VITAMIN E SUPPLEMENTATION AND THE RETINOPATHY OF PREMATURITY

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This paper will describe a series of studies on the relationship of vitamin E nutrition on the incidence, severity, and long-term outcome of retrolental fibroplasia (RLF) and the further effect of treatment with vitamin E at varying dosage levels and time schedules. The work was done from 1968 to 1980, over which time period the incidence of clinically significant vitamin E deficiency anemia among premature infants (birth weight under 2,000 g) raised on standard formulas and multivitamin drops decreased from something in the range of 20% to less than 1%. The studies involved the premature and intensive-care nurseries of Pennsylvania Hospital under the late Thomas R. Boggs and the outpatient ophthalmology practice of Drs. David Schaffer and Graham Quinn at the Children's Hospital of Philadelphia. Statistical consultation over the 12-year period was provided by Donald Goldstein, Mari Jo Mathis, and Chari Otis. Because it is now generally agreed that RLF, especially in its more common, low-grade form, is more properly referred to as a retinopathy of prematurity, that term and the abbreviation ROP will be used in this paper.

METHODS AND APPROACH

Development of Classification and Scoring Systems for Relating Visual Outcome to Acute Stage Disease, Neonatal Risk Factors, and Therapeutic Interventions

Since 1968, Dr. David Schaffer has been following all premature infants cared for in the Pennsylvania Hospital nurseries with weekly or biweekly eye exams using the indirect ophthalmoscope. Since about 1972, he has supervised a similar surveillance program in the nurseries of the Children's Hospital of Philadelphia and the Hospital of the University of Pennsylvania. At each exam, a description of the retinal findings as well as an assessment of the stage of retinal maturity or grade of ROP was recorded. Examinations were continued until complete regression of active disease or retinal vascular maturity was documented.

If necessary, the surveillance program was continued in the outpatient department. On the basis of this large body of clinical experience, Dr. Schaffer, and later Dr. Quinn, who joined his program in 1977, modified the original classification of Reese, King, and Owens[1,2] to include findings in far peripheral

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disease and clearly define the characteristics of low-grade ROP for both acute and cicatricial stages. The distinguishing feature of active grade 2 disease in their classification is the demarcation line of Flynn: that of active grade 3, extraretinal neovascularization (ERNV). An initial version of the classification for acute and cicatricial stages of the disease was included in our 1974 report on ROP at Pennsylvania Hospital from 1968 to 1972 and on preliminary results of the 1972 to 1974 clinical trial of the effects of prophylactic vitamin E.[1]

In 1979 the classification system per se was published in an updated version.[2] Two patterns of disease were described. One, by far the most common, is slowly progressive, does not usually exceed a severity of grade 2 (active), and often involves only part of the retina (segmental, as opposed to circumferential, disease). The other is uncommon and characterized by the presence of early dilatation and tortuosity of the posterior pole retinal vessels (i.e., during active grades 1 and 2), a tendency to circumferential involvement of the retina, and rapid progression to severe disease with extensive ERNV and retinal detachment. Disease of this more serious form is termed "plus" ROP, i.e., grade 2-plus active, grade 3-plus active, etc. The most advanced form of grade 2-plus active is defined by gross dilatation and tortuosity of posterior pole vessels and a circumferential (360°) demarcation line. The most advanced form of grade 3-plus active has these features plus extensive four-quadrant ERNV. Regression from grade 3-plus active ROP with extensive ERNV in two or more quadrants is very rare, an outcome of legal blindness or worse occurring in about 70% of victims. Active grades 4 and 5 ROP (partial and complete retinal detachment, respectively, or progressive fibrovascular overgrowth) are reached through the common pathway of 3-plus active disease.

An *incidence severity index* or mean severity ROP score was developed to describe, in one figure, the incidence and severity of acute stage ROP. In its simplest form, it consists of the sum of the highest grade of acute ROP, including 0, in the fellow eyes of each baby. This sum is divided by 1 to give the mean severity score for an individual and by the total number of babies to give the group mean severity. Plus ROP adds 0.5 to the numerical grade, e.g., grade 2 active = 2.0, and grade 2-plus active = 2.5.

The *cicatricial classification* has been addressed with similar care, since the degree of cicatrix is closely correlated with visual outcome. The higher grades of cicatricial (Cic) ROP—grade 3 Cic, in which there is a retinal fold, and grades 4 and 5 Cic, with retinal detachment and fibrous overgrowth—are unequivocal and easily recognized. So also are the more severe examples of grade 2 Cic (dragging of the retina toward a peripheral scar with varying degrees of macular heterotopia). Visual outcome with grade 2 Cic can vary widely depending on the extent and location of scarring. Grade 1-plus Cic, consisting of a far peripheral scar, is a hard finding, but the scar, if small, is easily missed. A wide range of refractive errors, often with anisometropia, is found in association with grade 1-plus Cic. Grade 1 Cic consists of several soft findings, no one of which is diagnostic of previous ROP and not all of which are necessarily present in any one infant. These consist of somewhat tortuous posterior vessels, irregular pigmentation in the region of the previous demarcation line, fine vitreoretinal adhesions, myopia (sometimes severe), and anisometropia. A checklist was worked out to define the constellations of findings that could be called grade 1 Cic ROP. This is important when addressing the matter of sequelae following "regressed" low-grade ROP. In our experience, when regression is complete, as defined by an absence of all cicatricial findings, there is no visual morbidity that can be distinguished from that in the general premature population in whom ROP did not develop in the nursery.[28]

TABLE 1

SCORING SYSTEM FOR LONG-TERM FOLLOW-UP OF ACTIVE ROP

	Right Eye	Left Eye
Grade cicatricial ROP	0 to 5	0 to 5
Refraction or visual acuity*		
Myopia (−0.25 to −6.00) (correctable to 20/30)	1 to 3	1 to 3
Visual acuity (corrected)		
20/40 to 20/200	4 to 8	4 to 8
Count fingers to NLP†		
Binocular—strabismus, anisometropia, amblyopia	0 to 3	

*Visual acuity deficit is estimated in children under age three years.
†NLP = no light perception.

Finally, an outcome or visual morbidity score was developed. This includes grade of cicatricial disease, refractive error or visual acuity, and presence or absence of anisometropia, amblyopia, and strabismus (TABLE 1). This score is a summation of findings in each eye. Therefore, a child who is blind in one eye but has useful vision in the other can be adequately described (see TABLES 1 and 2). In the more severe grades of active ROP, disparate findings in fellow eyes are not uncommon in both active and cicatricial stages.

The visual morbidity score has been useful in assessing the following:

1. The relationship of pattern and severity of acute stage disease to visual outcome.

TABLE 2

CLINICAL RANKING, MORBIDITY SCORE AND OPHTHALMOLOGIC FINDINGS AT ONE TO TWO YEAR FOLLOW-UP

Clinical Ranking	Morbidity Score	Representative Clinical Sequelae following Active ROP
A to C	0-8	Cicatricial ROP grade 1 to 1.5. Minimal to no visual handicap. Mild to moderate myopia.
D	9-11	Grade 1.5 to 2 cicatricial ROP; moderate to high myopia. Anisometropia/amblyopia/strabismus.
E	12-14	Grade 2 Cic with high myopia correctable to 20/20 vision. Anisometropia/amblyopia/strabismus.
F	15-17	Cicatricial ROP grade 2 to 3 with high myopia. Visual acuity correctable to only 20/200 in one eye. Anisometropia/amblyopia/strabismus.
G	18-20	Grade 2 to 3 Cic in one eye with corrected visual acuity 20/200. Fellow eye grade 4-5 cicatricial with light perception only.
H	21-22	Grade 4-5 Cic in one eye with light perception at best. Fellow eye grade 3 Cic with count finger vision.
I	23-26	Grade 4-5 Cic in both eyes with or without light perception.

