for vitamin E assay. Recovery studies with adult eye tissues have determined that the vitamin E extraction efficiency is greater than 96%. The remaining pellet was heated to 55° C under N_2 gas to remove acetone in the sample. The residue was dissolved in 1 N NaOH for protein determination (17). Two μg vitamin E acetate were added to the acetone supernatant as an internal standard. The solvent was removed using N_2 gas at 50° C and the residue redissolved in 100 μl methanol. Vitamin E was chromatographed by HPLC at 1 ml/min using a Waters Radial-Pak C8 cartridge column eluted with 94% methanol, 6% H_2O, and 2 g/liter tetraethyl ammonium perchlorate (Frederick Smith Chemical Co., Columbus, OH). Vitamin E was monitored using an ESA coulochem electrochemical detector with an oxidizing potential of +0.25 V. The vitamin E acetate was monitored on a Hitachi UV Spectrophotometer (at 292 nm) connected in series with the electrochemical detector. The HPLC retention times for α-tocopherol and the internal standard were 4.0 and 5.0 min, respectively. Since peaks for both agents were symmetrical, quantitation was made by comparison of peak heights. Detector response and the calibration curves were linear over 1 to 10 ng/ml range, and the minimal amount of α-tocopherol detectable at a mean signal to noise ratio of 3 was 1 ng/ml.

To assess the reproducibility and interassay variability, standard curves of retinal α-tocopherol were developed. At least 10 separate concentrations of α-tocopherol (0.001 to 10 μg) were added to the retinal tissue before processing to develop each standard curve. In Table 1 the linear regression analysis of the individual data points for five separate standard curves (the ratio of α-tocopherol peak height/internal standard peak height *versus* the concentration of α-tocopherol is shown. The mean slope was 1.01×10^{-4}, the intercept was -1.105×10^{-3}, and coefficient of determination (r^2) was 0.999.

Levels of α-tocopherol in the plasma, retina, vitreous, and choroid from individual groups were analyzed using a best curve fitting procedure (18). Intergroup comparison of retinal levels as a function of time was studied using a regression ANOVA (19).

Table 1. *Standard curves of α-tocopherol in retinal tissue**

Assay	n	a	b	r^2
1	10	-1.2×10^{-3}	9.44×10^{-5}	0.9998
2	10	-1.04×10^{-3}	1.04×10^{-4}	0.9999
3	10	-1.14×10^{-3}	1.10×10^{-4}	0.9999
4	10	-1.046×10^{-3}	1.03×10^{-4}	0.9999
5	10	-1.09×10^{-3}	9.35×10^{-5}	0.9999
Mean	10	-1.105×10^{-3}	1.01×10^{-4}	0.9999

* Linear regression analysis from five data sets using the peak height ratio of α-tocopherol/α-tocopherol acetate (Y) and concentration of α-tocopherol added to the tissue (X). Duplicate determinations of the n samples shown above were used for each calibration curve. a = Y intercept, b = regression slope, and r^2 = coefficient of determination.

Paired t test and a variance ratio test (20) were used to examine the difference in the retinal vitamin E levels between one eye to the other. A p value of 0.05 or less was required to declare the observed difference statistically significant.

RESULTS

Twenty-two kittens in the control, 24 in the IM, 21 in the IV, and 22 in the oral groups were studied.

Plasma α-tocopherol levels in three treated groups were significantly higher than in the control group; the latter showed no significant change over time (Fig. 1). In the IV and oral groups, the levels dropped as an exponential function of time. In the IV group the best fitting regression model for predicting plasma α-tocopherol level (Y in mg/dl) as a function of time was $Y = 110 \times X^{-0.91}$ (X = time in h, $r = 0.98$, $p < 0.001$) and in the oral group it was $Y = 25 \times X^{-0.4}$ ($r = 0.78$, $p < 0.001$). In the IM group the plasma levels increased up to 8 h followed by a drop up to 192 h. However, although significantly higher than the control group, these changes over time were not statistically significant.

