Personal paper**Retinopathy of prematurity: clinical implications of retinal development**

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Retinopathy of prematurity (ROP) is a potentially blinding ocular disorder that is unique to the preterm infant. The current epidemic affects the smallest and illest preterm survivors of modern neonatal intensive care units. With the prevention of neonatal asphyxia, judicious curtailment of oxygen, and adequate doses of vitamin E, ROP has become a disorder that is generally restricted to infants weighing less than 1001 g at birth. The size of the surviving population of infants with ROP dwarfs all previous predictions of absolute numbers because survival rates of infants weighing 501–750 g at birth are now approaching 60%, and those of infants weighing 751–1000 g are now approaching 90%.

The total incidence of ROP in infants weighing 501–750 g is almost 100% with severe ROP developing in about 30%. The total incidence of ROP in infants weighing 751–1000 g is almost 80% with severe ROP developing in about 10%. Whether the future total incidence of ROP and that of severe ROP will change is dependent on the unknown effects of the many new developments in the care of premature infants.

NORMAL RETINAL DEVELOPMENT

The immature retina has two blood supplies, the choroidal vessels and the inner retinal vessels (fig 1). The choroidal vasculature develops early, lies on the

Fig 1 Four interrelated and concomitant processes (arrows) that regulate normal retinal development. Note thinning of retina towards ora serrata, region of future shunt where ROP is first seen clinically, and region of future macula that will determine ultimate visual acuity.

outer surface of the retina, and is the sole supplier of nourishment to the thin, undifferentiated retina. The inner retinal vasculature develops late, lies within the inner surface of the retina, and nourishes the inner portions of the thick, maturing retina. This inner retinal vasculature develops from a peripheral migration of spindle cells from the optic disc. In utero, spindle cells in the thin, peripheral, avascular retina reside in a comparatively hypoxic environment.

PATHOLOGICAL EVENTS OF NEOVASCULARISATION AND TRACTION

After premature birth, spindle cells in the thin, peripheral, avascular retina are stressed by a comparatively hyperoxic environment. This stimulus for abnormal retinal development occurs because oxygen diffuses freely across the retina from the choroidal vasculature that cannot vasoconstrict. Spindle cells stressed by free radical damage stop migrating peripherally, stop forming inner retinal vessels by the process of canalisation, and begin to synthesise and secrete angiogenic factors. For about eight to 10 weeks these angiogenic factors induce abnormal inner retinal neovascularisation at the boundary between the vascular and avascular retina (fig 1). This boundary becomes a shunt that contains a large volume of rapidly flowing blood.¹⁻⁴ After this two month period myofibroblasts begin to differentiate from a stem cell line in the shunt, they form contractile sheets that invade the vitreous for about four months, and they may cause traction leading to retinal detachment.¹⁻⁴ This entire cascade of events can be interrupted and regress spontaneously, or can continue to progress relentlessly. In either case, the overall enlargement of the eye continues. This pathogenesis is different from other neovascularisations of the mature eye, such as those associated with diabetes and sickle cell disease.

Retinal development

There are four interrelated and concomitant processes that are important to an understanding of ROP (fig 1). These consist of a central/peripheral vector of retinal development that is coupled simultaneously with an inner/outer vector of retinal differentiation, a remodelling of the macular region, and overall ocular growth.

CENTRAL/PERIPHERAL AND INNER/OUTER VECTORS

Retinal development into the outer retina, connectors, and inner retina begins centrally at the optic disc and advances peripherally to the ora serrata. With respect to ROP there are two important central/peripheral developmental pro-

cesses: photoreceptors in the outer retina and spindle cells in the inner retina (fig 1).

Photoreceptors begin to differentiate from the outer neuroblastic retina and advance as a gradient of maturation (figs 1 and 2). There are at least two stimuli for the normal formation of inner retinal vessels. One stimulus is the thickening of the maturing retina that displaces the inner retina further and further from the choroidal vessels. Another stimulus is that as photoreceptors mature their metabolic rate increases, and they utilise more and more oxygen that diffuses from the nonvasoconstricting choroidal vasculature. Maturing photoreceptors secrete interstitial retinol binding protein into the space between the retinal pigment epithelium and the neural retina. This protein appears round the optic disc as early as 20 weeks' gestation and reaches the ora serrata at 29 weeks' gestation (a total transit time of nine weeks).⁵ Its presence in the subretinal space is critical to the potential retinal uptake of fat soluble vitamin E, and its extent establishes the area of retina that can be protected by vitamin E.⁶ After premature birth oxygen can diffuse easily from the non-vasoconstricting choroidal vasculature, can cause physiological vasoconstriction of the inner retinal vasculature (but not endothelial necrosis and retraction), and can create an abnormal hyperoxic environment for spindle cells. Interstitial retinol binding protein carries vitamin E (only in the preterm infant) into the connectors (Muller cells), and the connectors carry vitamin E across the retina to spindle cells.