2. The effect of treatment on visual outcome per se and as it relates to changes in pattern and severity of acute stage disease.
3. The relationship of visual outcome to the evolution of premature intensive-care techniques, survival of increased numbers of very immature infants, and the improved ability to recognize and supply the nutritional needs of the critically ill and growing premature.

Survey of Changes in Commercial Formulas Used to Feed Premature Infants—1968 to 1978: Relation to Measured Levels of Vitamin E in Serum in Premature Infants

Since 1968, records have been kept of product labels and manufacturers' brochures with regard to tocopherol, polyunsaturated fatty acid (PUFA), and iron content of commercial formulas used in our nurseries.

Since 1972, we have routinely measured the serum total tocopherol content of premature infants admitted to our nurseries soon after birth and at two- to three-week intervals thereafter. The micro method of Hashim was used.[3] Red blood cell (RBC) hydrogen peroxide fragility and RBC malondialdehyde (MDA) studies have been done in representative infants by the methods of Gordon and Stocks respectively.[4,5]

Clinical Studies

Since 1968, a surveillance of the incidence and severity of ROP at Pennsylvania Hospital nurseries has been maintained by Drs. Schaffer and Quinn by means of weekly or biweekly eye exams using the indirect ophthalmoscope. Examinations are continued until the retinal vasculature is mature or active retinopathy has regressed or stabilized at some degree of cicatrix. Infants with cicatrix are maintained in continuous long-term follow-up as suggested by Tassman.[6]

January 1, 1968 to February 1, 1972: Background Surveillance

The initial surveillance of incidence and severity of RLF in the Pennsylvania Hospital nurseries covering the period from January 1968 to February 1972 has been described in detail elsewhere.[1] Of the infants who developed active ROP (most of which appeared to regress completely), 40% returned for long-term follow-up between one and two years after a term birth date. It is this follow-up that is of particular importance to this paper.

February 1, 1972 to May 1, 1974: First Clinical Trial of Prophylactic Vitamin E for ROP

Single-blind, alternate infant enrollment, using the Hoffmann-La Roche preparation of parenteral *all-rac-α*-tocopheryl acetate or its placebo on investigational new drug (IND) permit. Weekly or biweekly ophthalmologic examinations with long-term follow-up.

All infants at Pennsylvania Hospital with birth weights under 2,001 g regardless of oxygen need and over 2,001 g with a gestational age under 36 weeks if

oxygen therapy were required for over 24 hours were eligible to enter the study. Twins were enrolled as twins even if the larger exceeded the birth weight limit of eligibility. Infants whose parents did not wish to participate in the study or who could not be approached for informed consent until after age 48 hours were admitted to a no-injection control group. Ophthalmologic information was therefore available on all infants. Once informed consent was signed, infants were enrolled within birth weight groups (≦ 1,000 g, 1,001–1,500 g, 1,501–2,000 g, > 2,000 g) and in order of admission to the nursery. Of infants with birth weight 2,000 g or less, 65 to 70% were inborn. Assignment to treatment group was made by nurse research assistants not involved in patient care by entrance of the infant's name on lined enrollment sheets, which they alternately assigned to medication "A" or "B" (vitamin or placebo respectively). If an infant died before the next succeeding line had been filled, that line was assigned to the same treatment (either A or B) as its predecessor. This was done in an effort to assure a more equal distribution of survivors between treatment groups among low birth weight infants whose chances for survival were still poor. (During the two-year period from 1971–72, the mortality rate at Pennsylvania Hospital among inborn and outborn infants was 60 and 70% respectively.) A similar adjustment was made for lines assigned to infants who died before receiving a first eye exam.

Pediatricians, parents, and, in particular, ophthalmologists were completely blinded as to treatment group. Orders were written for medication "A" or "B," which was packaged in identical, single-dose, dark-colored, appropriately labeled vials. Injections were given by the clinical care nursing staff.

Infants enrolled as uninjected controls (group C) were managed in exactly the same way as study infants from the standpoint of nursery routine and serial eye exams. Serum E analyses, however, were done only as indicated on a clinical basis, i.e., once soon after admission to the nursery and at two- to three-week intervals thereafter, when other hematologic tests were being run.

Extensive data were abstracted from the hospital charts for all three groups of babies, including birth weight, gestational age, parity, Apgar score, clinical diagnoses at admission, extent and duration of oxygen and ventilator therapy, arterial blood gas measurements, volume of transfused blood required, complications during the hospital stay, etc.

The dosage of intramuscular medication was 15 mg (0.3 ml) per kg, the initial injection almost always being given within 24 hours of birth. This was repeated at 6- to 12-hour intervals until a serum E level of 1.5 mg/dl was obtained (usually by age 48 hours). A serum E level in the range of 1.5 to 2.0 mg/dl was then maintained by intramuscular (IM) injections given every one, two, or three days as needed. The details of this dosage schedule have been previously published.[1] Placebo-injected infants received comparable volumes at comparable time intervals. When oral feedings were well established, oral medication was used to supplement and then supplant intramuscular medication. However, *no* oral placebo was available. Treatment was continued until the eyes were mature or active retinopathy had regressed. Treatment was not continued after hospital discharge. Serum E analyses were performed prior to the first injection of study medication, daily until stable, and then twice weekly until hospital discharge. The microcolorimetric method of Hashim,[3] which required between 50 and 100 λ of serum or plasma, was used. Red blood cell H_2O_2 fragility studies were done on the same sample of blood at weekly or biweekly intervals by the method of Gordon.[4]

Ophthalmologists, who were in the nursery only once a week early in the morning, could not distinguish between infants receiving injections and those who did not (i.e., between A and B versus C babies) and had no idea of treatment

group assignment with regard to vitamin versus placebo. Nurses giving the injections may have surmised the treatment group assignment, however, since the code was simple. For these reasons the importance of keeping parents and ophthalmologists unaware of treatment group assignment, i.e., "blinded," was repeatedly stressed to the nurses by the research staff. We know of no instance of "unblinding" of the ophthalmologists during either the intake or follow-up phase of the study.

Parents were asked to return for a follow-up exam between their infant's ages one and two years. This procedure was followed whether or not an infant had had ROP in the nursery. However, a consistent effort to maintain the sample was made only among infants who had had signs of retinopathy while in the nursery, since this group was smaller in number and more important.

The incidence and severity of acute stage ROP (severity being defined as sum of ROP grade for fellow eyes) and the visual morbidity at age one to two years were used to compare treatment groups. The mean severity score for a group is defined as the ROP grade sum for each baby, including 0, divided by the number of babies in the group and is therefore a measure of both incidence and severity. The visual morbidity score is defined in TABLE 1.

Intake to this first study, including intake to group C (uninjected controls), was stopped on May 1, 1974, the last four months having been used primarily to enroll infants to this latter group, which was to be used to address the question of vehicle effect. Two reasons dictated this termination. *First*, a new batch of Similac formula containing half again the previous amount of vitamin E had been received in the nursery and old supplies were nearly exhausted. These latter were being used to feed already enrolled babies. New admissions to the nursery were placed on the new formula. *Second*, the number of infants enrolled in the no-injection control group was almost equal to those in the placebo and vitamin groups. The pattern of serum E levels during the nursery stay in these infants was indistinguishable from that of placebo infants, and neither had changed throughout this study period. It was decided therefore to consider subsequent admissions as belonging to a no-injection control group for a future study that would evaluate the effectiveness of a new, more biologically available preparation of parenteral α-tocopherol free alcohol.[7] *Third*, a Bourns respirator had recently been purchased and was beginning to be put to clinical use. This extended our ability to offer intensive care to the sick, very small premature and, as in other nurseries, was to be associated with an increased survival rate among infants at highest risk for ROP.