Retinal α-tocopherol levels were significantly higher in all three-treated groups as compared to the controls, in whom no change occurred over time (Fig. 2). By 4 h following administration in the IV group, the retinal concentration of α-tocopherol had increased more than 400% of control group: 173 ± 39 (SEM) *versus* 40 ± 7.5 μg/g of protein ($p < 0.001$). However, in the IM and oral groups comparable retinal concentrations of α-tocopherol were noted at 24 and 72 h after therapy, respectively.

In the IV group, at 1 h following treatment, the retinal α-tocopherol levels were two times the control value (72.5 ± 21.0 *versus* 35 ± 2.5 μg/g of protein, $p < 0.001$). High retinal levels ranging from three to five times the control were maintained without any statistically significant change over time. In the IM and oral groups, however, the retinal concentrations increased as an exponential function of time. The best fitting model for predicting retinal α-tocopherol level (Y, in μg/g of protein) following one dose of IM vitamin E, as a function of time (X, in h) was $Y = 48 \times X^{0.24}$ ($r = 0.36$, $p < 0.05$) and for oral group it was $Y = 23 \times X^{0.41}$ ($r = 0.66$, $p < 0.001$).

Intergroup comparison of retinal levels, the route of administration, and time interactions revealed that the rate of rise and the final retinal concentration in the oral group was significantly higher than in all other groups (ANOVA, $p < 0.001$).

No statistically significant correlation was found between the plasma and retinal α-tocopherol levels in any treated group. When analyzed in an ANOVA regression model (which included the three treated groups, time, and interaction effects) the plasma levels explained 2–4% variance in the retinal levels: an increase of r^2 by 0.02 in the IV, 0.03 in the IM, and 0.04 in the oral groups, respectively (p = NS).

Variation in the retinal α-tocopherol levels noted between one eye to the other in 56 pairs of eyes are shown in Table 2. Although

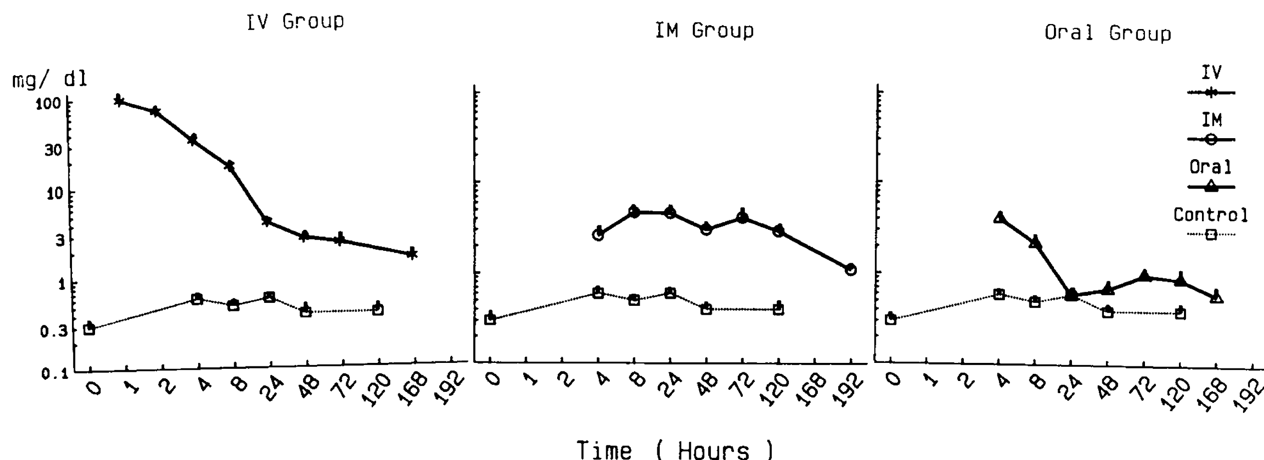

Fig. 1. Plasma α-tocopherol levels (mean ± SEM) in the IV, IM, and the oral groups shown in *bold lines*. The control data (*dotted lines*) are plotted in all three *panels* of the figure for comparison. The levels were significantly higher in all three treated groups as compared to the control. Both in the IV and oral groups the plasma levels declined as an exponential function of time, whereas no change occurred in the IM group. Regression equations are given in the text.