Spindle cells migrate in the inner retina from the optic disc to the ora serrata (figs 1 and 2). Spindle cells appear round the optic disc at 16 weeks' gestation and reach the ora serrata at 29 weeks' gestation (a total transit time of 13 weeks). After 29 weeks' gestation the spindle cell apron continually decreases in its linear extent so that the spindle cell apron has completely disappeared and the inner retinal vasculature is completely formed at 40 weeks' gestation. In the smallest infants the proximal portion of the spindle cell apron is intermingled with the formed vessels, and a few spindle cells may even remain in the adventitia of the hyaloid artery at the optic disc; in larger infants the spindle cell apron is not intermingled with the formed vessels, and spindle cells do not remain at the optic disc. A small region of the spindle cell apron anterior to the last formed vessels continues the inner retinal vasculature by canalisation.

The transretinal geometry between the slowly migrating spindle cells (13 weeks transit time) and the faster developing photoreceptors (nine weeks transit time) that are secreting interstitial retinol binding protein changes with gestational age.⁷ In

Fig 2 Gradient of maturing photoreceptors in outer retina (top left to right) is associated with inner retina (bottom left to right) with migrating spindle cells (arrow), canalising spindle cells (arrow head), capillaries (curved arrow), and mature vessels (open arrow). Top: transmission electron micrographs (magnification of 11 000). Note increasing mitochondria (M) in photoreceptors and increasing space (asterisk) between the retinal pigment epithelium and the neural retina. Bottom: light micrographs (magnification of 320).

infants weighing less than 1001 g at birth (27 weeks' gestation or less), most of the spindle cell apron is transretinal to photoreceptors that are not secreting interstitial retinol binding protein, and therefore these spindle cells are less likely to receive adequate protection from vitamin E. Thus even if vitamin E is given and oxygen is judiciously curtailed ROP may still be severe in the iller infants weighing less than 1001 g at birth. This morphological fact explains the clinical reality that vitamin E supplementation to eliminate plasma deficiency, even supplementation to pharmacological plasma concentrations, cannot protect all surviving infants from ROP.

In infants weighing 1001–1500 g at birth (28–32 weeks' gestation), most of the spindle cell apron is transretinal to photoreceptors that are

secreting interstitial retinol binding protein, and therefore these spindle cells can receive adequate protection from vitamin E. Thus if vitamin E is given and oxygen is judiciously curtailed, ROP is usually not severe in these infants. Without vitamin E, however, ROP may be severe in the iller of the infants weighing 1001–1500 g at birth. Thus in analysing the results of trials of vitamin E for protection against ROP, a multivariate analysis with birth weight as one variable must be used rather than a univariate analysis. In infants weighing more than 1500 g at birth (more than 32 weeks' gestation), fewer and fewer spindle cells remain within the retina. Thus whether or not vitamin E is given, but if oxygen is judiciously curtailed, severe ROP does not usually develop.

Fig 3 Extent of the vascularised retina (horizontal vascularised retinal length (cm) at about 10 weeks) and zones (international classification of ROP at about 10 weeks of age) is associated with birth weight (g), appropriate gestational age + about 10 weeks of life, and zones at about 10 weeks of age. Horizontal lines denote area of obligate zone I ROP. Vertical lines denote area of obligate zone II ROP. Combined horizontal and vertical lines denote area of zone I or II ROP (area of biological variability).

The extent of inner retinal vascularisation is directly associated with gestational age and birth weight (fig 3). The clinical importance of this association is that if severe ROP develops eight to 10 weeks after birth in infants appropriate for gestational age, those weighing 600 g or less will have ROP in zone I and those weighing 801 g or more will have ROP in zone II. Those infants weighing 601–800 g may have ROP in either zone I or zone II.⁸ This reflects the normal biological variability seen in all developmental processes. Thus the inner retinal vasculature establishes the area of retina that equates to the zones in the international classification of ROP,^{9,10} that does not secrete angiogenic factors,⁴ and that may be rescued by cryotherapy to the peripheral retina where the spindle cells reside. Cryotherapy entails placing a cold probe on the external surface of the eye to destroy all the living cells between the external surface and the vitreous. Retinal development along the central/peripheral vector stops after cryotherapy, but further retinal development associated with the inner/outer vector continues.

MACULAR REMODELLING

The macula is a specialised region of the posterior retina that is responsible for all fine visual acuity (fig 1). It is an avascular region in which the inner retina is displaced circumferentially 360° so that light can impinge perpendicularly on the perfectly parallel discs of the cone photoreceptors. The commitment to macular development and its subsequent remodelling must be triggered by an interplay between the inner/outer vector. This region is incompletely developed until three months after birth in an infant born at full term. Clinically the macula appears immature before this time—that is, it does not have a dimpled appearance.