May 1, 1974 to February 1, 1976: Second Clinical Trial of Prophylactic Vitamin E for ROP

Single (ophthalmology) blind, nonrandomized, no placebo, E treatment periods interspersed with no-injection control period. Parenteral tocopherol free alcohol (or acetate) and oral E alcohol (E-OH) used under Roche IND restrictions to achieve blood E level of 3 mg/dl, ophthalmology protocol and eligibility requirements unchanged.

Our goals for this second study period were:

1. Gain clinical experience with Roche's new E-OH parenteral preparation related to clinical effectiveness and possible deleterious side effects.

2. Verify the findings of the first study.
3. Extend our experience to include larger numbers of small, sick babies in the 1,250-g birth weight range.
4. Gain information on the hypothesis that serum E levels in excess of the usual normal range might be associated with a further decrease in mean severity of ROP.

In collaboration with the Hoffmann-La Roche Department of Medical Research, the following guidelines for this study were established:

1. IM injections of placebo in infants would probably not be justified in infants with birth weights in the range of 1,250 g or less, especially in view of the apparently beneficial effect of vitamin E. Therefore no parenteral placebo should be used.
2. IM injection of a promising, but unproven, therapy such as high-dosage vitamin E was not justified in infants weighing under 1,000 g. In 1974, mortality rates in such infants were in the range of 80% and trauma of any kind was of course a further jeopardy.
3. Since protocol with regard to target serum E level could not be maintained, it would be necessary to exclude infants with birth weights of 1,000 g or less if they required early intensive care and were judged unable to take oral medication for an extended period. This decision would need to be made within 48 hours of hospital admission. (One set of twins met this criterion of exclusion during 1974-75.)
4. Informed consent would be asked only of parents whose infants were born during periods assigned to E treatment; all other infants would fall into an informal control group. Management of E-treated infants would be identical to that of controls except for more frequent monitoring of serum E levels in the former to regulate dosage of the vitamin. The target serum E level in treated infants would be 3.0 mg/dl and was to be achieved through a combination of oral and parenteral treatment by the least number of injections possible.

In addition, it was decided to use the summer months (May through September) for entering control infants and abstracting clinical data from hospital charts on infants enrolled in the just-completed trial using α-tocopheryl acetate. This would maximize the efficiency of our much-curtailed research staff.

Finally, our initial experience with parenteral free E alcohol indicated that, compared to parenteral E acetate, this preparation is moderately irritating as an intramuscular injection. This required us to limit the size and frequency of E alcohol injections to a maximum of 0.4 to 0.5 ml per site and to stipulate that it could be repeated in smaller infants only after three to four days and used only in the thigh muscles. The resulting dosage regimen included use of both parenteral E acetate and E alcohol in small infants requiring parenteral feedings for extended periods. In retrospect, this latter would not really have been necessary since E alcohol is very effective in raising serum E levels and proved to be well tolerated under the new guidelines.

Between ages one and two years, infants cared for in this second treatment period were recalled for ophthalmologic exams and assessment of visual morbidity (see TABLE 1). Effect of treatment was evaluated on the basis of acute stage eye findings and long-term visual sequelae. Admission to this study ended February 1, 1976 and was dictated by loss of research personnel and limitation of funds. Details of admission to this second study are being published elsewhere.

Late 1976 to Mid-1978: Late Treatment of Already Established Severe ROP (Active Grade 3-Plus)

In late 1976, a respirator-dependent two-month-old infant (birth weight, 960 g) was found to have advanced grade 3-plus active ROP at the first ophthalmologic exam. Her condition had been too critical to allow for an eye exam earlier. The apparent response of this high-risk infant to high-dosage intramuscular and oral vitamin E therapy was striking and prompted us to offer this treatment to other infants. For admission to this study, we required that the patient be referred prior to retinal detachment and have the following retinal findings: a 360° demarcation line, extensive ERNV in two or more quadrants, and prominent posterior pole dilatation and tortuosity. After informed consent was signed, the serum E level was raised as quickly as possible to 5 to 6 mg/dl by a combination of parenteral and oral therapy. It was maintained in that range until the progression of active disease had stopped and retinopathy had clearly begun to regress. This occurred in two to four weeks time in most infants. The dosage was then decreased so as to maintain a level of 3 to 4 mg/dl for another month. A serum E level in the range of 2.0 to 2.5 mg/dl was then maintained until age one year. This level could usually be achieved with an oral dose of 25 to 75 mg/day (0.5 ml one to three times a day).

Discussion and Results

1968-71 ROP Survey: Sequelae of Low-Grade ROP at Age One to Two Years

The incidence and severity of acute stage retinopathy as distributed between birth weight groups in the Pennsylvania Hospital nurseries from 1968 through 1971 are shown in Table 3. In considering the finding presented here, it is important to remember that most very low birth weight (under 1,200-g birth weight) survivors at that time were small-for-gestational-age infants who did not require intensive care by present-day standards. They often needed little or no

Table 3

Incidence of Active Retinopathy of Prematurity: Pennsylvania Hospital (1968-1971)*

Birth Weight (grams)	Number of Babies	Number with ROP	Active Stages of ROP					Incidence of ROP
			1	2	3	4	5	
759-1000	8	6†	3	0	3	0	0	75.0%
1001-1500	50	16	9	3	3	1	0	32.0%
1501-2000	52	9	5	4	0	0	0	17.3%
2001-2500	37	2	1	1	0	0	0	5.4%
Over 2500	29	0	0	0	0	0	0	0.0%
Totals	176	33	18	8	6	1	0	18.8%

*The study population consisted of all infants with birth weights of 1,500 grams or less regardless of oxygen therapy and all infants with birth weights of greater than 1,500 grams who needed supplemental oxygen. Serum E levels in these infants were in the range of 0.3 mg/dl throughout the hospital stay.

†One of these infants received no O_2; one received 22 hours of 26% O_2.

TABLE 4

SEQUELAE OF LOW-GRADE RESOLVED RETINOPATHY OF PREMATURITY AT AGE ONE TO TWO YEARS*

Birth Weight (grams)	Grade of Acute ROP		Morbidity Ranking (score)	Grade of Cic ROP		Myopia (none to severe)		Amblyopia	Strabismus	Anisometropia
	od	os		od	os	od	os			
D 1940	1	1	A (0-2)	0	0	0	0	0	0	0
M 1130	1	1	A (0-2)	1+	0	0	0	0	0	0
G 1250	1	1	C (6-8)	1	0	3	2	0	0	1
H 1500	1	1	C (6-8)	1+	1+	1	1	0	1	0
C 1890	2	2	B (3-5)	1+	1+	1	1	0	0	0
S 1070	2	2	B (3-5)	1+	1+	1	1	0	0	0
MA 1720	2	2	C (6-8)	1+	1+	1	1	1	1	0
MB 1600	2	2	D (9-11)	1+	1+	2	2	1	1	1
R 1247	2	2	D (9-11)	1+	1+	1	2	1	1	1
O 1140	2	2	D (9-11)	2	2	2	2	0	0	1

*Pennsylvania Hospital, infants born 1968-1971. The standard formula for premature infants during the period 1968 through 1971 was Similac with iron, which provided per liter 12 mg iron, 4 IU of *all-rac*-α-tocopherol, and an E:PUFA ratio of 0.3; od is right eye; os is left eye.

oxygen during their stay in the nursery and tolerated cautious, early oral feedings. In contrast, a significant proportion of survivors in the 1,500-g birth weight range had severe respiratory distress and required artificial ventilation. In our nurseries, this was usually accomplished with a negative tank respirator.

