THE ELECTRORETINOGRAM AND VITAMIN A IN PRETERM INFANTS

by

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"Knowledge advances by steps, and not by leaps"

Thomas Babington Macaulay 1800-1859.

PREFACE

It was my privilege to spend two years between September, 1985 and August, 1987 as a Research Assistant in the Department of Child Health at the University of Missouri-Columbia Health Sciences Center, Columbia, Missouri, U.S.A., under the guidance of Calvin W. Woodruff, M.D., Professor of Child Health. Dr. Woodruff is a recognised expert in the field of Paediatric Nutrition, and his unbridled enthusiasm and ever enquiring mind prompted many challenging discussions.

Except where acknowledged otherwise, I personally carried out all of the research here described, and the writing of this thesis was completed entirely by myself.

I offer this work as a tribute to the many babies who took part.

Helen Mactier

Dundee, 1987.

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SUMMARY

The electroretinogram (ERG) records an electrical potential arising from cells within the retina in response to stimulation by light. As an objective measure of retinal function, electroretinography has potential clinical application in neonates. However, technical difficulties inherent in recording low amplitude responses from unco-operative subjects have limited work in this field, and to date little is known about the normal development of the ERG during the early weeks of life.

Plasma concentrations of retinol in the majority of preterm infants are marginal or deficient by adult standards, and there is indirect evidence that this reflects depleted liver stores of vitamin A. At present the functional significance of these observations is unclear. The earliest sign of vitamin A deficiency in adults and older children is impaired dark adaptation, and this is associated with changes in the ERG prior to the onset of subjective nightblindness.

Thus, it was anticipated that the development of a reliable method for recording the ERG in the neonatal period might help to determine vitamin A requirements in preterm infants.

A technique is described by which the ERG could be studied soon after birth in extremely low birthweight infants.

The success rate in recording an interpretable averaged ERG was 96% in term infants and 92% in preterm infants. Results with this method in adult controls were comparable to results obtained by standard techniques. Continuous observation of the infant and assessment of the degree of eye opening was achieved by video monitoring. Minimal restraint was necessary, and no major incidents or complications were encountered.

Electroretinograms were recorded successfully in 50 term infants aged between 7 and 148 hours. The amplitude of the ERG was less and the a- and b-wave latencies were longer than in adult controls. Eyelid closure during recording of the ERG significantly increased the a-wave latency and diminished the amplitude of the response. Both the a- and b-wave latencies shortened with advancing postnatal age.

Fifty-nine preterm infants were studied at ages ranging from 7 hours to 87 days. The amplitude of the ERG was less and the a-wave latency was longer than in term infants of comparable postnatal age. The ERG was absent initially in two of the most immature infants although each subsequently demonstrated a clearly defined response. Eyelid closure did not have a significant effect upon any of the ERG parameters in preterm infants. The b-wave latency decreased after 48 hours' exposure to light ($p < 0.01$) and subsequently was related inversely to postconceptional age. Longitudinal and cross-sectional observations both showed a reduction in the a- and b-wave latencies and an increase in the amplitude of the

ERG over time. Maturation of the ERG in preterm infants appears to be mediated, at least in part, by exposure to light.

The preterm infants had significantly lower plasma levels of retinol, retinol-binding protein, prealbumin and α-tocopherol than term infants of comparable postnatal age. Plasma concentrations of retinol were below accepted normal levels for older children in all but three preterm infants, and did not change over time. The rise in plasma retinol concentration following an oral dose of 5000 IU retinol (the retinol dose response) suggested that low circulating levels of retinol in preterm infants reflect reduced hepatic reserves of vitamin A. Tocopherol levels were adequate in the majority of preterm infants after the fifth day of life and were higher in infants of all gestational ages fed with own mother's milk. Standard oral vitamin supplementation did not affect plasma levels of either retinol or α-tocopherol.

A relationship was not demonstrated between any of the averaged ERG parameters and either the plasma concentration of retinol or the retinol dose response.

The electroretinographic threshold was measured in 12 preterm infants. Allowing for eye opening, there was a significant reduction in the threshold over time. The logarithm of the electroretinographic threshold correlated with the retinol dose response, but was not related significantly to the predose plasma concentration of retinol.

This work suggests that retinal stores of vitamin A in apparently healthy preterm infants are reduced in conjunction with depletion of hepatic reserves. Current recommendations for vitamin A supplementation of preterm infants may be inadequate and require review subsequent to further studies of vitamin A tissue function.

PART I

REVIEW AND RATIONALE

CHAPTER 1. THE ELECTRORETINOGRAM

1.1 HISTORY OF ELECTRORETINOGRAPHY

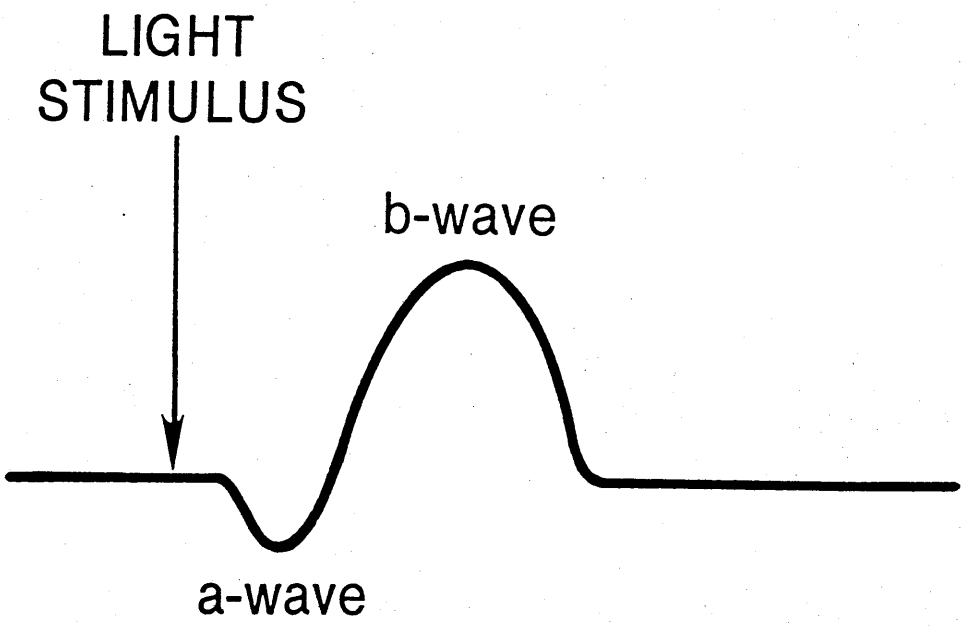
The discovery of the electroretinogram (ERG) is accredited to Holmgren (1) who, in 1865, observed a change in the resting potential of the excised frog eye when light fell upon the retina. Dewar in Scotland (2) succeeded in measuring the human ERG for the first time in 1877, but it was not until the development of the contact lens electrode by Riggs in 1941 (3) that electroretinography became established as a useful clinical tool.

1.2. DEFINITION

The ERG is a record of the transient action potential which arises from the outer layers of the retina in response to light stimulation.

As measured using surface electrodes, the ERG consists of an initial corneal negative deflection, the "a-wave", followed by a larger positive "b-wave" (4) (Figure 1). The duration of the response is of the order of 0.1 - 0.3 seconds.

FIGURE 1. THE ELECTRORETINOGRAM



1.3. CELLULAR ORIGINS OF THE ELECTRORETINOGRAM

Intraretinal recording has contributed to an, as yet incomplete, understanding of the cellular origins of the components of the ERG. Absorption of quanta of light by molecules of visual pigment gives rise to a wave of (in vertebrates) hyperpolarisation of the photoreceptors, which is measured as the a-wave (5). This initiates extracellular potassium changes in the inner nuclear layer, and the b-wave is postulated to reflect resultant electrical activity in the Muller (glial) cells of the retina (6,7). The ganglion cells and the fibres of the optic nerve do not contribute to the generation of the ERG; consequently the ERG should be regarded as a test of retinal function, rather than of vision.

1.4. PRINCIPLES OF ELECTRORETINOGRAPHY

In practice, the change in electrical potential across the retina is measured between two surface electrodes. A contact lens (3,8) or conductive thread (9) located on the anterior surface of the eye provides the active electrode, and a reference electrode is located in the periocular region, commonly on the forehead or temple.

Any light stimulus entering the eye is scattered by media opacities and reflected unevenly to receptors in all parts of the retina (10). Since the ERG is a summation of responses

arising from all receptors excited by the stimulus, it is important that the stimulus provides uniform illumination of the retina. Full-field or Ganzfeld stimulus conditions are achieved by the use of a diffusing sphere to elicit similar, synchronised responses from the entire retina (11).