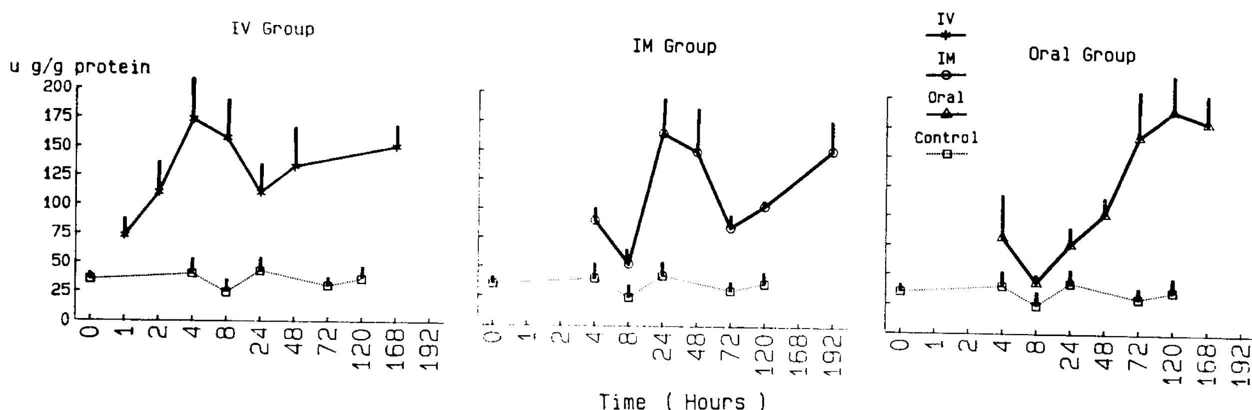

Fig. 2. Retinal α-tocopherol levels in the IV, IM, and the oral groups, and in the control. High retinal levels were achieved in the IV group by 2 h and no significant change occurred over time; in the IM and oral groups the levels increased as an exponential function of time. (Regression equations are in the text. Group symbols and abbreviations are as in Fig. 1.)

Table 2. *Differences in retinal α-tocopherol levels between eyes**

	Control	IV	IM	Oral
No. of pairs of eyes	11	17	15	11
Mean Difference (± SD) (μg/g of protein)	21.9 ± 13	64 ± 51	37.1 ± 29	38.4 ± 40

* The difference in the retinal α-tocopherol levels between one eye to the other in individual animals was significant in all four groups (paired *t* test, $p < 0.05$). The above mean values in the vitamin E-treated groups (IV, IM, and oral) were significantly larger than that of the control mean difference (ANOVA and Newman-Keuls test, $p < 0.05$). The variances in the treated groups were also significantly larger than the variance of the control group [variance ratio test (20), $p < 0.05$].

there was a statistically significant difference between one eye to the other even in the control group, the difference was higher and the variances larger in the vitamin E-treated groups. The SD values were 79.7, 78.2, and 104.2% of the mean difference in the IV, IM, and oral groups, respectively, as compared to 59.4% of the mean difference in the control group ($p < 0.005$).

As shown in Figures 3 and 4, the choroidal and vitreal levels of α-tocopherol were significantly higher in all the treated groups as compared to the control ($p < 0.001$). In the IM group the change over time occurred as an exponential function of time. The best fitting model for predicting vitreous level, (Y, in μg/g of protein), was $Y = 113 \times X^{0.005}$ (X = time in h, $r = 0.58$, $p < 0.001$). The best fitting model for predicting the choroidal levels was $Y = 24.26 \times X^{0.006}$ ($r = 0.56$, $p < 0.001$). The changes in the α-tocopherol concentrations in the choroid and vitreous over time in the IV and oral groups, however, were not statistically significant.

DISCUSSION

The natural antioxidant properties of vitamin E has prompted its clinical use in premature infants. Despite controversy about its efficacy, however, the oral form of this drug is used as a routine in many centers for the prevention of severe ROP. Although the optimum retinal level for antioxidant effect is not known, a need for monitoring plasma levels has been stressed to minimize the associated complications (21–23).