As can be seen from TABLE 3, ROP was more common and more severe in the lower birth weight groups. Though present in over one-third of low birth weight infants, it was usually mild in degree and regressed spontaneously. These have been consistent findings by all workers in the field and have not changed over the past 10 to 15 years in spite of advances in neonatal care. What has changed since 1968-72, at least in our nurseries, is the pattern of visual morbidity to be found between 1 and 2 years of age among infants who have had this more common, low-grade form of ROP. Before about 1972, significant visual morbidity, though mild in degree, was the rule among such infants, even though active disease had apparently regressed completely (see TABLE 4). The abnormalities noted at the 1-year follow-up were of the type commonly found among premature infants. Had no scrutiny of the peripheral retina been part of their newborn care, there would have been no reason to attribute the myopia and strabismus found in these infants to ROP save the inconspicuous peripheral findings of low-grade cicatricial disease. These might well have gone unrecognized in another setting. In contrast, FIGURE 1 shows the long-term visual morbidity among infants who had had low-grade ROP in the nursery during the years 1972 to 1976 and compares it with the visual morbidity found among similar infants cared for during the earlier time period. Healing from ROP had been more complete in these later born infants who comprised the study population for our first trial of the effect of prophylactic vitamin E on ROP. This is shown by the highly significant decrease in sequelae of ROP that was found among control and E-treated infants alike. Among E-treated infants, no sequelae whatsoever could be attributed to ROP,

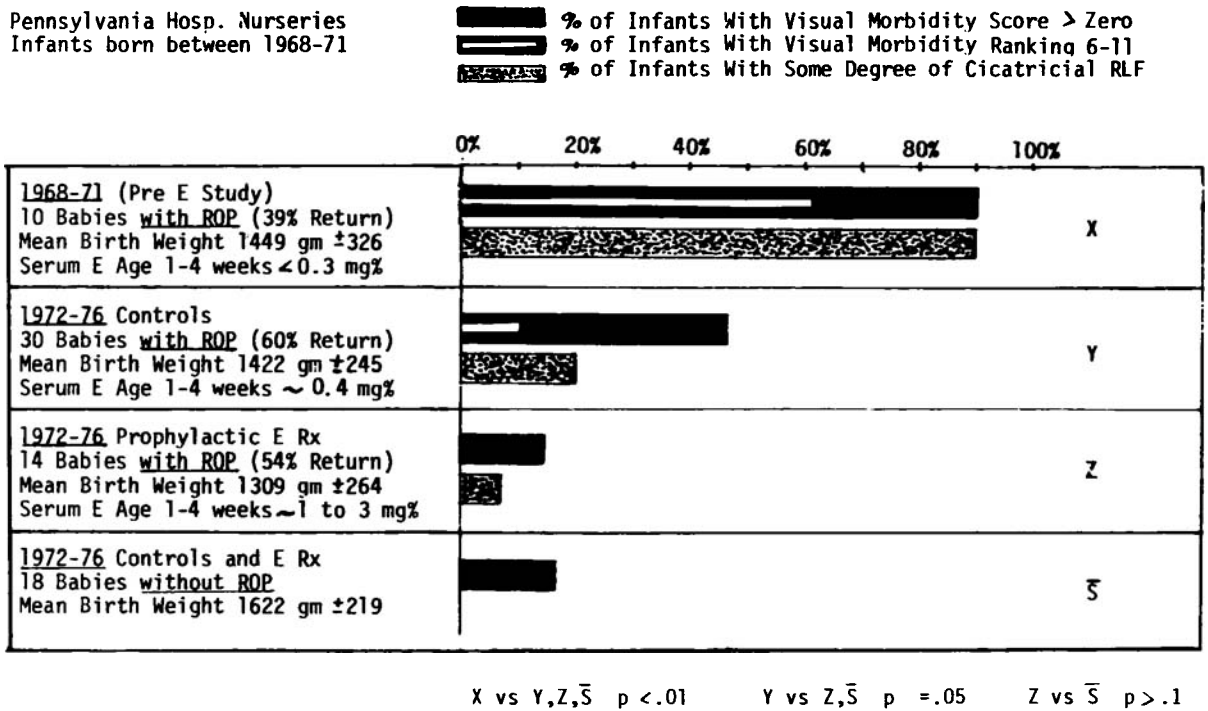

FIGURE 1. Visual morbidity score and incidence of cicatricial ROP at follow-up exam—age one to two years. Findings in infants with mild resolved ROP versus no ROP. Comparison between time periods—effect of high-dosage E treatment.

since they did not differ at age 1 year from infants who had not developed any retinopathy while in the nursery. (The number of returning infants in this no-ROP group is small and embraces treated and untreated infants. It is in all likelihood, however, a representative group.)

We believe the difference in healing between the two time periods in treated and untreated infants alike is primarily due to the growth-modulating and healing properties of vitamin E, which expressed themselves in association with only modest increments in serum E level. Certainly the most important difference in nutrition of premature infants born before 1972 as compared to those born subsequently related to vitamin E. During the mid-1960s, it was common practice to use formulas that were rich in iron, high in polyunsaturated fatty acids, and low in vitamin E throughout the nursery course as well as after discharge. This feeding practice aggravated the subclinical state of E deficiency that is present in the human newborn as well as in the newborn of many mammalian species. This deficiency state is evidenced by meager body stores of the vitamin, low serum E levels, and an increased fragility of RBC membranes on oxidant challenge. The latter is one of the more accurate and readily available biochemical markers of E

TABLE 5

CHANGES IN THE COMPOSITION OF SIMILAC (S-20) FROM 1968 TO 1978

Similac*	E (IU/L)	Percent of Fatty Acids as Linoleic Acid	Fe (mg/L)	E:PUFA Ratio†
1968-72	5 IU	32%	12	0.3
1972-74	9 IU	32%	trace	0.50
1974-75	12 IU	32%	trace	0.66
1975-76	15 IU	32 → 23%	trace	0.7 to 1.1
1977-78	15 IU	23%	trace	1.1

*Similac was the predominant formula used at the Pennsylvania Hospital premature nurseries from 1968 to 1978.

†E:PUFA ratio = mg *RRR*-α-tocopherol to polyunsaturated fatty acids (linoleic plus linolenic); 1 mg *RRR*-α-tocopherol = 1.5 IU of vitamin E; 1 mg *all-rac*-α-tocopheryl acetate = 1.0 IU of vitamin E.

deficiency in humans and is present long before clinical signs of anemia, encephalopathy, or myopathy appear, if indeed they ever do.

Though generally unrecognized until the work of Hassan, Oski, Melhorn, and others,[8-12] clinical E deficiency in the mid-1960s was common among growing premature infants, especially those with birth weights below 1,500 g.[11,12] It reached its peak at about eight weeks after birth and was associated with variable degrees of anemia, especially if iron supplements were started soon after birth.[13] It slowly corrected itself as fat absorption improved and growth rate decreased.

The response of commercial suppliers of infant feedings (and vitamin drops) to this new information was prompt and commendable. The generous E:PUFA ratios of the newer formulas have largely done away with clinical evidence of E deficiency in the premature. (Breast milk from a well-nourished mother also provides a generous supply of E relative to PUFA in most instances.)