The distance between the temporal rim of the optic disc and the centre of the macula is constant in all normally developing or developed human eyes. With the development of severe ROP the distance between the temporal rim of the optic disc and the centre of the macula may be increased by dragging, whether the ROP regresses spontaneously or is treated successfully by cryotherapy.

OCULAR GROWTH

As the retina develops along the central/peripheral and inner/outer vectors with macular remodelling, the overall diameter of the globe increases rapidly with only 15% of the increase in the diameter of the globe occurring after 40 weeks' gestation (figs 1 and 4). Unless phthisis (ocular death with shrinkage) occurs, the globe continues to enlarge after the development of severe ROP, with or without surgical intervention.

Whether ROP is regressing or progressing, this concept of ocular growth is important when retinal detachment caused by severe traction (from substantial invasion by myofibroblasts) occurs.

Neovascularisation (spindle cells)

INITIATION

After preterm birth normally migrating and canalising spindle cells can become stressed by free radicals. The probability that this stress to spindle cells stimulates the development of severe ROP is greatest in the smallest and illest preterm infants. The stress is seen ultrastructurally as early as the fourth day of life as a formation of extensive gap junctions between adjacent spindle cells uniformly throughout the apron (fig 5a, 5c). Shortly afterwards these gap junction linked spindle cells contain an extensive volume of rough endoplasmic reticulum (fig 5b, 5d). This indicates increased communication between cells (gap junctions), and increased protein synthesis and secretion (rough endoplasmic reticulum). Increased amounts of angiogenic factors have been detected only from homogenates of the peripheral retina when gap junction linked spindle cells are present.^{4,7} This state exists for about eight to 10 weeks.^{1–4}

After eight to 10 weeks of synthesis and secretion of angiogenic factors, extensive gap junctions between adjacent spindle cells disappear uniformly throughout the apron, the extensive volume of rough endoplasmic reticulum decreases, and all spindle cells stack within the inner retina. The stimulus for the stacking of the spindle cells is unknown; this subclinical event indicates, however, that peripheral spindle cells are no longer the stimulus for neovascularisation and that severe ROP will either regress or progress for unknown reasons.^{1–4}

KINETICS

At all gestational ages the moment of premature birth usually initiates the development of ROP. The development may, however, be initiated before birth by an intrauterine catastrophe, in which case the disease may be seen earlier than eight to 10 weeks after birth. Similarly, the initiating event may occur weeks after premature birth if the oxidant/antioxidant balance is upset before completion of the inner retinal vasoformation with disappearance of all spindle cells, in which case the disease may be seen eight to 10 weeks after the delayed imbalance. Development of severe ROP is a predictable cascade of events triggered by stressed spindle cells and their subsequent synthesis and secretion of angiogenic factors for a given length of time.

Conceptional age (gestational age at birth plus weeks of life) should not be applied to the timing of the development of severe ROP or to the timing of surgical intervention. Conceptional age, however,

may be applied to the concept of a given posterior area of vascularised inner retina (ROP stage 3+) that can be dragged by an actively contractile sheet of myofibroblasts, by a fixed inactive remnant

Fig 4 Size of eyes (horizontal diameter of globe (cm) at birth) is associated with birth weight (g) and appropriate gestational age (weeks) at birth. The solid box indicates ocular size at birth. The dashed box indicates the predicted ocular size 10 weeks after birth. The horizontal lines, vertical lines, and combined horizontal/vertical lines are the same as in fig 3. Closed circles are preterm infants. Open circles are term infants and adults. The eyes with zone I ROP treated at about 10 weeks have more growth yet to occur than similar eyes with zone II ROP.

Fig 5 Migrating spindle cells (A and B) have adjacent plasma membranes (arrows) with no gap junctions and minimal rough endoplasmic reticulum (curved arrows). In contrast, stressed spindle cells (C and D) have adjacent plasma membranes (arrows) with gap junctions and moderate rough endoplasmic reticulum (curved arrows). Transmission electron micrographs: A and C magnification of 140 000, B and D magnification of 32 000.