Several aspects of our results raise questions relevant to the clinical use of vitamin E. Although exogenous vitamin E clearly increased retinal levels in all groups, the peak retinal concentration and its change over time were highly influenced by the route of administration. Most rapid increase in the retinal levels occurred in the IV group; three times the control within 2 h and four times the control by 4 h of administration. These high concentrations remained steady up to 192 h. Comparable concentrations were achieved in the IM and oral groups at much later times; by 24 and 72 h after administration, respectively. If

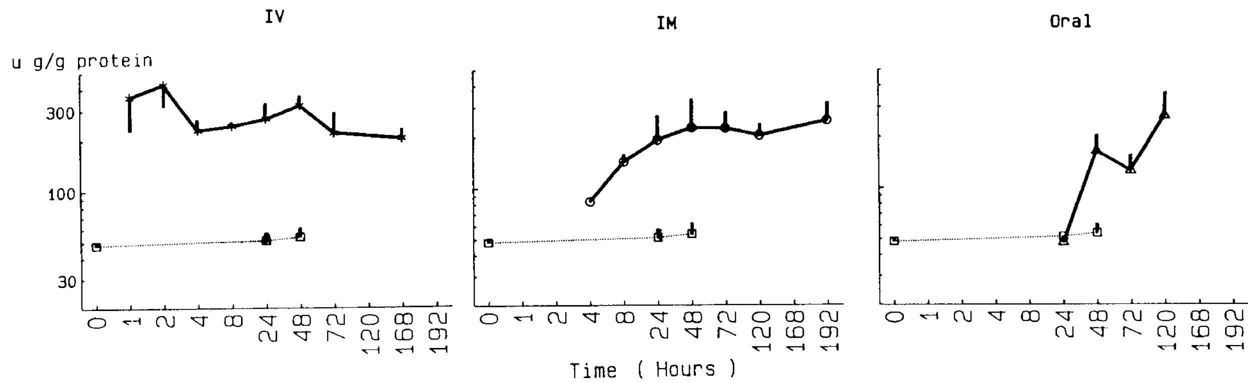

Fig. 3. Choroidal α-tocopherol levels in the IV, IM, and the oral groups, and in the control. The levels were significantly higher in the treated groups as compared to the control. The changes over time were statistically significant in the IM group (regression data in the text) but not in the IV and oral groups. (Symbols and abbreviations are as in Fig. 1.)

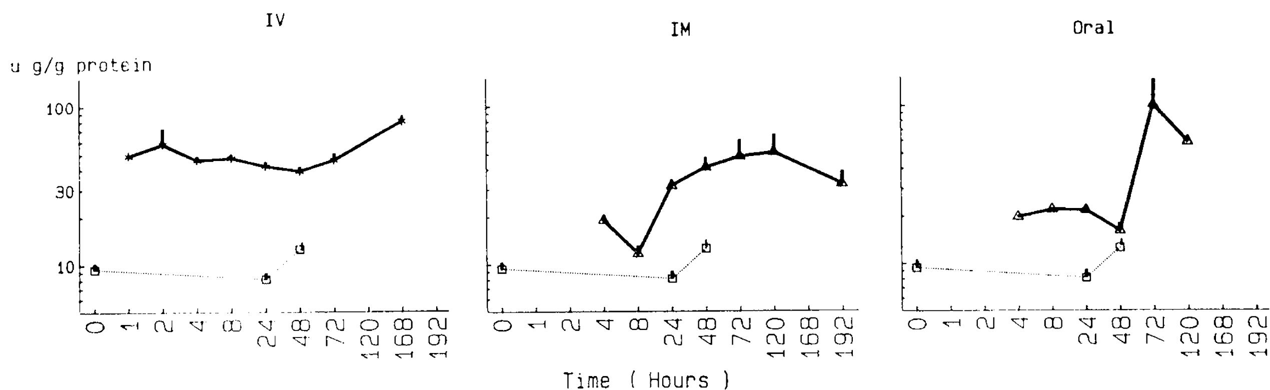

Fig. 4. Vitreal α-tocopherol levels in the IV, IM, and oral groups, and in the control. The levels were significantly higher in the treated groups as compared to the control. The changes over time were statistically significant in the IM group (regression data in the text) but not in the IV and oral groups. (Symbols and abbreviations are as in Fig. 1.).