The course of formula changes over the 10-year period from 1968 to 1978, using Similac as a representative formula, is presented in TABLE 5. The effect of

these changes on serum E levels in healthy prematures born at Pennsylvania Hospital who were able to tolerate early oral feedings is shown in FIGURE 2. Of note are the higher serum E concentrations at birth in babies born in 1978 as compared to 1972. They indicate that there had been an increase in the E content of maternal diets. However, the content of polyunsaturated fat in maternal diets must also have increased, because the RBC membranes of the newborn in 1978 were still abnormally susceptible to oxidative attack as measured by standard hydrogen peroxide assays. Therefore, though presumably endowed with better

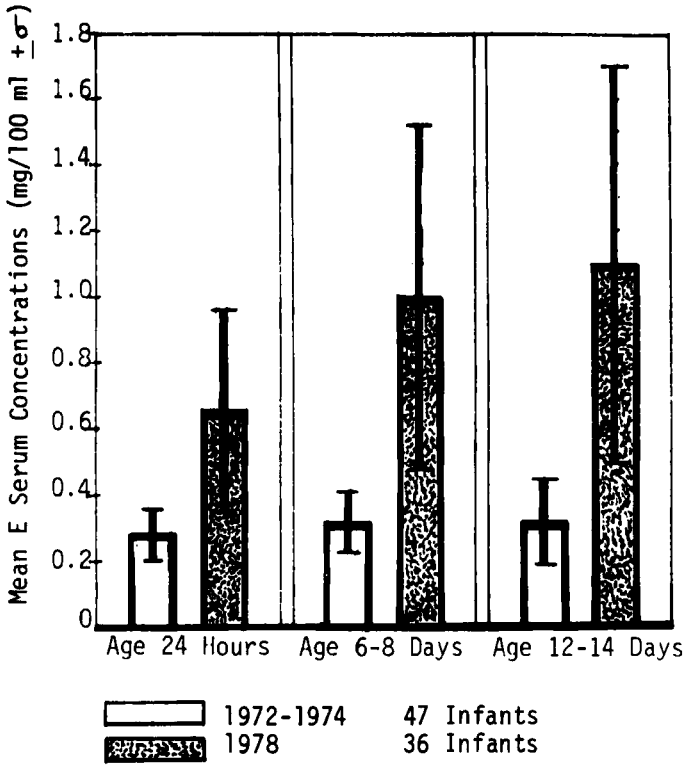

FIGURE 2. Infants with birth weights less than 2,000 g who were not treated with supplemental vitamin E and were able to take early oral feedings.

stores, these more recently born infants would still seem to need the kind of initial boost of vitamin E that is provided by the colostrum of mammalian milk.[29]

The remarkable richness of colostrum and early milk with regard not only to absolute content of α-tocopherol but also of α-tocopherol relative to PUFA is shown in TABLE 6, which is a compilation from work by Quaife, Harris, Herting, Abderhalden, and Jansson.[14-18] Nutritional research continues to demonstrate how well the milk of the mother is adapted to the needs of her newly born.

TABLE 6

CONCENTRATION OF TOTAL TOCOPHEROL RELATIVE TO POLYUNSATURATED FATTY ACID CONTENT OF COLOSTRUM, TRANSITIONAL, AND MATURE MILK*

	α-Tocopherol (IU/L)	Linoleate (% of fatty acid)	E:PUFA (mg/g)
Human colostrum			
Quaife-Harris[14,15] 1947 & 1950	1.3 to 36	—	—
Jansson *et al.*[18] 1981 (mean)	10.0 ± 5.5	12.1 ± 1.0	6.23 ± 3.9
Human transitional milk			
Abderhalden[17] 1947	4.0 to 18.5	—	—
Jansson *et al.*[18] 1981 (mean)	4.8 ± 1.8	12.8 ± 2.8	1.43 ± 0.66
Human mature milk			
Quaife-Harris[14,15] 1947 & 1950	1.0 to 5.0		
Jansson *et al.*[18] 1981 (mean)	3.2 ± 1.8	12.9 ± 2.2	0.78 ± 0.28
Cow colostrum			
Herting & Drury[16] 1969 (mean)	4.33 to 2.41		2.16 ± 1.43
Cow milk (mature)			
Herting & Drury[16] 1969	0.56 to 0.32		0.21 ± 0.08

*Values are given as range or mean ± standard deviation (SD).

Clinical Trials of Prophylactic Vitamin E for ROP

The results of the two clinical trials of prophylactic vitamin E treatment for ROP are shown in TABLES 7 through 13. TABLE 7 shows incidence and mean severity of ROP for vitamin E, placebo, and no-injection control groups for study 1 infants with birth weights of 2,000 g or less. Vitamin E treatment was associated with a reduction in both incidence and severity of ROP, with the difference in mean severity between groups being significant at $p < 0.02$ (analysis of variance). From February 1972 to May 1974, no instance of either severe or moderately severe ROP (grade 3 active or worse) was encountered in our nurseries. Therefore the differences seen between E-treated and control infants represent an effect on low-grade retinopathy.

During study 1, the mean serum E level during the nursery stay was 0.36

TABLE 7

EFFECT OF VITAMIN E ON INCIDENCE AND SEVERITY OF ROP: FINDINGS IN INFANTS WITH COMPLETE ACUTE STAGE EYE DATA*

	Vitamin E	Placebo	No Injection
Number of infants	49	46	41
Number with ROP	11	16	14
Incidence of ROP	22%	35%	34%
Mean severity	0.55	1.02	1.19
Analysis of variance Mean severity		$t(134) = -2.3$ $p = 0.012$	

*Only infants with birth weights of 2,000 g or less. Pennsylvania Hospital, February 1972 to May 1974.

mg/dl ± 0.143 (standard deviation) for placebo and 0.34 mg/dl ± 0.085 (SD) for no-injection control infants. For E-treated infants, it was 2.0 mg/dl ± 0.478 (SD). Birth weight, gestational age, and requirement for oxygen and transfused blood were similar between groups (TABLE 8). Treatment was associated with a significant decrease in mean severity of ROP ($p < 0.02$). TABLE 9 shows that this is also true when both study periods are considered together (1972–76).

Considering the second study alone, the same downward trend in incidence and severity of ROP was seen, but as shown in TABLE 10, the differences between groups did not reach the level of statistical significance. Again birth weight, gestational age, and requirement for blood and oxygen were similar between groups. However, in comparison to study 1 infants, infants cared for during the later time period had a somewhat lower mean birth weight (1,582 g vs. 1,662 g) and required much more intensive care. This was reflected in a doubling of the mean number of days of oxygen treatment required and a doubling of the volume of

TABLE 8

EFFECT OF VITAMIN E ON MEAN SEVERITY OF ROP: FINDINGS IN INFANTS WITH COMPLETE ACUTE STAGE EYE DATA*

	Birth Weight (mean; grams)	Gestation (mean; weeks)	F_{IO_2} >Room Air (mean; days)	Blood Transfused (ml/kg)	ROP Mean Severity (score)
Treated ($n = 58$)	1681	33.8	2.46	23	0.50
Not treated ($n = 103$)	1652	33.6	2.91	23	0.94
t-test	0.82	0.14	0.69	0.01	2.10
p (two tailed)	0.604	0.885	0.488	0.991	0.037
p (one tailed)					0.019

*Pennsylvania Hospital, February 1972 to May 1974. Infants with birth weights under 2,000 g, or over 2,000 g if gestational age 36 weeks or less and >24 hours O_2 treatment required. Mean serum E level of treated infants = 2.03 ± 0.478 (SD) from day 4 to discharge.

transfused blood administered per kilogram of body weight. We interpreted this as posing too great an oxidant stress for the vitamin E provided.

Among study 2 E-treated infants, the mean serum E level was 3.1 mg/dl ± 0.958 (SD) as compared to 0.5 mg/dl ± 0.236 (SD) in control infants ($p < 0.0001$). It is interesting to note that, as shown in TABLE 11, the mean serum E levels in study 2 control infants were significantly higher ($p < 0.001$) than those of control infants enrolled in study 1.