Pupillary dilatation minimises differences in pupil size and consequent variation in the intensity of light falling upon the retina (12). Furthermore, artifacts caused by pupillary movement are eliminated.

Even with optimal shielding, some background electrical activity or "noise" inevitably will be recorded, which can make interpretation of the ERG difficult. For this reason computer averaging of responses may be used when recording predictably low amplitude responses, as in infants and young children or adults with diffuse retinal pathology (13,14). When numerous individual responses are averaged, the random electrical noise is gradually reduced towards zero and the ERG, being a constant signal, may be distinguished more easily. The signal to noise ratio is increased by the square root of n, where n = number of averaged responses (13).

1.5. PROPERTIES OF RODS AND CONES

The human retina contains approximately 6 million cones and 100 million rods, and the relative contribution of each type of photoreceptor to the mass response is determined by

the conditions under which the ERG is recorded (15). The different properties of rods and cones are summarised in Table I.

The rods have a lower stimulus threshold and may be stimulated selectively by weak light stimuli of short (blue) wavelength under dark adapted conditions. Higher intensity stimuli will excite both types of photoreceptor; the ERG is then the algebraic summation of rod and cone responses. In the presence of significant background light rod function is suppressed, and a strong red light stimulus excites only the cones. Cone function may also be isolated by using flickering light stimuli since the rods have a longer refractory period and cannot respond at stimulus rates of greater than 20 Hertz (Hz). Compared to the cone response, the b-wave of the rod ERG is of longer latency (time from stimulus onset to b-wave peak) and greater maximum amplitude (height of the b-wave).

The sensitivity of all the photoreceptors is enhanced by dark adaptation, and the amplitude of the ERG is increased. A similar effect upon the ERG is produced by intensifying the light stimulus.

Clearly, therefore, both the state of adaptation of the retina and the characteristics of the stimulus must be taken into account when recording the ERG.

TABLE I - PROPERTIES OF RODS AND CONES

	Rods	Cones
Rate of dark adaptation	Slow	Fast
Sensitivity to light stimuli	High	Low
Sensitivity to background light	High	Low
Wavelength of maximum sensitivity (colour)	Short(blue)	Long(red)
Refractory period	Long	Short
b-wave latency	Long	Short
Maximum amplitude of response	Large	Small
Sensitivity to retinopathic agents/hypovitaminosis A	High	Low

CHAPTER 2. THE ELECTRORETINOGRAM IN INFANCY

2.1. ANATOMICAL AND PHYSIOLOGICAL ASPECTS

Compared to other vertebrate species, the human retina is in a relatively advanced state of development by the time of birth. However, recent evidence suggests that at term the retina may be less mature, both in structure and function, than believed previously.

At 22 weeks of gestation cones are present in the fovea (16), and by the seventh intrauterine month mitosis has ceased and both rods and cones can be distinguished in the peripheral retina (17). Between the sixth and ninth gestational months the retinal surface area increases twofold, with a further 50% increase occurring before the second postnatal year (18). Morphologically, with the exception of the fovea, the retina of the newborn term infant closely resembles that of the adult. However, migration within the retina of neural and glial cells as well as photoreceptors is not complete until the third or fourth year of life (16). Full development of the fovea once was thought to be achieved by the sixth month of extrauterine life (19), but probably also does not take place until the third or fourth year (16). The functional significance of this is not yet clear. Since the fovea subtends only about 5 degrees of

visual angle, immaturity in that region will not have a significant influence upon the full-field ERG (20).

The development of the ERG in human infants may be explained by observations on the neurotransmitter properties of the retina on the first day of life (21). The content of dopamine, the predominant catecholamine in the vertebrate retina, is twenty times less in the newborn than in the adult retina (21). Dopamine release and the activity of its bio-synthetic enzyme, tyrosine hydroxylase, are increased by light (22,23), and this increase in dopamine synthesis is related to light intensity (22). Furthermore, dopamine depletion in the frog retina results in a lengthening of the b-wave latency and a reduction in the amplitude of the ERG (24).

2.2. ANIMAL STUDIES

The earliest component of the ERG which can be elicited from the developing vertebrate retina is the a-wave, and its appearance coincides with formation of the outer segments of the photoreceptors (25-28). As mature synapses develop between the photoreceptors and second order neurones, the b-wave appears and quickly becomes the predominant component of the ERG (26,27). In the maturing rat retina, increases in both the maximum amplitude of the ERG and the retinal sensitivity to light are much greater than would be anticipated from the concomitant increase in rod outer

segment length and retinal surface area (28).

The ERG cannot normally be recorded from newborn kittens until after the fifth postnatal day. In a study of kittens born and reared in complete darkness, the appearance of the ERG was delayed and the latency of the b-wave was prolonged during the first six weeks of life (29). This corresponded with a reduction in the intensity of staining for sulphydryl groups in the retina (30). Regardless of light exposure, the ERG had developed in all kittens by four weeks after birth.

Thus, there is evidence that the ERG is influenced by the maturation of neural mechanisms in the developing retina, and that this in turn is determined, at least in part, by exposure to light.

2.3. EARLY HUMAN STUDIES

Recording of the ERG from a newborn baby was first attempted by Zetterstrom in 1951 (31). Unaware that the retina of the young infant has a much higher threshold of stimulation than that of the older child or adult (32,33), she concluded initially that the ERG was absent during the first couple of days of life in healthy term infants. This was in keeping with the then current theory that human infants were blind at birth, although it was at odds with animal studies

showing ERG responses from the relatively immature retina of the 15 day old dog (34). In 1960 Winkelman and Horsten demonstrated that all normal newborns of at least 34 weeks' maturity will have a measurable ERG as soon as 30 minutes after birth, provided that the retina is dark adapted adequately and stimulated with a sufficiently intense light source (34,35). Furthermore, allowing for reduced retinal sensitivity, the ERG in response to rapidly flickering stimuli was similar to that of the adult (36). They concluded that both cone and rod function was present within a few hours of birth in infants of greater than 32 weeks' gestation, and this has been confirmed by study of the ERG in premature and newborn term infants using different coloured light stimuli (37-39).

2.4. FEATURES OF THE INFANT ELECTRORETINOGRAM

In fullterm infants at all stimulus intensities the latencies of the ERG are longer and the amplitude is smaller than in adults (31,38,39,40). The mean amplitude of the dark adapted ERG in the first 38 hours of life has been reported to be six to ten times less than that of the adult, although the b-wave latency was only slightly longer (39).

In term infants, at least from six weeks of age, there is a steady change in the b-wave latency and amplitude of the ERG towards adult values, which continues into the second

year of life (33).

Hrbek and Mares (40) noted longer a- and b-wave latencies and reduced amplitude in the ERGs of premature infants on the first day of life, compared to mature infants of the same age. However, the differences were not significant. In subsequent recordings from the premature infants the latencies were shorter and the amplitude of the response was greater.

The sensitivity of the retina to light in preterm infants is probably less than in term infants, and related to maturity (41), but this has never been measured accurately. Psychophysical (32) and electroretinographic (33) studies are in agreement that the retina normally attains adult sensitivity by six months of age.

2.5. CLINICAL APPLICATION

The ERG is an objective measure of retinal function and thus has potential as an adjunct to ophthalmological assessment in neonates, and perhaps even as an indicator of gestational maturity in preterm infants (42). However, only two studies to date have pursued the clinical application of electroretinography in the neonatal period.

The ERG in the third week of life in infants who had received phototherapy with eye shielding did not differ from that of age-matched controls. Only infants of greater than 36 weeks' gestation were included in this study (43).

Taurine deficiency in cats causes ERG changes before structural alterations in the photoreceptors become apparent (44,45), and ERG amplitude and retinal taurine concentration show parallel changes in rats (46). However, supplementing the diets of healthy, very-low-birthweight, formula-fed infants with taurine did not have a significant effect upon the ERG at 37 postmenstrual weeks (47).

Thus, while some information exists about the ERG in the early weeks of life, there are major gaps in current knowledge. It is not known how the retina of the extremely preterm infant responds to light, if at all, nor how the ERG subsequently is modified by increasing age and maturity, exposure to light or, indeed, other potentially adverse environmental influences such as hypovitaminosis A.