in human infants the retinal disposition of vitamin E is similar, oral administration on the 1st day of life may not raise the retinal level up to 3 days; a time period perhaps with the greatest need for optimal antioxidant effect of vitamin E. Although the most critical time for achieving appropriate retinal concentrations of α-tocopherol level is unknown, Kretzer *et al.* (24) suggest that to be efficacious, vitamin E supplementation must be initiated at birth. The recommendation was based on their observation of rapidly increasing retinal gap junctions, within 4 days of exposure to oxygen in premature infants. Other than to suggest that in the kitten, parenteral administration of vitamin E did result in earlier peak retinal concentrations as compared to the oral route, we cannot make definitive clinical recommendations based on the results of our studies. Human studies examining the effect of route of administration on the retinal tissue disposition are needed to establish clinical guidelines, and in addition, at present there is no vitamin E preparation approved for parenteral use.

We are unable to explain the reasons for the influence of the route of exogenous administration on the retinal concentrations of vitamin E. It is possible that the measured retinal levels may in part reflect vitamin E in the retinal capillaries containing high plasma levels during the first 8 h following IV administration. However, the retinal levels remained steady despite falling plasma levels in the IV group. Further, despite steady plasma concentrations in the IM group and falling levels in the oral group the retinal concentrations continued to rise, suggesting that variations in plasma levels cannot fully explain the reason for the differences in retinal concentrations in the three treated groups.

From the postmortem evaluation of eyes in human infants we have previously demonstrated that retinal levels were high in babies treated with vitamin E as compared to untreated infants (11). However, no definite correlation between the plasma and the retinal concentrations could be established. The lack of relation between the plasma and retinal levels in the present study corroborates this finding. Monitoring plasma concentrations may help prevent toxic complications, but since the disposition of vitamin E in the retina cannot be estimated from its plasma concentration, neither the adequacy nor the duration of treatment can be determined from the plasma levels.

The striking variation in the retinal levels noted between the pairs of eyes may suggest that the metabolic patterns may vary between the individual eyes. Similar variations in α-tocopherol concentrations were also noted between the pairs of eyes in our clinical study (11). Whether different degrees of ROP between the pairs of eyes often found in infants can be due to a variation in the extent of vitamin E uptake is an interesting speculation that requires further studies for confirmation.

The clinical significance of high concentrations of vitamin E in the choroid and vitreous is yet unknown. However, rich blood supply to the retina as opposed to the vitreal tissue and the presence of a high amount of polyunsaturated fatty acids on the retinal photoreceptor membranes can explain why the retinal levels were much higher than the vitreal levels (25). In a recent study the cellular incorporation of α-tocopherol was shown to be dependent on the transport of low density lipoprotein (26), suggesting that an active mechanism may be involved in the uptake of vitamin E by the retina. This may explain high retinal levels of α-tocopherol achieved in all three treated groups.

Although we chose 100 mg/kg vitamin E to match a single daily dose used in human infants, our study design differs in many aspects from the routine clinical use. Preterm infants

receive the total daily dose of vitamin E in three to six divided doses; the drug is used daily for several weeks, and perhaps most importantly, as opposed to healthy kittens, the preterm infants are likely to be quite ill during the first days of life. Because these factors could significantly influence drug absorption from the gut and the muscle, the retinal tissue disposition of vitamin E in babies could be different from the pattern noted by us in the newborn kittens. Therefore we suggest caution before extrapolating our results to newborn infants. More studies are needed to further our understanding of vitamin E kinetics in the human retina.

Acknowledgment. We greatly acknowledge the assistance given by Dr. H. N. Bhagwan from Hoffman-LaRoche, Inc Nutley, NJ.

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