Table 1 Risk factors for retinopathy of prematurity

Clinical fact	Clinical event	Effect on spindle cells
Immaturity:		
Increased survival of infants weighing <1000 g	Low birth weight, low gestational age, surfactant	The smaller the infant, the larger the area of spindle cells in the inner retina with insufficient interstitial retinol binding protein in the outer retina
Free radical damage:		
Acidosis	Acute hypothermia, acute hypoxia, chronic hypotension (dopamine)	Subsequent reperfusion of retinal tissue results in increased oxygen consumption
Large amounts of oxygen given	Better ventilation, pneumothorax, bronchopulmonary dysplasia, patent ductus arteriosus, apnoea	More oxygen free radicals trigger increased formation of gap junctions between spindle cells
Increased amount of oxygen released to tissues by adult haemoglobin	Transfusion of blood for intraventricular haemorrhage, hyperbilirubinaemia, clinical laboratory monitoring	Replacement of fetal haemoglobin by adult haemoglobin shifts the dissociation curve so that more oxygen is released to the retina through the choroidal vessels
Microbiologically confirmed sepsis	Bacterial, fungal, viral systemic infections	Ingestion of pathogens by macrophages leads to the release of oxygen free radicals by macrophages
High light intensity	Unusually high, continuous light in the modern neonatal intensive care unit	Continuous, high light damages the developing photoreceptors so that they consume less of the oxygen that is arriving through the choroidal vessels and more oxygen reaches the spindle cells

Table 2 Comparison of four randomised masked controlled clinical trials that evaluated the effect of prophylaxis with vitamin E on the suppression of development of severe ROP

	Hittner et al ¹²	Finer et al ¹³	Johnson et al ¹⁴	Phelps et al ¹⁵
Study design:				
When study was conducted	November 1979 to December 1980	July 1978 to April 1981	January 1979 to May 1981	December 1980 to August 1983
Year that results were published	1981	1982	1988	1987
Initial route of administration	Oral	Intramuscular	Slow intravenous infusion, or intramuscular	Rapid intravenous infusion
Form of vitamin E used	Alcohol	Acetate	Alcohol	Alcohol
Mean vitamin E level in study (mg%)	1.2	4.6	5.0	3.5
Infants studied (all weighed <1500 g at birth)—control/treatment groups:				
Mean birth weight (g)	1050/1050	1203/1185	1156/1162	1205/1181
No receiving first dose of vitamin E within 24 hours of birth	75/75	64/62	194/186	147/140
No of infants whose eyes were examined when they were >8 weeks of age	51/50	50/47	147/141	99/97
No of infants that died	24/24	14/15	47/45	29/31
No of infants excluded	0/1	0/0	0/0	19/12
Toxic effects reported	None	None	Necrotising enterocolitis, sepsis	Intraventricular haemorrhage, retinal haemorrhages
Outcome of ROP —control/treatment groups:				
ROP ≥stage 3+, weighing ≤1500 g at birth	5/0	3/0	7/3	1/1
ROP ≥stage 3+, weighing ≤1000 g at birth	4/0	2/0	5/3	1/1
p Value	0.01	0.01	0.05	NS

myofibroblast ridge, or by ocular growth not accompanied by further retinal development, along the central/peripheral vector. Thus loss of retinal function by dragging without detachment, or by retinal detachment, is an event that is often delayed by months or years and is associated with continuous traction for some time.

RISK FACTORS

The risk factors of ROP are those clinical events that stress spindle cells. These events fall into two groups: immaturity and free radical damage (table 1).¹⁻⁴ Additionally, maternal angiogenic factors caused by diabetes or hyperthyroidism may initiate the development of severe ROP that follows a time sequence indicating an intrauterine onset. A combination of genetic factors may also induce a clinical appearance that mimics severe ROP but which is histologically distinct.¹¹

ANTIOXIDANT: VITAMIN E SUPPLEMENTATION

There have been four randomised, masked controlled clinical trials to evaluate the effect of prophylaxis with vitamin E on the suppression of the development of severe ROP (table 2).¹²⁻¹⁵ The protocol for each trial was designed so that vitamin E should be given from the first day of life and should be continued without interruption until inner retinal vascularisation was complete. Each used different vitamin E preparations, achieved different plasma vitamin E concentrations, used a different initial route of administration, evaluated ROP by different classification systems, set different end points for analysis, and had different percentages of infants lost to follow up.

The three clinical trials in which prophylaxis with vitamin E was shown to be effective may be explained by the spindle cell/myofibroblast pathogenesis of ROP. Early and continuous vitamin E supplementation resulted in sufficient retinal uptake to stabilise spindle cell membranes against free radical damage in almost all infants weighing 1001–1500 g at birth. This supplementation also partially protected most extremely high risk infants (those weighing 1000 g or less at birth) unless several risk factors were present. Johnson *et al* in their initial report took one year as their end point, which allowed exclusion of a few totally blind infants in the control group who died before 1 year of age.¹⁶

The single trial in which prophylaxis with vitamin E was not shown to be effective¹⁵ may also be explained by the spindle cell/myofibroblast pathogenesis of ROP. The initial, rapid intravenous infusion may have resulted in delayed uptake into the retina caused by sequestration by the liver of the transiently high plasma vitamin E concentrations.