One infant in study 2 developed blinding ROP, a control infant with a birth weight of 1,640 g who suffered a severe anoxic insult at birth. In addition, one of a pair of 1,000-g twins developed blinding ROP. This set of twins was removed from the study before age two days because it was clear that maintaining treatment protocol would not be possible in the male, who had been assigned to the E group. The male died with bronchopulmonary dysplasia (BPD) at about two months of age. The little girl survived, but with extensive anoxic brain damage. She had originally been assigned to the control group.

TABLE 9

EFFECT OF VITAMIN E ON MEAN SEVERITY OF ROP: FINDINGS IN INFANTS WITH COMPLETE ACUTE STAGE EYE DATA*

	Birth Weight (mean; grams)	Gestation (mean; weeks)	F_{iO_2} >Room Air (mean; days)	P_{aO_2} >100 mm Hg (mean; hours)	Blood Transfused (ml/kg)	ROP Mean Severity (score)
Treated (n = 105)	1648	33.5	4.6	8.3	37.5	0.65
Not treated (n = 164)	1619	33.3	4.4	8.8	34.4	1.04
t-test	0.70	0.44	0.13	0.52	0.31	2.17
p (two tailed)	0.485	0.661	0.900	0.601	0.754	0.031
p (one tailed)						0.016

*Pennsylvania Hospital, February 1972 to February 1976. Infants with birth weight under 2,000 g, or over 2,000 g if gestational age 36 weeks or less and >24 hours O_2 treatment required.

TABLE 10

EFFECT OF VITAMIN E ON MEAN SEVERITY OF ROP: FINDINGS IN INFANTS WITH COMPLETE ACUTE STAGE EYE DATA*

	Birth Weight (mean; grams)	Gestation (mean; weeks)	F_{iO_2} >Room Air (mean; days)	Blood Transfused (ml/kg)	ROP Mean Severity (score)
Treated (n = 47)	1607	33.1	7.16	56	0.83
Not treated (n = 61)	1562	32.6	6.91	54	1.21
t-test	0.72	1.24	0.08	0.07	1.21
p (two tailed)	0.475	0.219	0.934	0.941	0.229†
p (one tailed)		0.11			0.12

*Pennsylvania Hospital, May 1974 to February 1976. Infants with birth weight under 2,000 g, or over 2,000 g if gestational age 36 weeks or less and >24 hours O_2 treatment required. Mean serum E level of treated infants = 3.10 ± 0.958 (SD) from day 4 to discharge.

†If a weighted severity score is used that reflects more accurately the clinical implications of severe versus low-grade ROP, then t = 1.85, p = 0.033 (one tailed).

The experience with this pair of twins convinced us that any definitive study of the effect of vitamin E on the pathogenesis of ROP would require the option of being able to administer parenteral medication by intravenous infusion. The protocol for our three-hospital, double-blind, clinical trial, sponsored by the National Eye Institute, has included this capability. (Intake to this trial ended in May 1981.)

TABLE 12 shows the results of data from the combined trials (studies 1 and 2) using a multiple regression technique. Predictor variables are entered into the equation in order of their contribution to outcome variance. Very weak predictors are not entered at all. The cumulative R^2 value represents the fraction of total outcome variance that can be accounted for by the predictors selected for entrance. Birth weight is by far the most important predictor. Volume of blood received per kilogram of body weight and then treatment group are selected for entrance into the equation. Both have p values in the highly significant range. It is important to note however that the contribution of these two variables to R^2 is

TABLE 11

CHANGING MEAN CONCENTRATIONS OF TOTAL TOCOPHEROL IN SERUM OF UNTREATED INFANTS AT PENNSYLVANIA HOSPITAL*

	February 1972 to May 1974	May 1974 to February 1976	t-test
Day 0 to 1	0.29 ± 0.118	0.33 ± 0.148	t = 1.806 p = 0.05
Day 5 to discharge	0.35 ± 0.110	0.51 ± 0.236	t = 5.965 p <0.001

*Values represent means ± SD in infants with birth weights of 2,000 g but requiring over 24 hours of oxygen treatment. Serum tocopherol is given in mg/dl ± 1 SD.

TABLE 12

PREDICTION OF INCIDENCE AND SEVERITY (MEAN SEVERITY) OF ROP: PENNSYLVANIA HOSPITAL, FEBRUARY 1972 TO FEBRUARY 1976

Predictor Variable	R^2	F	p
Birth weight	0.30824	93.587	<0.001
Blood (ml/kg)	0.33438	10.102	<0.001
Type of treatment	0.34545	4.667	<0.001
Days $F_{i_{O_2}}$ over room air	0.34955	2.147	<0.05
Shock	0.35344	1.239	NS
Apnea	0.35625	1.268	NS
Pregnancy complications	0.35389	0.868	NS

*Multiple regression analysis. The dependent variable is mean severity of ROP for all infants. The seven predictors account for only 36% of outcome variance. Includes only infants with complete acute stage eye data (n = 269) and birth weights of 2,000 g or less, or over 2,000 g but under 36 weeks gestation and requiring oxygen for over 24 hours.

small in absolute terms and that even with all significant predictors entered, only about 35% of outcome variance is accounted for.

TABLE 13 shows results of the regression analysis for babies with birth weights of 1,500 g or less. Again birth weight is the most powerful predictor followed by treatment group, blood requirement, and shock. When infants are preselected for moderately low birth weight, only 30% of outcome variance is accounted for. Most known risk factors for ROP [e.g., apnea, Apgar, $P_{a_{O_2}}$ sepsis, necrotizing enterocolitis (NEC)] were included in the research data base. Hypercarbia, acidosis, and anoxia were also included but not in the detailed way that recent work suggests they should be.[19-21] However, these problems were not prominent among survivors in the study populations described and their more rigorous inclusion probably would not have added much to the ability to predict mean severity of ROP in an individual infant. The data speak strongly for the existence of individual resistance (or susceptibility) factors and are consistent with the great variability in degree of pathology that results from a given hyperoxic exposure as previously observed in both human and animal studies.[22,23] In recent work with

TABLE 13

PREDICTION OF INCIDENCE AND SEVERITY (MEAN SEVERITY) OF ROP: PENNSYLVANIA HOSPITAL, FEBRUARY 1972 TO FEBRUARY 1976*

Predictor Variable	R^2	F	p
Birth weight	0.17714	11.180	<0.001
Type of treatment	0.21931	6.482	<0.001
Blood (ml/kg)	0.25995	6.032	<0.001
Shock	0.27963	6.072	<0.001
Pregnancy complications	0.29363	1.748	NS
Days $F_{i_{O_2}}$ over room air	0.29407	0.076	NS
Apnea	0.29446	0.047	NS

*Multiple regression analysis. The dependent variable is severity of ROP in infants ≤1,500 g birth weight. The seven predictor variables account for 29.5% of the total variance. Includes only infants with complete acute stage eye data (n = 95) and birth weights of 1,500 g or less. The contribution of birth weight to outcome variance is relatively less when only infants with birth weights of 1,500 g or less are considered.

the kitten model, Phelps and Rosenbaum reported a very high correlation between the litter a kitten belonged to and the appearance of the retina two weeks after hyperoxic exposure.[24] This "queen effect" emphasizes the importance of genetic differences in the pathogenesis of ROP.

Maintenance of serum E levels in the 1.5 to 3.0 mg/dl range was not associated with apparent untoward side effects. The incidence of NEC and culture-proven sepsis during 1972-76 was not different between treatment groups ($\chi^2 = 0.39$, $p > 0.3$). The incidence of BPD was very low and also did not differ between treatment groups ($\chi^2 = 0.17$, $p > 0.5$).