CHAPTER 3. VITAMIN A AND THE ELECTRORETINOGRAM

3.1. VITAMIN A METABOLISM AND FUNCTION

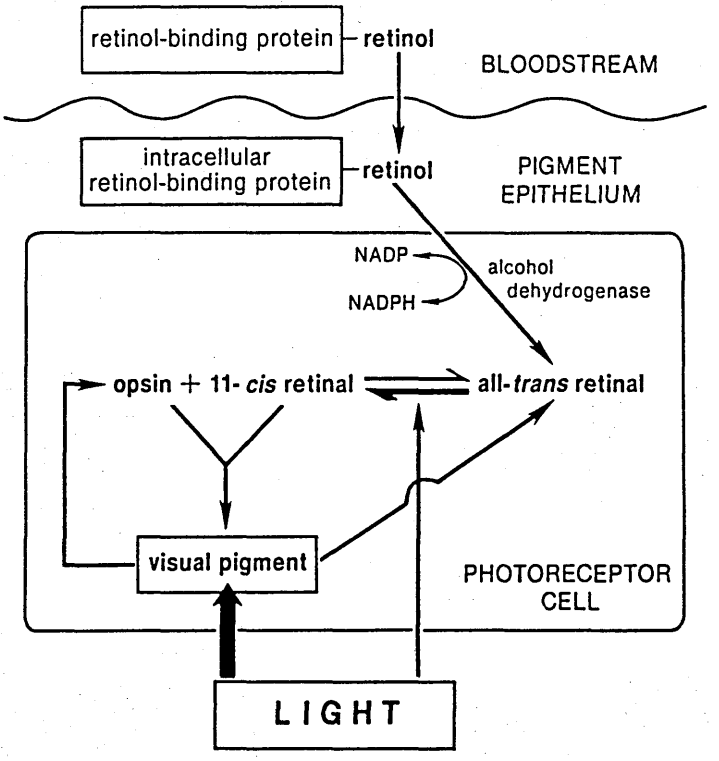
Fat soluble vitamin A is a multifunctional vitamin whose mechanism of action is not yet understood fully. Dietary retinyl esters and carotenoid precursors are converted to retinol in the intestinal mucosa, re-esterified with long chain fatty acids and transported via the lymphatics to the liver (48,49). Retinol is mobilised from hepatic stores bound to a specific carrier protein, retinol-binding protein, which normally circulates in a 1-1 molar complex with prealbumin. Since over 90% of the body's reserves of vitamin A are stored in the liver, the plasma concentration of retinol reflects the total body content of vitamin A only in severe depletion or in hypervitaminosis A. Vitamin A is necessary for the maintenance of differentiated epithelia (50) and mucus secretion (51), for reproductive function (52,53), for the regulation of osteoblastic activity (54), for the release of insulin from the islet cells of the pancreas (55) and as a precursor of visual cell pigments (56). The metabolism and function of vitamin A have been reviewed comprehensively (57).

3.2. THE VISUAL CYCLE

The ancient Egyptians recognised that eating liver could alleviate nightblindness (58), but it was not until 1924 that "fat soluble A factor" was identified as the "vital amine" necessary for vision (59). Vitamin A deficiency in rats was found to be associated with impaired regeneration of visual pigment (60), and could be relieved by retinal extract of pigs (61). In 1935 Wald described the roles of vitamin A and rhodopsin in the visual cycle (62) (Figure 2), and vitamin A was later named retinol in recognition of its first identified site of action (56).

Specific uptake of retinol from the plasma into the pigment epithelium is mediated by intracellular retinol-binding protein (63,64), and retinol is oxidised to all-trans retinal in the outer segments of the photoreceptors. Isomerisation of all-trans retinal yields 11-cis retinal which combines with specific proteins called opsins to form visual pigments. There are four visual pigments in the human retina; the abundant rod pigment, rhodopsin, and three less well understood cone pigments (63). The molecular configuration of retinal is fundamental to the action of light upon the photoreceptor. Bleaching of visual pigment by light results in isomerisation of 11-cis retinal to the all-trans form, and the visual pigment molecule dissociates to all-trans retinal and opsin. Light-induced reisomerisation of all-trans retinal occurs in the

FIGURE 2. THE VISUAL CELL CYCLE



pigment epithelium (65), and 11-cis retinal is made available for renewed synthesis of visual pigments. Thus, the retinal stores of vitamin A are in continuous interchange with circulating retinol via the vascular pigment epithelium.

The vitamin A content of the pigment epithelium and choroid is twelve times greater than that of other tissues in the body, with the exception of the liver (66). Nevertheless, less than 1% of the body's stores of vitamin A is concerned with visual function (57,63). Autopsy data and controlled depletion studies suggest sparing of retinal stores of vitamin A in hypovitaminosis A (67,68).

3.3. ANIMAL STUDIES

When the albino rat is raised on a vitamin A deficient diet, a steady depletion of liver stores of vitamin A occurs, and then the blood concentration of retinol falls precipitously (69). Coincident with the fall in serum retinol is a decline in the rhodopsin content of the eye which is dependent upon the intensity and duration of light to which the animal is exposed (70). Vitamin A deficient rats reared in darkness show no depletion of rhodopsin (70). As the rhodopsin content of the eye falls, the amplitude of the ERG (in particular the a-wave component) decreases, and there is a concomitant rise in the dark adapted electroretinographic threshold (69,70). The logarithm of the electroretinographic threshold is related

linearly to the concentration of rhodopsin in the eye (69). After prolonged vitamin A deficiency structural alterations occur in the retina, with rod cell degeneration preceding degeneration of the cones (69,71). Electroretinographic changes cannot then be reversed completely with vitamin A replacement therapy (69).

3.4. HUMAN STUDIES

The first sign of vitamin A deficiency in human subjects is impaired dark adaptation (49,72). This manifests as nightblindness and begins to occur with serum retinol values of 20-30 μg/dl (72). Young children are most prone to the effects of vitamin A deficiency (73), and the prevalence of nightblindness in preschool children having serum retinol values of less than 20 μg/dl has been estimated at 9.7% (49).

In adults with vitamin A deficiency the dark adapted threshold of the ERG is raised, and the amplitude of the response is reduced, prior to the onset of subjective nightblindness (74-77). As measured by electroretinography, the rods are more susceptible than the cones to the effects of hypovitaminosis A (75-77) (Table I), and in severe cases changes in the ERG associated with vitamin A deficiency may not be reversed completely with appropriate replacement therapy (74,75,78-80).

The amplitude of the ERG correlated with the serum

vitamin A concentration in Indonesian and Thai children aged from five to nine years (80). This confirmed a previously observed reduction in the amplitude of the ERG in undernourished children with signs and symptoms of vitamin A deficiency (81). The ERG has been recorded in children with serum vitamin A levels as low as 1.0 μg/dl (80); an earlier finding of frequently absent ERGs in children with nightblindness and low plasma concentrations of vitamin A (73) is attributable, at least in part, to insensitive recording equipment.

In electroretinographic studies of rod function, Fulton (33,82) has reported a reduction in both the sensitivity of the retina and the maximum amplitude of the dark adapted ERG in a five month old infant with cystic fibrosis and low serum retinol, compared to normal infants of the same age.

CHAPTER 4. THE VITAMIN A STATUS OF PRETERM INFANTS

Preterm infants have significantly lower plasma concentrations of retinol, retinol-binding protein and prealbumin at birth than term infants (83-86). The placental transport of vitamin A is understood poorly, but in healthy term infants cord blood levels of retinol and retinol-binding protein are only about half of those in maternal blood (85-88). In preterm infants cord blood levels of retinol and retinol-binding protein do not correlate with gestational age (83-86,89), and it appears that accumulation of vitamin A in the human fetus occurs over a relatively short space of time, probably during the eighth gestational month (83,85). This is consistent with studies in the developing rat fetus (90).

The plasma retinol concentration remains steady or tends to fall during the first few weeks of life in preterm infants (91-93) and, despite routine vitamin A supplementation or doubling the supplement to 3000 IU daily in formula-fed infants (91), the majority of preterm infants have circulating levels of retinol considered marginal (10-19 μg/dl) or deficient (less than 10 μg/dl) in adults and older children (91-94). Autopsy studies have suggested that this reflects reduced liver stores of vitamin A (89,95), a situation confirmed *in vivo* by

the rise in circulating retinol - retinol-binding protein complex following an oral dose of retinol (the retinol dose response) (96).

The clinical significance, however, of low circulating levels of retinol in preterm infants remains unclear. An hypothesised relationship with necrotising enterocolitis (83) is unproven, and xerophthalmia has not been reported in this population. Fluorescein-positive superficial punctate keratopathy will be present in approximately 7.5% of preschool children with serum retinol levels less than 20 μg/dl, although corneal xerosis is rare until the serum vitamin A concentration falls below 15 μg/dl (49). Preterm infants who develop bronchopulmonary dysplasia have lower plasma levels of retinol at birth than those who do not develop bronchopulmonary dysplasia (93), and continue to have reduced circulating levels of retinol throughout at least the first two months of life (92,93). Moreover, there is evidence that early vitamin A supplementation in preterm infants may reduce the incidence and severity of bronchopulmonary dysplasia (97).