This may have left the spindle cells in an unfavourable oxidant/antioxidant balance for long enough to induce formation of gap junctions. Some of the treated infants received doses intended for infants in the control group in error; this interrupted the prophylaxis. Thus even if there was initial stabilisation of the spindle cell membranes, spindle cells may have been left in an unfavourable oxidant/antioxidant balance for long enough to induce formation of gap junctions. The incidence of intraventricular haemorrhage was increased in those infants receiving treatment. There is a close association between intraventricular haemorrhage and ROP; thus the incidence of ROP in the infants receiving treatment may have been artificially increased, which would have masked any effect of vitamin E on the suppression of the development of severe ROP. This increased incidence of intraventricular haemorrhage is not in accordance with the results of Speer *et al*¹⁷ or Sinha *et al*,¹⁸ and may have been caused by the initial rapid intravenous infusion. Vitamin E may have displaced vitamin K at its binding site.

The issue of vitamin E supplementation to suppress the development of severe ROP was recently reviewed by the Institute of Medicine of the National Academy of Sciences.¹⁹ The committee concluded that there was no definite evidence of either benefit or harm from prophylaxis with vitamin E against ROP, and that the risks of giving vitamin E seem to be minimal for premature infants provided that the doses are kept low enough to achieve a blood concentration of no higher than 3 mg%.

Our personal clinical experience since 1979 with vitamin E prophylaxis is more favourable. No infant who weighed greater than 1001 g at birth, who received vitamin E according to our protocol, and who did not have severe hypoxia during delivery or severe hypotension with subsequent reperfusion, has developed severe ROP. Thus a sufficient retinal uptake of plasma vitamin E must be stabilising most of the spindle cell apron and halting the cascade of events that may ultimately result in traction and retinal detachment. The development of severe ROP in infants weighing 1000 g or less at birth is delayed. Insufficient retinal uptake of plasma vitamin E caused by there being insufficient amounts of interstitial retinol binding protein in the subretinal space may only transiently suppress formation of gap junctions between spindle cells in the inner retina. Thus in the smallest and illest infants, prophylaxis with vitamin E may ultimately fail to prevent sufficient spindle cell membrane damage. Severe ROP develops about two weeks later than in similar infants not receiving vitamin E.

The overall incidence of all stages of ROP is not decreasing because the protection given to plasma membranes by vitamin E is not an 'all or nothing' effect, and is by no means a panacea. Vitamin E prophylaxis is drastically decreasing the severity of ROP in infants weighing between 1001 and 1500 g at birth.

The ultimate clinical question is whether vitamin E prophylaxis to decrease the development of severe ROP can be justified for all infants weighing 1500 g or less at birth. The issue of its safety has been at the root of the controversy. With our protocol we have not seen a single case of toxicity from vitamin E prophylaxis in our centre since we began giving it in 1979. All reported toxic reactions²⁰ have resulted from high peaks of plasma vitamin E given by rapid intravenous administration (up to 15 mg%), or high, sustained plasma vitamin E concentrations achieved by slow intravenous infusion or intramuscular injection (more than 8 mg% for several days), or the high osmolality of oral vitamin E preparations (more than 2000 mOsm), or the use of improper carriers in small amounts to make intramuscular preparations soluble (sesame seed oil, Emulphor E1-100, and polysorbate 80), or the use of toxic polysorbate carriers in large amounts in E-ferol. E-ferol is a drug that was illegally sold in the United States for a short time from late 1984 to early 1985; it was recalled after many deaths had been reported, for which civil and criminal actions were taken.

OUR CURRENT RECOMMENDATIONS CONCERNING VITAMIN E

To suppress the development of severe ROP, we give 100 mg/kg/day of *dl*- α -tocopherol (or *dl*- α -tocopheryl acetate) orally in a medium chain triglyceride solution from the first hours of life until retinal vascularisation is complete. To suppress the development of severe intraventricular haemorrhage,^{17 18} we give 15 mg/kg intramuscularly or 5 mg/kg intravenously over eight hours as soon as possible after delivery, and 10 mg/kg intramuscularly or 3 mg/kg intravenously over eight hours on the second day of life. To maintain mean plasma

vitamin E concentrations between 1.2 and 3.5 mg% until oral feedings can be started (or if oral feedings are interrupted) we give 10 mg/kg intramuscularly every third day or 3 mg/kg/day intravenously over eight hours. We use only *dl*- α -tocopherol in water.

ANTIOXIDANT: SELENIUM SUPPLEMENTATION

Because vitamin E provides incomplete, age dependent, antioxidant protection against the development of severe ROP, future research must focus on antioxidant systems that are already endogenous to the retina of the infant weighing 1000 g or less at birth and that can be stimulated by water soluble substances. Vitamin C is an antioxidant system that meets these criteria; the system is, however, already operating maximally in infants of all gestational ages.⁶ Two independent laboratories have documented high retinal concentrations of selenium dependent glutathione peroxidase in all premature infants weighing 160 g or more at birth.^{21 22} Perhaps selenium supplementation from the first hours of life will maintain the activity of this antioxidant system and protect spindle cells in all infants who are likely to survive.