Treatment of Already Established Severe ROP

Spontaneous regression from midstage grade 3-plus active ROP (characterized by a 360° demarcation line, obvious dilatation and tortuosity of blood vessels of the posterior retina, and prominent ERNV in two or more quadrants) is rare and incomplete at best. The outlook for vision is poor, with about a 70% chance of legal blindness (best corrected vision 20/200) or worse. More advanced grade 3-plus active ROP invariably goes on to some degree of retinal detachment and has an even worse prognosis.

Against this dismal background, the results of our preliminary work with high-dosage vitamin E treatment of already established midstage grade 3-plus active ROP is very encouraging. During 1976, 1977, and 1978, we treated 10 such infants, first in the Pennsylvania Hospital nurseries and later, on request of the infants' doctors, at Children's Hospital and the Hospital of the University of Pennsylvania. The findings in these 10 infants at the one to two year follow-up are presented in TABLE 14. The visual morbidity score as previously described is used to define outcome. Also presented are the findings at follow-up in 14 untreated infants born during the same time period and cared for in the same nurseries. E treatment had either not been requested or was requested only after major retinal detachment had occurred, which was too late to begin treatment by our protocol. All eye examinations were done by Drs. Schaffer and Quinn, and all infants had been under their ophthalmologic care throughout their hospital course. Infants who exhibited significant tractional phenomena (which is occasionally seen while active disease is still present) or who developed any degree of retinal detachment, whether on E treatment or not, were referred to Dr. William Tassman for consultation and possible surgical intervention. None of the 24 infants in TABLE 14 were treated surgically prior to the one to two year follow-up visit.

A significantly improved visual outcome was associated with high-dosage E treatment ($p < 0.02$). The incidence of legal blindness (rank G) or worse was decreased from 71% to 40%. A good visual outcome (defined by ranks A through D or scores 0 through 11) was found in 40% of E-treated but in only 14% of non-E-treated infants. One of the E-treated infants had no cicatricial findings whatever and a one-year visual morbidity score of only 2. An intermediate outcome (ranks E and F) was found to be about equally distributed between groups. No adverse side effects other than an occasional sore injection site were associated with E treatment.

Cryotherapy, photocoagulation, and scleral buckling are the only other treatments presently available for severe progressive ROP. Opinions differ as to the optimal timing for these procedures and the value of treatment.[25,26] Certainly the

results reported here for high-dosage E treatment compare favorably with results of these other approaches.

It is important to remember that ROP is an anoxic retinopathy. Only the initial injury to the primitive vasoformative tissues of the retina is hyperoxic in nature. The pivotal work of Drs. Kretzer and Hittner reported at this meeting has greatly advanced our understanding of the nature of this hyperoxic insult, especially as it occurs today under conditions of controlled oxygen therapy. It has also provided important insight into the role of vitamin E in modulating the response of vasoformative tissues to changes in oxygen tension.

It is doubtful that vitamin E exerts its beneficial effects in the anoxic stages of ROP in the same way as in the preceding period of hyperoxic insult. The optimal

TABLE 14

VISUAL MORBIDITY SCORE AT AGE ONE TO TWO YEARS IN 24 CHILDREN WHO HAD ACTIVE GRADE 3-PLUS ROP OR WORSE IN THE NURSERY*

Visual Morbidity	E Treatment 1976-1978 (10 infants)†	No E Treatment 1976-1978 (14 infants)†
Rank A-B Score 0-5	1 (10.0%)	0 —
Rank C-D Score 6-11	3 (30.0%)	2 (14.3%)
Rank E-F Score 12-17	2 (20.0%)	2 (14.3%)
Rank G-H Score 18-22	1 (10.0%)	1 (7.1%)
Rank I Score 23-26	3 (30.0%)	9 (64.3%)
Mean birth weight	1070 g	1176 g

*Infants were cared for in the nurseries of the University of Pennsylvania Neonatal Complex (Hospital of the University of Pennsylvania, Children's Hospital of Philadelphia, and Pennsylvania Hospital). All ophthalmologic examinations were performed by Drs. Schaffer and Quinn.

†Distribution by rank. $p < 0.02$ (analysis of variance).

serum levels (i.e., dosage regimen) may also be different. That it does function both to decrease the hyperoxic insult and to decrease the extent of neovascularization as a response to anoxia is clear from the kitten work of Phelps and Rosenbaum.[24,27] Extent of neovascularization and tendency to regression and repair versus the tendency to progression and fibrous outgrowth are the main determinants of eventual pathology and are greatly influenced by individual and species differences. Vitamin E appears to be able to favorably influence these phenomena, perhaps through its capacity to modulate growth, decrease scar formation, and promote healing. At the peak of severe acute stage disease, when the eye appears congested and inflamed and there is vascular stasis with leakage of fluid and exudate from abnormal vessels, the functions of vitamin E as

antiinflammatory agent and modulator of prostaglandin balance may be important. It should be emphasized, however, that vitamin E, used to raise serum levels well above the physiologic range, is a pharmacologic agent with potential toxic side effects. It should be used on a study protocol and with careful monitoring of serum E levels.

In conclusion, beneficial effects of vitamin E therapy have been demonstrated at several points along the pathogenetic pathway of ROP. These need to be verified and extended to include infants of lower birth weights. The optimal levels of serum vitamin E to be sought at different points of the disease pathway need to be clarified so that preventive and therapeutic regimens can be developed. The improved E nutrition provided by present-day infant formulas appears to have already decreased the incidence of sequelae from low-grade ROP.

Summary

The effect of high-dosage E treatment (Rx) initiated at the stage of 3-plus active disease (target serum E levels, 5–6 mg/dl) was evaluated by a standardized scoring system of visual morbidity at the one to two year eye exam among infants cared for in the University of Pennsylvania Neonatal Complex (1976–1978). The incidence of legal blindness in both eyes or worse was decreased from 71 to 40% in E Rx ($n = 10$) as compared to non-E Rx ($n = 14$) infants, and the number of infants with minimal visual morbidity was increased. Pilot studies (1972–76; target serum E level, 1.5 and 3.0 mg/dl) of the prophylactic effect of E Rx from birth on showed a decrease in mean severity of acute stage disease and a decrease in sequelae at one to two years. A striking difference in visual morbidity following resolved low-grade ROP was seen when prestudy infants (1968–72) who were fed early iron supplements and given formulas with low E:PUFA ratios were compared to non-E Rx as well as to E Rx 1972–76 infants. Vitamin E seems to exert a beneficial effect at all stages of ROP, perhaps because of its broadly based regulatory role.

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References

1. Johnson, L., D. Schaffer & T. Boggs. 1974. The premature infant, vitamin E deficiency and retrolental fibroplasia. Am. J. Clin. Nutr. **27**: 1158.