HYPOTHESIS

In the majority of preterm infants plasma levels of retinol are low, reflecting reduced liver stores of vitamin A. At present, the clinical significance of this observation is unclear. Determination of acceptable plasma levels of retinol in preterm infants requires an *in vivo* measure of vitamin A tissue function.

From studies in animals and older humans it might be anticipated that the earliest sign of a functional vitamin A deficiency state in preterm infants would be changes in the ERG. However, normative ERG data for preterm infants have not been documented.

This thesis addresses these issues.

PART II

THE ELECTRORETINOGRAM IN TERM AND PRETERM INFANTS

CHAPTER 5. MATERIALS AND METHODS

5.1. PATIENTS

Infants were recruited from the neonatal intensive care unit or postnatal wards of the University of Missouri-Columbia Hospital and Clinics. Informed, written consent was obtained from one or both parents (usually the mother) on a form approved by the hospital ethical committee. The gestational age of the infant was estimated from the maternal obstetric records and from clinical examination (98). When calculating postnatal age the day of birth was considered day 1, regardless of the time of birth.

5.2. RECORDING OF THE ELECTRORETINOGRAM

In order to create full-field stimulus conditions, a light-proof reflective covering for an Armstrong isolation incubator (Ohio Medical Products, Madison, Wisconsin) was constructed from a canvas hood lined by an infant warming blanket of metallic fabric (a "space blanket"). The stimulus was a white flash of 0.1 millisecond (msec) duration, delivered by a Grass PS22 photostimulator (Grass Instrument Company, Quincy, Illinois) at maximum intensity. This was placed in a

corner of the incubator at the infant's feet and directed obliquely upwards. Sufficient red background light was provided within the covered incubator by a flashlight to allow continuous video monitoring of the infant via a sleeve in the top of the reflective covering (Figure 3).

All ERG recordings were made using a C4 electrodiagnostic system (Nicolet, Madison, Wisconsin). The band pass filters were set at 1 Hz and 1000 Hz, and the reject mode allowed artifactual responses, such as those caused by blinking, to be discarded.

There was no appreciable difference in signal quality between ERGs recorded either in an electrically shielded neurophysiology laboratory or in the neonatal intensive care unit; consequently most of the ERG recordings were made in the nursery.

Soon after feeding and changing, the infant was swaddled and placed supine in the incubator with a padded head restraint (Figure 4). Continuous heart rate monitoring was continued in those preterm infants deemed to be at risk from apnoea or bradycardia. All recordings were made from the left eye following local anaesthesia (proparacaine 0.5%) and pupillary dilatation (2 drops tropicamide 1%). The Dawson-Trick-Litzkow silver thread electrode (9) was placed under the lower eyelid, and reference and ground electrodes were attached to the left temple and forehead, respectively (Figure 5).

FIGURE 3. LIGHT-PROOF, REFLECTIVE INCUBATOR COVER WITH VIDEO CAMERA IN POSITION.

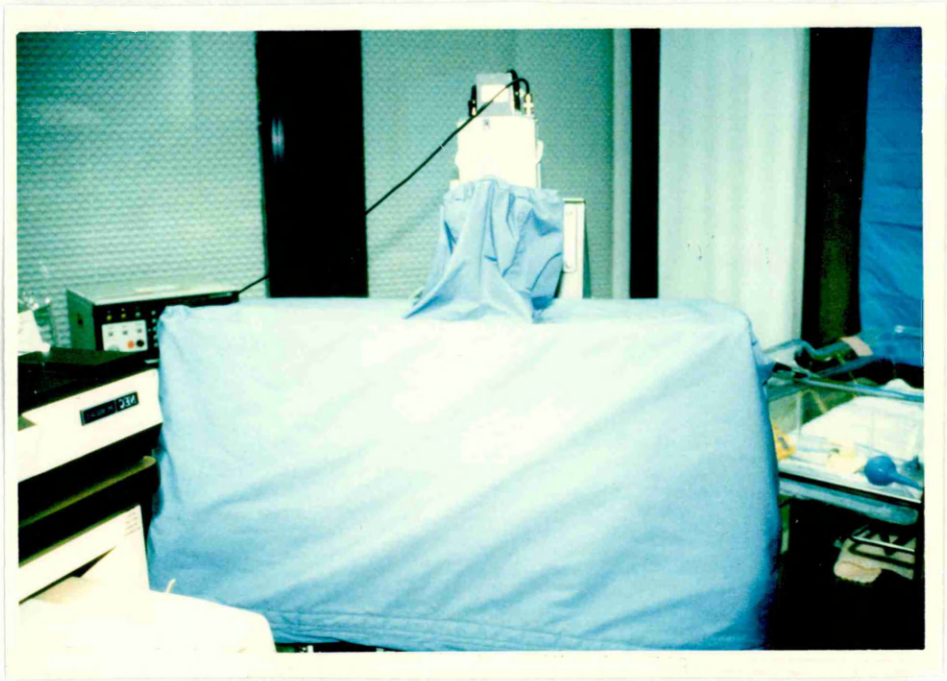


FIGURE 4. PRETERM INFANT POSITIONED FOR RECORDING OF THE ELECTRORETINOGRAM.

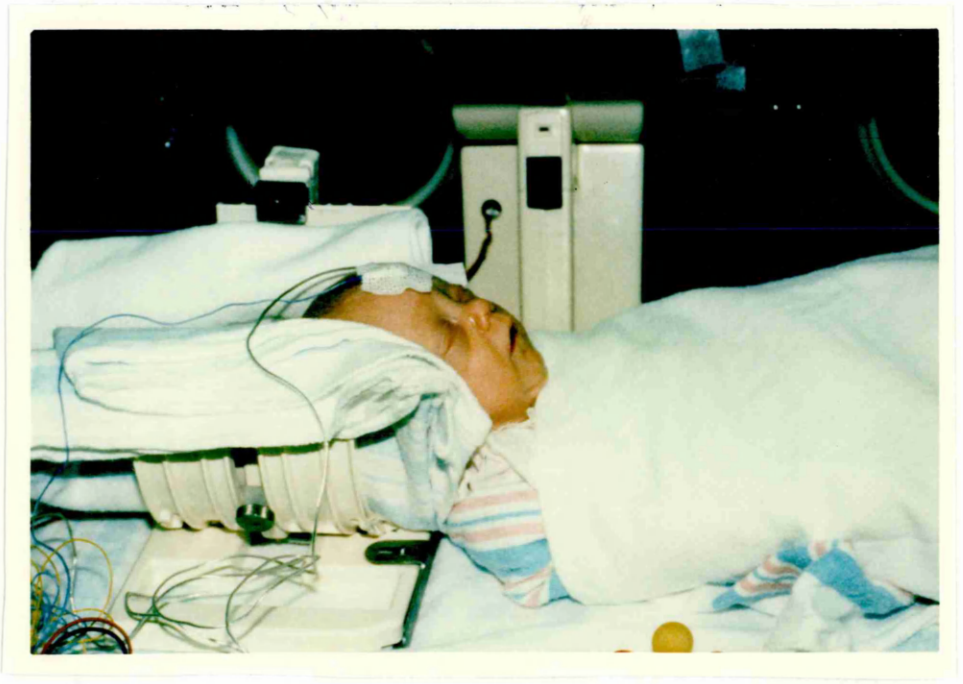
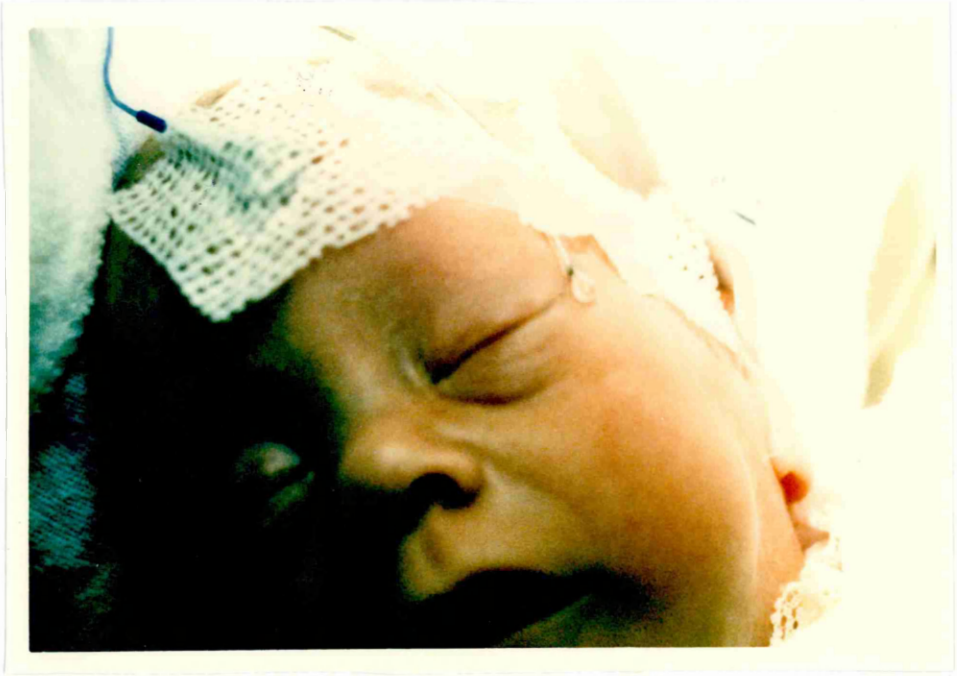


FIGURE 5. ELECTRODE PLACEMENT IN A PRETERM INFANT



The infant was adapted to the dim red background light for 15 minutes before being light adapted to the stimulus at a rate of 1.1 Hz for 2 minutes. One hundred responses were then averaged and amplified. This was the number of averaged responses found to be necessary to achieve an interpretable ERG in the majority of preterm infants.