Traction (myofibroblasts)

REGRESSION OF STAGES 1, 2, OR MILD STAGE 3 ROP

When regression of mild ROP (international classification of ROP stages 1, 2, or mild stage 3) occurs, synthesis and secretion of angiogenic factors stop, gap junctions between adjacent spindle cells disappear uniformly throughout the apron, the extensive volume of rough endoplasmic reticulum decreases, and spindle cells remain as an apron. When regression of mild ROP occurs, inner retinal vasoformation continues by canalisation and reaches the ora serrata. Peripheral and posterior retinal and vascular changes tabulated by international classification of ROP¹⁰ do not follow stages 1 or 2 ROP, and there is no evidence of any remnants of myofibroblasts. These changes are minimal after mild stage 3 ROP, and there are minimal remnants of myofibroblasts, but usually no appreciable

Fig 6 Top: preoperative appearance at the age of 10 weeks of right retina of an infant weighing 650 g at birth with zone II stage 3+ ROP. Note the extent of vascularised retina (distance between the pair of three vertical dashes equals 2.5 cm of retina); and immature macula (circle); and the distance between the temporal rim of the optic disc (two vertical dashes) and the centre of the macula equals 0.5 cm of retina). Moderate plus disease and moderate invasion of myofibroblast are present. Bottom: postoperative appearance of the same eye at the age of 10 months. Note extent of viable retina (distance between the pair of three double vertical dashes equals 1.9 cm of retina); temporally displaced macula (circle); and distance between the temporal rim of the optic disc (two vertical dashes) and centre of the macula equals 0.7 cm of retina. Dragging of posterior vessels and the region of full thickness cryotherapy are visible. Fixation is unsteady (nystagmus) in this myopic (-9.00) eye at the age of 21 months. Comparison of these two retinal montages shows that cryotherapy to the avascular retina and shunt alone do not guarantee a favourable outcome with respect to macular function.

dragging of the larger retinal vessels, or of the macula.

REGRESSION OF SEVERE STAGE 3 ROP

When regression of severe ROP (international classification of ROP moderate or severe stage 3) occurs, synthesis and secretion of angiogenic factors stop, gap junctions between adjacent spindle cells disappear uniformly throughout the apron, the extensive volume of rough endoplasmic reticulum decreases, and all spindle cells stack within the inner retina. The stimulus for the spindle cell stacking is unknown; this subclinical event indicates, however, that peripheral spindle cells are no longer the stimulus for neovascularisation and that severe ROP will either regress or progress for unknown reasons. When regression of severe ROP occurs, inner retinal vasoformation may occur between the stacked spindle cells, but this vasoformation is not by canalisation and does not reach the ora serrata. In some cases of regression of severe ROP, greyish remnants of the contractile myofibroblast sheets that are at the site of the former shunt are left behind along the retina vitreous surface as the only sequelae of the active ROP tabulated by international classification of ROP as mild peripheral, and posterior retinal and vascular changes.¹⁰ In other cases these greyish remnants exist in sufficient numbers to cause dragging of the larger retinal vessels and of the macula. This is usually seen clinically as temporal dragging of the vessels and macula, tabulated by international classification of ROP as moderate peripheral, and posterior retinal and vascular changes.¹⁰

PROGRESSION

When progression of severe ROP occurs, synthesis and secretion of angiogenic factors stop, and all spindle cells stack. Clinically the posterior retinal vessels become dilated and tortuous (which is termed 'plus disease' according to international classification of ROP⁹), the shunt becomes engorged, and the myofibroblast sheets become vascularised and eventually haemorrhage into the vitreous (fig 6

top, fig 7 top, fig 8 top). Histologically a surge of myofibroblast sheets differentiate from the shunt and invade the vitreous (fig 9).

THE CONCEPT OF CRYOTHERAPY

Cryotherapy for ROP entails the application of a destructive cold probe to the scleral surface. The cold application must destroy both the spindle cells in the inner retinal layer and the myofibroblasts on the retina/vitreous surface. Cryotherapy destroys all living ocular tissue between the sclera and the retina/vitreous surface round the complete circumference of the eye. Fibroblasts from adjacent living sclera invade the dense scleral connective tissue residue, and allow anterior enlargement of the globe after cryotherapy.