2. SCHAFFER, D., L. JOHNSON, G. QUINN & T. BOGGS. 1979. A classification of retrolental fibroplasia to evaluate vitamin E therapy. Ophthalmologica **86:** 1749.
3. HASHIM, S. & G. SCHUTTRINGER. 1966. Rapid determination of tocopherol in macro and micro quantities of plasma. Am. J. Clin. Nutr. **19:** 136.
4. GORDON, H., H. NITOWSKY & M. CORNBLATH. 1966. Studies on tocopherol deficiency in infants and children. I. Hemolysis of erythrocytes in hydrogen peroxide. Am. J. Dis. Child. **92:** 164.
5. STOCKS, J., E. OFFERMAN, C. MODELL & T. DORMANDY. 1972. The susceptibility to autooxidation of human red cell lipids in health and disease. Br. J. Hematol. **23:** 713.
6. TASSMAN, W. 1968. Juvenile retinal detachment. J. Pediatr. Ophthalmol. **5:** 160.
7. NEWMARK, H., W. POOL, *et al.* 1975. Biopharmaceutic factors in parenteral administration of vitamin E. J. Pharm. Sci. **64:** 655.
8. HASSAN, H., S. HASHIM, T. VANITALLIE & W. SEBRELL. 1966. Syndrome in premature infants associated with low plasma vitamin E levels and high polyunsaturated fatty acid diet. Am. J. Clin. Nutr. **19:** 147.
9. OSKI, F. & L. BARNESS. 1967. Vitamin E deficiency: a previously unrecognized cause of hemolytic anemia in the premature infant. J. Pediatr. **70:** 211.
10. MELHORN, D. & S. GROSS. 1971. Vitamin E-dependent anemia in the premature infant. I. Effects of large doses of medicinal iron. J. Pediatr. **79:** 569.
11. MELHORN, D. & S. GROSS. 1971. Vitamin E-dependent anemia in the premature infant. II. Relationships between gestational age and absorption of vitamin E. J. Pediatr. **79:** 581.
12. LO, S., D. FRANK & W. HITZIG. 1973. Vitamin E and hemolytic anaemia in premature infants. Arch. Dis. Child. **48:** 360.
13. WILLIAMS, M., R. SHOTT, P. O'NEAL & F. OSKI. 1975. Role of dietary iron and fat on vitamin E deficiency anemia of infancy. N. Engl. J. Med. **292:** 887.
14. QUAIFE, M. 1947. Tocopherols (vitamin E) in milk: their chemical determination and occurrence in human milk. J. Biol. Chem. **169:** 513.
15. HARRIS, P., M. QUAIFE & P. O'GRADY. 1952. Tocopherol content of human milk and of cow's milk products used for infant feeding. J. Nutr. **46:** 459.
16. HERTING, D. & E. DRURY. 1969. Vitamin E content of milk, milk products, and simulated milks: relevance to infant nutrition. Am. J. Clin. Nutr. **22:** 147.
17. ABDERHALDEN, R. 1947. Vitamin E content of human milk and cow milk. Biochem. Z. **47:** 318.
18. JANSSON, L., B. AKESSON & L. HOLMBERG. 1981. Vitamin E and fatty acid composition of human milk. Am. J. Clin. Nutr. **34:** 8.
19. LUCEY, J., J. HORBAR & M. ONISHI. 1981. Cerebral and retinal hypoperfusion as a possible cause of retrolental fibroplasia—hypothesis to explain non O_2 related RLF. (abst.). Pediatr. Res. **15:** 670.
20. PHELPS, D. & A. ROSENBAUM. 1982. Effect of hypoxemia on recovery from oxygen induced retinopathy in the kitten model. (abst.). Pediatr. Res. **16:** 303A.
21. FLOWER, R., D. BLAKE, *et al.* 1981. Retrolental fibroplasia: evidence for a role of the prostaglandin cascade in the pathogenesis of oxygen-induced retinopathy in the newborn beagle. Pediatr. Res. **15:** 1293.
22. KINSEY, V. 1956. Retrolental fibroplasia: cooperative study of retrolental fibroplasia and the use of oxygen. Arch. Ophthalmol **56:** 481.
23. ASHTON, N. 1979. Symposium, retrolental fibroplasia. Ophthalmologica **86:** 1695.
24. PHELPS, D. & A. ROSENBAUM. 1977. The role of tocopherol in oxygen-induced retinopathy: kitten model. Pediatrics **59**(suppl.): 998.
25. KALINA, R. 1980. Treatment of retrolental fibroplasia. Surv. Ophthalmol. **24:** 229.
26. BEN-SIRA, I., I. NISSENKORN, E. GRUNWALD & Y. YASSUR. 1980. Treatment of acute retrolental fibroplasia by cryopexy. Br. J. Ophthalmol. **64:** 758.
27. PHELPS, D. & A. ROSENBAUM. 1979. Vitamin E in kitten oxygen-induced retinopathy. II. Blockage of vitreal neovascularization. Arch. Ophthalmol. **97:** 1522.
28. SCHAFFER, D., G. QUINN & L. JOHNSON. 1982. Sequelae of arrested, mild retinopathy of prematurity. Paper presented at the 13th Biannual Meeting of the Gonin Society, Cordoba, Argentina, April.

29. JOHNSON, L., C. DALIN, R. DWORANCYZK & B. HARRISON. 1982. Functional significance of serum vitamin E levels. Pediatr. Res. **16:** 167A.

DISCUSSION

R. J. SOKOL (*Children's Hospital Medical Center, Cincinnati, Ohio*): Could you tell me if there was any change in the way you monitored $P_{a_{O_2}}$ in these patients? Was there any significant difference in the average $P_{a_{O_2}}$ for the groups of patients?

L. JOHNSON: The last patient studied was born in 1976. We did not have a transcutaneous monitor in the nursery at that time. These were all arterial blood gases, and there was no difference in the amount of exposure to $P_{a_{O_2}}$ over 100 mm Hg in each group.

R. J. SOKOL: Were you drawing blood gases as frequently from 1972 to 1976 as you were from 1976 to 1978?

L. JOHNSON: Yes.

D. L. PHELPS (*University of California, Los Angeles, Calif.*): Can I ask a question. When Dr. Tassman says 70% legal blind on your stage 3-plus, does that include his late retinal detachments? In other words, is this a lifetime prognosis?

L. JOHNSON: No. The 70% legally blind figure applies to early visual outcome (age one year or less) and involves almost entirely the infant with grade 3 Cic ROP or worse. Infants with moderate to severe grade 2 Cic ROP, with severe myopia and marked traction on the retina, are the ones at risk for late retinal detachment. As shown in our data, they usually have a visual acuity of 20/20 (best corrected) at age one to two years.

W. A. PRYOR (*Louisiana State University, Baton Rouge, La.*): I know there's quite a literature on RLF, and I was delighted to hear your study. Are there data on the effect of varying amounts of E?

L. JOHNSON: We have been trying to address that problem in our sequential clinical trials. In the larger double-blind trial sponsored by the NEI, for which intake was recently completed, a very high serum E level was maintained to try to definitively learn whether "more E is better" and what side effects may be expected. Our earlier trials, and that of Dr. Hittner, were testing the effect of E in a more physiologic range.

P. M. FARRELL (*University of Wisconsin, Madison, Wis.*): The data you showed on cord blood tocopherol levels in the recent assessment being higher than the earlier values are interesting. I wanted to ask if you would elaborate on this. There seems to be a large standard deviation, so there must have been some infants with very high tocopherol levels.

L. JOHNSON: The large standard deviation probably reflects differences in both maternal diet and placental function. Some infants nowadays do have surprisingly high day-zero serum E levels (up to 0.9 mg/dl). Others, especially those born to mothers with toxemia or placental insufficiency, still have levels of 0.3 mg/dl or less.

H. J. MEVWISSEN (*Albany Medical College, Albany, N.Y.*): How do you explain the fact that one eye is bad and the other eye is not so bad?

L. JOHNSON: I think that local conditions in the eye, probably largely hemody-

namic in nature, influence the retinal response both to hyperoxia and to primary and secondary hypoxia. The degree of scarring and fibrous overgrowth seems to be considerably influenced by anoxia secondary not only to degree of endothelial hyperoxic insult but to differences in tissue perfusion as well. Difference in degree of disease between fellow eyes is much more common in severe ROP. The mechanism of action of E at this stage of the disease may be different from its action in the preceding hyperoxic stage, which, in most infants, causes only a mild, reversible, secondary, anoxic retinopathy.