Via the video monitor, the frequency of eye opening during recording of the ERG was noted and scored as follows:

0 = eyes closed throughout

1 = intermittent eye opening

2 = eyes mostly open.

The fundus and ocular surface were examined after removal of the recording electrode. In no case was there any evidence of irritation or abrasion. The entire procedure occupied about 30 minutes.

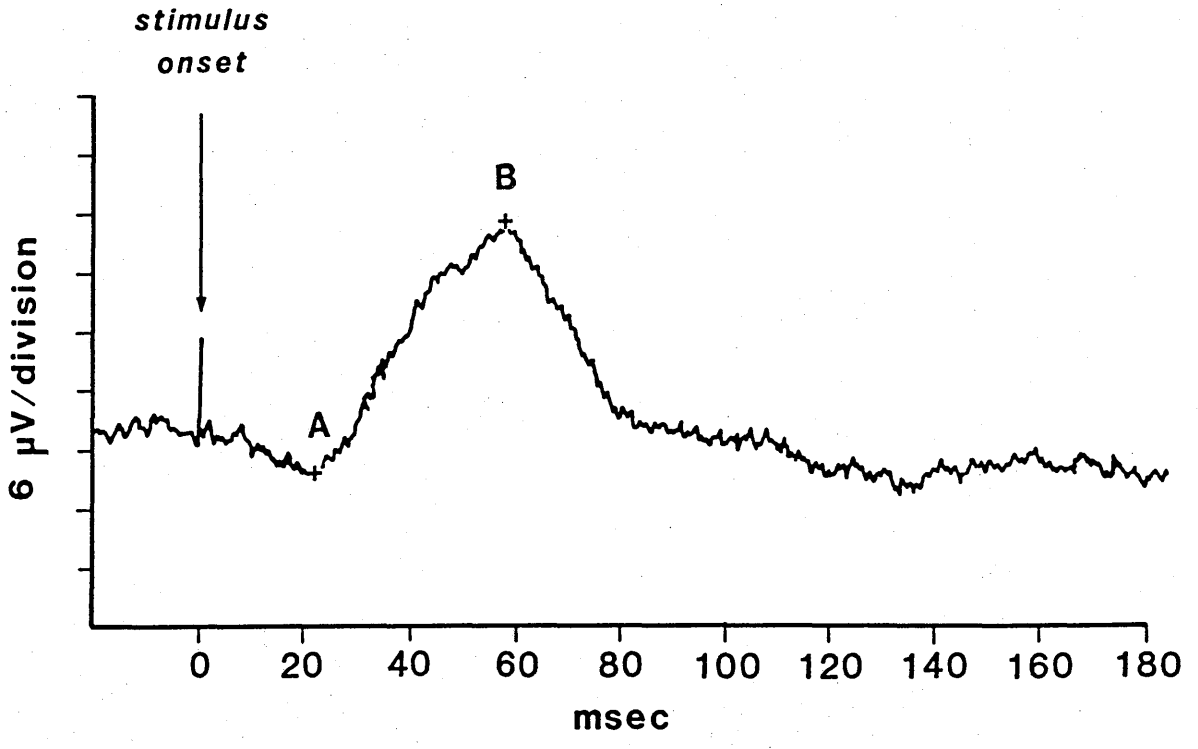
Figure 6 shows a representative averaged ERG from a preterm infant. The following measurements were made from each ERG:

a-wave latency - time from stimulus onset to a-wave trough (msec)

b-wave latency - time from stimulus onset to b-wave peak (msec)

amplitude - from a-wave trough to b-wave peak (microvolts (μV)).

FIGURE 6. REPRESENTATIVE ELECTRORETINOGRAM FROM A PRETERM INFANT.



The a-wave latency is the time interval from stimulus onset to point A, the b-wave latency is the time interval from stimulus onset to point B and the amplitude is the voltage difference between point A and point B.

The infant was aged 32 days (gestational age 32^{+2} weeks).

All of the ERG measurements were reviewed by an independent observer without knowledge of the maturity or postnatal age of the infant.

5.3. STANDARDISATION OF EQUIPMENT

To ensure that the retina was in a steady state of light adaptation at commencement of recording the ERG, a series of four ERGs in rapid succession, consisting of 25 averaged responses each, was made from one infant following 2 minutes' adaptation to the flashing light stimulus (Table II). The a-wave and b-wave latencies were constant and the amplitude, although variable, did not show a trend over time. There was intermittent eye opening throughout the recording session.

The equipment was standardised against a Ganzfeld diffusing sphere (Nicolet, Madison, Wisconsin) at high intensity in four adult volunteers. This was achieved by removing the top of the incubator together with the reflective covering and placing them upon the floor. The adult then positioned his head inside the incubator top in a position similar to that occupied by the infants. The conductive thread electrode was used throughout, and all recordings from one adult were made on the same day in random order. Both "eyes open" and "eyes closed" states were studied in order to examine the effect of eyelid closure upon the ERG (Table III). There were no significant differences in either the latencies or amplitude of

TABLE II - SERIES OF 4 ELECTRORETINOGRAMS MADE IN RAPID SUCCESSION FROM A FEMALE INFANT (GESTATIONAL AGE 41^{+2} WEEKS, AGED 19 HOURS).

ERG number	a-wave latency (msec)	b-wave latency (msec)	Amplitude (μV)
1	26.0	56.5	18.1
2	26.0	55.6	30.2
3	26.0	55.2	30.8
4	26.0	56.0	23.4

Each electroretinogram is the average of 25 responses.

There was intermittent eye opening throughout the recording session.

TABLE III - COMPARISON OF GANZFELD AND INCUBATOR ELECTRORETINOGRAMS IN 4 CAUCASIAN ADULTS.

		Eyes open	Eyes closed	p*
GANZFELD	a-wave latency (msec)	15.4±0.5	24.9±1.0	<0.01
	b-wave latency (msec)	37.5±1.5	56.0±3.9	<0.01
	Amplitude (μV)	127.0±15.4	82.4±17.8	NS
INCUBATOR	a-wave latency (msec)	16.1±1.0	23.8±1.9	<0.05
	b-wave latency (msec)	41.2±2.8	52.5±5.7	<0.05
	Amplitude (μV)	99.6±15.3	86.2±3.8	NS

Values are expressed as mean ± SEM.

*p values obtained by Student's paired t-test.

There were no significant differences between Ganzfeld and incubator values.

the ERG between the incubator and the Ganzfeld diffusing sphere. Eyelid closure significantly increased the a- and b-wave latencies for all adult subjects, but did not consistently affect the amplitude. For both recording techniques, there was a large interindividual variation in the amplitude of the response.

In one preterm infant the ERG was recorded on two successive days. The same ERG latency and amplitude values were obtained on both occasions.

5.4. STATISTICAL METHODS

All results were analysed using either PC Statistician (99) or Statistical Analysis Systems (100) programmes. Differences between mean values were evaluated by Mann-Whitney U test, and Spearman Rho or multiple linear regression techniques were used to determine correlations. Changes over time were analysed by Friedman's two-way nonparametric ANOVA or by Student's paired t-test. All results are expressed as mean $\pm$ SEM, and p values < 0.05 were considered significant.

5.5. DISCUSSION

Recording of the ERG in neonates requires considerable modification of standard techniques. The procedure should afford a minimum of disturbance or discomfort to the infant

and yet permit reliable results to be obtained. Since visual fixation cannot be achieved, the use of full-field stimulus conditions is imperative. By utilising a reflective covering for an incubator and a standard light source, full-field stimulus conditions were created which were comparable to those used for routine electroretinography in adults. Furthermore, since the incubator cover was light-proof, the infant could be adapted to controlled ambient light conditions prior to recording of the ERG.