CRYOTHERAPY TO THE AVASCULAR RETINA FOR STAGE 2 OR MILD STAGE 3 ROP

Cryotherapy to the avascular retina when only neovascularisation is present, (that is when spindle cells are still secreting angiogenic factors and myofibroblasts have not separated from the shunt) has been suggested. Theoretically, obliteration of the peripheral inducers would stop the progression of the disease completely. Such early cryotherapy, however, may cause problems in the smallest infants. Many sessions of cryotherapy would be needed to obliterate spindle cells that were intermingled with the formed vessels at the time of the initial cryotherapy but which continue to migrate into the treated areas later, and there would be destruction of an appreciable amount of the peripheral visual field and possibly macular dragging in an eye that might have undergone spontaneous regression. Thus early cryotherapy (at ROP stage 2 or mild stage 3) is not recommended.

CRYOTHERAPY TO THE AVASCULAR RETINA FOR MODERATE OR SEVERE STAGE 3 ROP

The National Eye Institute of the National Institutes of Health in the United States of America sponsored a clinical trial to evaluate the effect of cryotherapy to the avascular retina for ROP in eyes randomised

Fig 7 Top: preoperative appearance at the age of 10 weeks of the right retina of an infant weighing 880 g at birth, with zone II stage 3+ ROP. Note the extent of vascularised retina (distance between the pair of three vertical dashes equals 2.4 cm of retina); and immature macula (circle); and the distance between the temporal rim of the optic disc (two vertical dashes) and the centre of the macula equals 0.5 cm of retina. Severe plus disease and an area of haemorrhage adjacent to severe invasion of myofibroblasts are visible. Bottom: postoperative appearance of the same eye at the age of 20 months. Note temporally displaced, abnormally pigmented macula (circle), the distance between the temporal rim of the optic disc (two vertical dashes) and the centre of the macula equals 0.6 cm of retina, and the anterior extent of the therapeutic scleral buckle (wide solid circular line). Fixation is unsteady (nystagmus) in this myopic (−9.50) eye. Comparison of these two retinal montages shows that cryotherapy to the avascular retina and shunt alone did not prevent retinal detachment, and that a therapeutic scleral buckle does not permit a favourable outcome with respect to macular function.

Fig 9 Transmission electron micrograph of a myofibroblast with actin filaments (arrows) criss crossing the cytoplasm and ending in peripheral densities (curved arrows) that are analogous to Z lines of striated muscle (magnification of 48 000).

in symmetrical disease or in randomised infants in asymmetrical disease. Threshold disease was the development of five or more contiguous or eight cumulative clock hours of stage 3 ROP in zone I or II in the presence of 'plus' disease. This clinical trial found that cryotherapy to the avascular retina (as determined by fundus photographs of outcome three months after treatment) reduced the number of posterior retinal detachments, retinal folds affecting the macula or retrolental tissue, by 50%.²³ Of 156 treated eyes, 22% had an unfavourable outcome (78% had a favourable outcome), and of 149 untreated eyes, 43% had an unfavourable outcome (57% had a favourable outcome). Thus neither vitamin E nor cryotherapy is a panacea.

Though this collaborative trial evaluated the

effect of cryotherapy for ROP, many questions still remain unanswered. The mean birth weight for the study was 801 g and only 12 eyes (8%) had zone I ROP. It is unfortunate that the study will generate long term data only for comparatively large infants (92% had zone II ROP). According to the spindle cell/myofibroblast pathogenesis of ROP, if vitamin E supplementation for prevention of severe ROP had been given, many of the larger preterm infants might not have required cryotherapy, and the ratio of zone I : zone II disease would have been more equal. With increasing survival of the smallest preterm infants, however, the dilemmas of zone I disease cannot be addressed by the data base of this trial. Unfortunately the outcomes of zone I, which are more difficult to treat, were grouped with those

Fig 8 Top: preoperative appearance at the age of 10 weeks of the right retina of an infant weighing 760 g at birth with zone II stage 3+ ROP. Note the extent of vascularised retina (distance between the pair of three vertical dashes equals 2.4 cm of retina); and immature macula (circle); and the distance between the temporal rim of the optic disc (two vertical dashes) and the centre of the macula equals 0.5 cm of retina. Severe plus disease and an area of haemorrhage adjacent to severe invasion of myofibroblasts are visible. Bottom: postoperative appearance of the same eye at the age of 17 months. Note undisplaced, normally pigmented macula (circle), the distance between the temporal rim of the optic disc (two vertical dashes) and the centre of the macula equals 0.5 cm of retina, and the anterior extent of the prophylactic scleral buckle (wide dashed circular line). Fixation is steady (no nystagmus) in this myopic (-6.25) eye at the age of 18 months. Comparison of these two retinal montages shows that cryotherapy to the avascular retina and shunt combined with a prophylactic scleral buckle does permit a favourable outcome with respect to macular function.

of zone II. The study reported treatment that ranged from 6.7 to 23.9 weeks. The timing of cryotherapy was at a mean of 11.4 weeks, however, which coincides with the window of treatment (generally 8 to 10 weeks, but occasionally 6 to 15 weeks) predicted by the spindle cell/myofibroblast pathogenesis of ROP. At the later time points (15 weeks or more), it is not stated how many eyes were treated or what the percentage of favourable outcomes was. Unfortunately the study grouped the percentage of favourable outcomes without regard to the timing of cryotherapy. Thus it is unknown how many eyes were treated primarily to prevent traction associated with myofibroblasts. Despite a protocol designed to avoid the shunt, appreciable haemorrhages occurred in 30 eyes (19%). This high incidence was most likely caused by accidental cryotherapy to the shunt while it was still engorged with blood. Though it was important to expedite publication of the effectiveness of cryotherapy, only 45 infants had been followed for one year. Because of the developmental process of ocular growth (fig 4), the spindle cell/myofibroblast pathogenesis of ROP would predict that many of the reported successes will become failures with long term follow up. Tasman's editorial about the study has already alluded to this problem by describing rhegmatogenous retinal detachment in the better eye of two patients (at 1 and 4 years of age).²⁴ He attributes these retinal detachments to the intolerable stress associated with ocular growth that occurs after cryotherapy caused by a firm peripheral chorioretinal adhesion in small eyes. Our centre had a similar retinal detachment in the better eye of a patient (at 6 years of age).²⁵

We realise that when the remnant myofibroblast sheets produce minimal traction, the single procedure as recommended by the collaborative group may be sufficient; the larger retinal vessels and macula can, however, be appreciably dragged by ocular growth, and visual acuity may be as low as 20/200. Frequently, when myofibroblast sheets produce appreciable traction, this single procedure is not sufficient to counterbalance the forces generated by an active contraction of myofibroblast sheets, by a fixed remnant myofibroblast ridge, or by ocular growth, and retinal detachment can occur. To obtain retinal reattachment, a therapeutic scleral buckle is required (often with drainage of subretinal fluid). Scleral buckling entails placing a thin (0.75 mm) but broad (3.5 mm) silicone band round the equator of the eye under the four rectus muscles. Even when the retina is successfully reattached anatomically, the macular region has a distorted clinical appearance, and the best visual acuity that can be anticipated is 20/400.

OUR CURRENT SURGICAL PROTOCOL

With our long experience of cryotherapy to the avascular retina (since 1974) and with the unfavourable visual outcome of most of the patients caused by macular dragging or retinal detachment, we have advocated since 1987 the following surgical operations based on both the spindle cell/myofibroblast pathogenesis of ROP and the reality of ocular growth.

Destroy the spindle cell apron that is the source of angiogenic factors. This part of our current protocol is identical to that recommended by the collaborative study except that we treat both eyes when symmetrical disease is present. Clinically, within a day or two after cryotherapy to the avascular retina, the posterior retinal vessels become less dilated and tortuous, the shunt empties of blood, and the sheets of myofibroblasts atrophy.

Destroy the shunt and sheets of myofibroblasts that are the source of traction leading to macular dragging or retinal detachment. This second cryotherapy to the shunt and sheets of myofibroblasts (optimally some three to seven days after the initial cryotherapy to the avascular retina) will produce less haemorrhage than might be produced if the shunt and sheets of myofibroblasts are inadvertently treated during the initial cryotherapy. The two treatments may be sufficient to prevent retinal detachment in some cases, but do not prevent dragging of the macula (fig 6 bottom). The two cryotherapies, however, may not in many cases be sufficient to prevent retinal detachment, and a therapeutic scleral buckle may be required to reattach the retina (fig 7 bottom).

Place a prophylactic scleral buckle at the site of the former shunt at the time of the second cryotherapy. With continued growth of the anterior portion of the eye, this band changes the general shape of the eye from a sphere to an ellipse so that the posterior portion of the eye in which the macula resides is not dragged. Additionally, this band counterbalances tractional forces from the active contraction of sheets of myofibroblasts or from a fixed inactive remnant myofibroblast ridge that can lead to retinal detachment even when the shunt has been obliterated.

This staged procedure seems to allow the macula to develop maximally without distortion from dragging or detachment, and visual acuity is usually better than 20/40 (fig 8 bottom). When myofibroblasts have completely disappeared from the vitreous, complete quiescence of ROP has been obtained. When ocular growth is complete (at about 18 months), it is usually safe to remove the scleral buckle and permit the eye to return to its normal spherical shape.

As successful as these drastic operations may be,

this also is not a panacea, and is not the ultimate solution to the continuing saga of ROP. Thus the challenge remains to stabilise spindle cells before the initiation of the cascade of events recounted here—the spindle cell/myofibroblast pathogenesis of ROP as modified by ocular growth.

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