The conductive thread electrode is easier to use than the contact lens electrode, particularly in extremely small eyes, and there is less risk of corneal irritation or abrasion (9). The signal quality is comparable to that of the contact lens electrode, with less intraindividual variability (9). No ocular complications were encountered in our studies.

Computer averaging of responses was necessary to achieve consistently interpretable ERGs because of the low amplitude of the responses. By averaging one hundred responses, the signal to noise ratio was improved tenfold (13). Rejection of artifactual responses, such as those caused by periocular muscle movement, further enhanced the signal quality of the averaged ERG.

The first in a series of light flashes results in immediate disadaptation of the retina and so it is important to allow the retina to reach a steady state of adaptation to the stimulus before beginning to collect responses for averaging (101). The

time taken for the retina to achieve steady state varies with the intensity and frequency of the stimulus and may be shorter in infants than in adults (102). With our recording technique the average of the first 25 collected responses did not differ from the average of the final 25 collected responses. Thus, a two minute period of adaptation to the stimulus was sufficient for the retina to reach steady state. Variability in the amplitude of the serial ERGs may be accounted for by the infant's irregular, intermittent eye opening.

Eyelid closure during recording of the ERG in adult controls significantly increased both the a- and b-wave latencies and decreased the mean amplitude. These findings are in agreement with previous studies in adults (103) and children (104), and are compatible with attenuation of the light stimulus by closure of the eyelids (4,38,105,106). In two of our adult controls the amplitude of the ERG was unchanged or slightly increased by eyelid closure. An increase in the amplitude of the averaged ERG in response to diminishing the intensity of the stimulus has been described (101) and can be attributed to the retina being in a less light adapted state when averaging of responses is commenced (15,107). By means of a video camera it was possible to document the degree of eye opening during recording of the ERG in infants.

This technique, in conjunction with continuous heart rate monitoring, subsequently allowed the ERG of even very small

preterm infants to be studied without compromising their care and safety.

CHAPTER 6. THE ELECTRORETINOGRAM IN TERM INFANTS

6.1. PATIENTS

Fifty-two term (defined as $\geq$ 37 completed weeks of gestation) infants were studied, including 3 postmature (> 42 completed weeks) infants (Table IV). There were 29 males and 23 females. They had a mean gestational age of 39.6 $\pm$ 0.2 (SEM) weeks and, excluding one severely growth retarded infant (1750 grams at 37^{+0} weeks), a mean birthweight of 3347 $\pm$ 70 grams. By the criteria of Lubchenco et al (108), 5 infants were small for gestational age, 3 were large for gestational age and 44 were appropriately grown. The group comprised 45 Caucasian infants and 7 black infants. There were no major congenital anomalies, and all infants had normal fundi. The age at recording of the ERG ranged from 7 to 148 hours (mean 37 $\pm$ 4 hours).

6.2. RESULTS

The ERG was recorded successfully in 50 (96%) term infants. In 2 black infants low signal to noise ratio precluded interpretation of the ERG. These infants were aged 13 and 15

TABLE IV - TERM INFANTS.

Patient number	Sex	Gestational age (weeks)	Birthweight (grams)	Postnatal age (hours)	Race*	Degree of eye opening#	a-wave latency (msec)	b-wave latency (msec)	Amplitude (μV)
1	F	37^{+0}	4560	17	C	0	24.4	47.5	12.7
2	M	37^{+0}	1750	134	C	0	20.0	53.1	16.2
3	F	37^{+0}	2930	7	C	0	30.8	56.0	8.5
4	F	37^{+3}	3180	24	C	0	24.0	49.6	7.8
5	M	37^{+3}	2860	31	C	1	24.0	52.4	20.8
6	F	37^{+4}	2800	27	C	0	26.4	64.8	14.9
7	M	37^{+6}	3020	29	C	0	18.1	50.0	15.1
8	M	38^{+0}	3170	14	C	0	29.2	62.0	8.7
9	F	38^{+0}	3460	76	C	1	24.0	57.2	16.4

*C = Caucasian B = Black

#0 = Continuous eyelid closure 1 = Intermittent eye opening 2 = Eyes mostly open

TABLE IV - TERM INFANTS (cont.).

Patient number	Sex	Gestational age (weeks)	Birthweight (grams)	Postnatal age (hours)	Race*	Degree of eye opening#	a-wave latency (msec)	b-wave latency (msec)	Amplitude (μV)
10	M	38^{+0}	3420	31	C	1	28.0	48.4	28.8
11	M	38^{+1}	2980	77	C	1	20.4	48.0	43.0
12	M	38^{+2}	3080	69	C	0	20.8	61.6	16.1
13	M	38^{+3}	3180	42	C	0	26.4	59.2	15.6
14	M	38^{+3}	2180	38	C	0	22.5	54.8	14.6
15	F	39^{+0}	3030	25	C	0	22.5	54.4	15.5
16	F	39^{+0}	3300	23	C	0	20.6	52.5	12.1
17	M	39^{+1}	3530	38	C	1	16.3	59.4	16.5
18	F	39^{+2}	2300	41	C	0	30.6	55.0	8.8
19	F	39^{+2}	3430	72	C	0	24.8	60.0	22.6
20	M	39^{+4}	3475	149	C	0	26.0	68.4	15.9

*C = Caucasian. B = Black.

#0 = Continuous eyelid closure. 1 = Intermittent eye opening. 2 = Eyes mostly open.

TABLE IV - TERM INFANTS (cont.).

Patient number	Sex	Gestational age (weeks)	Birthweight (grams)	Postnatal age (hours)	Race*	Degree of eye opening#	a-wave latency (msec)	b-wave latency (msec)	Amplitude (μV)
21	F	39^{+5}	3150	25	C	1	18.4	63.8	41.7
22	M	39^{+6}	4130	96	C	0	22.0	52.0	16.2
23	M	39^{+6}	3450	11	C	0	25.6	48.8	9.5
24	M	40^{+0}	3640	48	C	0	26.8	51.6	14.6
25	F	40^{+0}	3040	26	C	0	20.4	54.4	13.8
26	M	40^{+0}	3180	98	C	2	17.6	55.2	68.4
27	M	40^{+1}	3950	38	C	0	29.6	50.8	17.8
28	M	40^{+2}	3500	18	C	0	24.0	48.4	6.7
29	M	40^{+2}	3840	38	C	0	24.8	46.4	12.3
30	M	40^{+2}	3460	31	C	1	27.2	50.8	18.6
31	F	40^{+3}	3240	10	C	0	26.3	60.0	19.0

*C = Caucasian. B = Black.

#0 = Continuous eyelid closure. 1 = Intermittent eye opening. 2 = Eyes mostly open.

TABLE IV - TERM INFANTS (cont.).

Patient number	Sex	Gestational age (weeks)	Birthweight (grams)	Postnatal age (hours)	Race*	Degree of eye opening#	a-wave latency (msec)	b-wave latency (msec)	Amplitude (μV)
32	M	40^{+4}	3770	39	C	0	20.8	43.5	8.9
33	F	40^{+6}	2930	19	C	2	18.4	56.8	33.7
34	F	41^{+0}	3200	18	C	1	21.6	55.6	44.5
35	F	41^{+0}	2980	53	C	1	19.2	57.2	76.5
36	F	41^{+0}	3620	12	C	2	22.0	54.8	52.2
37	M	41^{+2}	3570	23	C	0	28.0	48.8	7.6
38	F	41^{+2}	4800	19	C	1	26.0	55.8	25.6
39	M	41^{+3}	3120	34	C	0	27.5	57.5	18.8
40	M	41^{+3}	3920	16	C	0	27.6	60.0	13.5
41	M	41^{+4}	4220	17	C	0	23.6	51.2	11.7
42	M	41^{+4}	2960	67	C	2	18.4	55.0	77.6

*C = Caucasian. B = Black.

#0 = Continuous eyelid closure. 1 = Intermittent eye opening. 2 = Eyes mostly open.

TABLE IV - TERM INFANTS (cont.).

Patient number	Sex	Gestational age (weeks)	Birthweight (grams)	Postnatal age (hours)	Race*	Degree of eye opening#	a-wave latency (msec)	b-wave latency (msec)	Amplitude (μV)
43	M	42^{+1}	3740	76	C	2	18.8	54.8	57.1
44	M	42^{+2}	4380	22	C	0	26.0	51.3	7.8
45	F	42^{+5}	2800	32	C	0	22.8	60.8	34.2
46	M	37^{+4}	2960	15	B	0	uninterpretable		
47	M	37^{+6}	3100	24	B	0	25.6	63.1	16.4
48	F	38^{+3}	2920	18	B	0	28.8	51.2	15.7
49	F	39^{+0}	3350	14	B	0	28.4	55.6	12.9
50	F	39^{+5}	3240	24	B	0	30.4	56.4	7.3
51	F	39^{+5}	3400	13	B	1	uninterpretable		
52	F	40^{+1}	3240	31	B	2	20.4	58.8	26.6

*C = Caucasian. B = Black.

#0 = Continuous eyelid closure. 1 = Intermittent eye opening. 2 = Eyes mostly open.

hours, with intermittent eye opening and continuous eyelid closure, respectively. No repeat attempt was made to record their ERG.

The mean ± SEM values of the ERG parameters by race and degree of eye opening are given in Table V.

6.2.1. FACTORS INFLUENCING THE ELECTRORETINOGRAM

i) RACE

For infants with eyes closed, the a-wave latency was significantly longer in black infants (Table V). The b-wave latency also tended to be longer in black infants, but there was no difference in the amplitude of the response.

Accordingly, only Caucasian infants were included in further statistical analyses.

ii) EYELID CLOSURE

Continuous eyelid closure increased the a-wave latency ($p < 0.01$) and decreased the amplitude ($p < 0.001$) of the ERG compared to eyes mostly open (Table V). ERGs recorded with intermittent eye opening did not differ significantly from those recorded with continuous eyelid closure.

The group of infants in whom the eyes were mostly open was also more mature ($p < 0.05$). Eye opening was the most significant variable influencing both amplitude and a-wave

TABLE V - TERM INFANTS: ELECTRORETINOGRAPHIC PARAMETERS BY RACE AND DEGREE OF EYE OPENING.

	Caucasian			Black	
	Eyes open (n=5)	Intermittent eye opening (n=10)	Eyes closed (n=30)	Eyes open (n=1)	Eyes closed (n=4)
a-wave latency (msec)	19.0 ± 0.8	25.5 ± 1.3	24.8 ± 0.6*	20.4	28.3 ± 1.0$^{\Delta}$
b-wave latency (msec)	55.3 ± 0.4	54.9 ± 1.6	54.5 ± 1.1	58.8	56.6 ± 2.5
Amplitude (μV)	57.8 ± 7.5	33.2 ± 5.9$^{\#}$	13.9 ± 1.0^{o}	26.6	13.1 ± 2.1
Gestational age (weeks)	41.1 ± 0.4	39.4 ± 0.5$^{\#}$	39.5 ± 0.3$^{\#}$	40.1	38.8 ± 0.4
Birthweight (grams)	3284 ± 167	3384 ± 174	3332 ± 115	3240	3153 ± 93
Postnatal age (days)	3.2 ± 0.7	2.6 ± 0.3	2.7 ± 0.3	2.0	2.0 ± 0
Postconceptional age (weeks)	41.4 ± 0.4	39.6 ± 0.4$^{\#}$	39.7 ± 0.3$^{\#}$	40.3	38.9 ± 0.3

Values are expressed as mean ± SEM. p values obtained by Mann-Whitney U test.

$^{\#}$ $p<0.05$ versus eyes open. * $p<0.01$ versus eyes open. ^{o} $p<0.001$ versus eyes open.

$^{\Delta}$ $p<0.05$ versus Caucasian infants with eyes closed.

latency. However, there was also an independent effect of advancing postconceptional age in increasing the amplitude of the ERG. The a-wave latency was shortened by both eye opening and advancing postnatal age, but was not influenced significantly by postconceptional age.

The amplitude of the ERG correlated with postnatal age in infants with continuous eyelid closure ($r = 0.38$; $p < 0.05$), but not in infants with eyes open.

Eyelid closure did not affect the b-wave latency in term infants.

iii) SEX

The b-wave latency was greater in female infants ($p < 0.05$) (Table VI). Neither the a-wave latency nor the amplitude of the ERG differed between the sex groups. The female infants were significantly younger ($p < 0.05$), and multiple linear regression analysis showed an independent effect of both sex and postnatal age upon the b-wave latency. The b-wave latency was not influenced significantly by any other variable.

iv) INTRAUTERINE GROWTH

None of the ERG parameters was different in infants either small or large for gestational age, compared to appropriately grown infants.

TABLE VI - CAUCASIAN TERM INFANTS: SEX DIFFERENCES IN THE ELECTRORETINOGRAM.

	Males (n=27)	Females (n=18)	p*
a-wave latency (msec)	23.7 ± 0.8	23.5 ± 0.8	NS
b-wave latency (msec)	53.5 ± 1.1	56.5 ± 1.0	<0.05
Amplitude (μV)	21.4 ± 3.5	25.6 ± 4.3	NS
Gestational age (weeks)	39.8 ± 0.3	39.5 ± 0.4	NS
Birthweight (grams)	3388 ± 111	3263 ± 140	NS
Postnatal age (days)	3.1 ± 0.3	2.2 ± 0.2	<0.05
Postconceptional age (weeks)	40.0 ± 0.3	39.7 ± 0.4	NS
% with eyes open	11%	11%	NS

Values are expressed as mean ± SEM

*p values obtained by Mann-Whitney U test

6.2.2. INTERRELATIONSHIP OF ERG PARAMETERS

The a-wave latency correlated inversely with the amplitude of the ERG ($r = -0.45$; $p < 0.005$) in Caucasian term infants, and the same relationship was true for both eyes open and eyes closed. In black infants the a-wave latency was related similarly to the amplitude ($r = -0.90$; $p < 0.05$).

The b-wave latency did not correlate with either the a-wave latency or the amplitude of the ERG in any group of infants.

6.2.3. COMPARISON WITH ADULT CONTROLS

The amplitude of the ERG was less and the b-wave latency was longer ($p < 0.05$) in newborn term infants than in adults tested by the same technique (Table VII). The a-wave latency was also longer in term infants, but the difference was not significant. Only subjects with eyes open were compared, since eyelids are thicker and presumably more opaque in adults.

6.3. DISCUSSION

Successful recording of the ERG in response to single flash stimuli using standard techniques requires a high degree of patient co-operation. The method described in Chapter 5 permitted an interpretable ERG to be recorded at the first

TABLE VII - COMPARISON OF THE ELECTRORETINOGRAM IN CAUCASIAN TERM INFANTS AND ADULTS.[#]

	Term Infants (n=5)	Adults (n=4)	p*
a-wave latency (msec)	19.0 ± 0.8	16.1 ± 1.0	NS
b-wave latency (msec)	55.3 ± 0.4	41.2 ± 2.8	<0.05
Amplitude (μV)	57.8 ± 7.5	99.6 ± 15.3	<0.05

Values are expressed as mean ± SEM

*p values obtained by Mann-Whitney U test

[#]All recordings were made with eyes open.

attempt in 96% of newborn term infants, without the need for general anaesthesia (73,102) or more than minor restraint.

In term infants, as in adults, the a-wave latency was significantly shorter when the eyes were open. Furthermore, the mean amplitude was increased over fourfold. Eye opening resulted in a much greater increase in the amplitude of the ERG in term neonates than in adult controls, suggesting that the visual pigment in infancy may be more resistant to, or recover more quickly from, bleaching by high intensity light stimuli (102). It is unclear why the b-wave latency was not affected by eyelid closure in term infants.

The correlation between amplitude and postnatal age in infants with continuous eyelid closure was not seen in infants with eyes open. This could be explained by the normal reduction in eyelid oedema over the first few days of life, which would make the eyelids more translucent.

Racial differences in the ERG have not been described previously. Greater attenuation of the light stimulus by pigmented eyelids would account for the prolongation of a- and b-wave latencies, but one would have expected a concomitant reduction in the amplitude of the response.

In adult subjects the amplitude of the ERG is greater in females than in males (109-112), but the latencies do not differ (109,112). A study of the ERG in 20 term infants found no consistent sex differences (39), nor have sex differences been reported in visual evoked responses from term and

preterm infants (39,113-115).

In newborn term infants, the averaged ERG was of lower amplitude and longer latencies than in adult controls. These results confirm the findings of others (31,33,38). Since the ERG is influenced both by the conditions of recording and by the nature of the stimulus, such comparisons can only be made between subjects tested by the same technique, in the same laboratory (15,112,116).

Even over the range of 1-7 days, both a- and b-wave latencies were shortened significantly by advancing postnatal age. Thus, the maturation of the ERG previously described from six weeks of age in term infants (33) commences very soon after birth. Large interindividual variability in the amplitude of the ERG is well documented (9,42,43,47,82,104, 105), particularly with the eyes closed (104), and may have contributed to the lack of correlation between amplitude and postnatal age in this study.

Since the b-wave is dependent upon the a-wave for its generation, the strong correlation observed between the a- and b-wave latencies of the ERG in normal adult subjects is not unexpected (112). During the first week of life, however, we saw no relationship between the b-wave latency and either the a-wave latency or the amplitude. The infant retina attains adult sensitivity prior to full maturation of the b-wave latency and amplitude of the ERG (33). These observations are compatible with the hypothesis that photochemical and neural

mechanisms in the retina develop, at least to some extent, independently of each other.

Clearly, quantitative measurement of the ERG in term infants requires that the eyes be open. In the unsedated infant, continuous eye opening may be achieved by the use of a speculum in conjunction with a contact lens electrode (31,38,39,42,43). However, particularly if the infant falls asleep, this does not prevent eye rolling (38), which is essentially equivalent to eyelid closure. All of our infants with eyes open were awake, and eye rolling was not observed.

The technique described above allows the ERG to be recorded soon after birth in term infants, with little inconvenience to the patient. If the degree of eye opening is noted, a reasonable assessment of the normality of the response can be made. However, only in an awake, co-operative infant can either accurate measurement of the a-wave latency or the amplitude of the ERG, or serial studies of these parameters, be obtained.

6.4. SUMMARY AND CONCLUSIONS

1. An interpretable ERG was obtained in 96% of newborn term infants using the technique described in Chapter 5.

2. The ERG was recorded as early as 7 hours after birth, and was of lower amplitude and longer a- and b-wave latencies than in adult controls.

3. Eyelid closure during recording of the ERG significantly increased the a-wave latency and decreased the amplitude, but did not affect the b-wave latency.

4. The a- and b-wave latencies of the ERG were longer in black infants than in Caucasian infants.

5. The b-wave latency was significantly longer in female infants.

6. The a- and b-wave latencies shortened with advancing postnatal age.

CHAPTER 7. THE ELECTRORETINOGRAM IN PRETERM INFANTS

7.1. PATIENTS

Fifty-nine preterm infants were studied, including 32 males and 27 females (Table VIII). The population comprised 53 Caucasian infants, 4 black infants, 1 Indian and 1 Japanese/ Caucasian mixed infant. Their gestational ages ranged from 25^{+4} weeks to 36^{+5} weeks, with a mean of 32.7 $\pm$ 0.3 (SEM) weeks. The mean birthweight was 1805 $\pm$ 74 grams (range 800-2980 grams). Four infants were small for gestational age (108) and the remainder were of appropriate birthweight for gestational age. There were four sets of like-sex twins (2 monozygous, 1 dizygous and 1 of undetermined zygosity). Down's syndrome was confirmed by chromosomal analysis in one pair of monozygous twins; there were otherwise no major congenital anomalies. Fundoscopy was normal in 56 infants, but revealed retinopathy of prematurity grade I in 1 and grade III in 2. Assisted ventilation was required in 27 (46%) of the infants for a mean of 11.6 $\pm$ 2.8 days (range 1-52 days) and a further 15 (25%) received a period of supplemental oxygen therapy. Recording of the ERG was not attempted until the infant was in a stable condition and breathing room air. Hyperbilirubinaemia was treated by phototherapy in 49 (83%)

TABLE VIII - PRETERM INFANTS.

Patient number	Sex	Gestational age (weeks)	Birthweight (grams)	Postnatal age at recording of first ERG (days)	Duration of exposure to light (days)	Race*	Respiratory therapy (days)	
							Assisted ventilation	Supplementary oxygen#
1	F	25^{+4}	850	57	49.3	C	37	5
2	F	27^{+0}	940	87	61.3	C	52	24
3	F	28^{+0}	800	85	78.8	C	46	27
4	M	28^{+4}	1020	68	55.6	C	1	55
5	M	28^{+6}	1170	5	1.0	C	-	1
6	M	29^{+0}	1330	35	23.0	C	6	20
7	F	29^{+3}	1020	23	10.2	C	1	1
8	F	29^{+3}	980	20	14.0	C	4	2
9	M	30^{+3}	1140	26	22.4	C	5	3
10	M	30^{+3}	1130	35	22.6	C	31	3
11	M	30^{+4}	1510	9	1.5	C	5	-

*C = Caucasian. B = Black. O = Other. # Unassisted ventilation.

TABLE VIII - PRETERM INFANTS (cont.).

Patient number	Sex	Gestational age (weeks)	Birthweight (grams)	Postnatal age at recording of first ERG (days)	Duration of exposure to light (days)	Race*	Respiratory therapy (days)	
							Assisted ventilation	Supplementary oxygen#
12	M	30^{+6}	1460	2	1.2	C	-	-
13	F	31^{+0}	1560	12	9.8	C	-	-
14	M	31^{+4}	1980	3	2.3	C	1	-
15	M	32^{+0}	1620	35	29.6	C	-	1
16	F	32^{+0}	1610	20	17.4	C	-	1
17	M	32^{+0}	1960	6	3.4	C	-	1
18	F	32^{+2}	1800	16	12.5	C	-	1
19	F	32^{+2}	1420	16	11.4	C	-	1
20	F	32^{+3}	1840	5	2.0	C	-	1
21	F	32^{+3}	1920	1	0.4	C	-	-
22	F	32^{+4}	1640	3	1.8	C	-	1
23	F	33^{+0}	2180	10	9.4	C	5	-

*C = Caucasian. B = Black. O = Other. # Unassisted ventilation.

TABLE VIII - PRETERM INFANTS (cont.)

Patient number	Sex	Gestational age (weeks)	Birthweight (grams)	Postnatal age at recording of first ERG (days)	Duration of exposure to light (days)	Race*	Respiratory therapy (days)	
							Assisted ventilation	Supplementary oxygen#
24	F	33^{+0}	1320	18	12.6	C	6	6
25	M	33^{+1}	2010	6	2.9	C	2	-
26	M	33^{+2}	2600	7	6.2	C	-	1
27	M	33^{+4}	1850	9	3.4	C	1	-
28	M	33^{+4}	2320	4	1.8	C	-	1
29	M	33^{+5}	1550	12	6.1	C	1	1
30	M	33^{+6}	2080	2	0.6	C	-	-
31	M	33^{+6}	1860	19	15.4	C	5	1
32	F	34^{+0}	2340	7	3.8	C	-	-
33	F	34^{+0}	1910	3	1.8	C	-	-
34	F	34^{+1}	2300	8	6.8	C	-	1
35	M	34^{+1}	1620	4	1.2	C	-	-

*C = Caucasian. B = Black. 0 = Other. # Unassisted ventilation.

TABLE VIII - PRETERM INFANTS (cont.).

Patient number	Sex	Gestational age (weeks)	Birthweight (grams)	Postnatal age at recording of first ERG (days)	Duration of exposure to light (days)	Race*	Respiratory therapy (days)	
							Assisted ventilation	Supplementary oxygen#
36	M	34^{+2}	2260	3	1.6	C	-	1
37	M	34^{+6}	2750	12	9.7	C	4	3
38	M	35^{+0}	2670	4	3.6	C	-	-
39	M	35^{+0}	1990	5	3.4	C	-	-
40	M	35^{+0}	1770	2	0.9	C	-	-
41	M	35^{+0}	1400	11	6.0	C	1	-
42	M	35^{+0}	2210	10	4.1	C	5	2
43	M	35^{+0}	1890	11	2.5	C	5	1
44	M	35^{+2}	1850	15	14.2	C	-	-
45	F	35^{+2}	2980	1	0.3	C	-	-
46	M	35^{+2}	2340	6	2.9	C	-	-
47	F	35^{+3}	1940	4	3.1	C	-	-

*C = Caucasian. B = Black. O = Other. # Unassisted ventilation.

TABLE VIII - PRETERM INFANTS (cont.).

Patient number	Sex	Gestational age (weeks)	Birthweight (grams)	Postnatal age at recording of first ERG (days)	Duration of exposure to light (days)	Race*	Respiratory therapy (days)	
							Assisted ventilation	Supplementary oxygen#
48	M	35^{+4}	2690	3	2.4	C	-	-
49	F	35^{+5}	2630	2	1.1	C	-	1
50	F	35^{+6}	2450	2	1.5	C	-	1
51	F	36^{+2}	2620	5	2.8	C	-	3
52	M	36^{+4}	2250	2	0.8	C	-	-
53	F	36^{+5}	2550	2	1.1	C	-	-
54	F	26^{+0}	800	71	62.0	B	28	40
55	F	29^{+2}	890	49	40.2	B	29	5
56	F	31^{+2}	1190	46	39.0	O	7	-
57	F	32^{+0}	1510	20	3.4	O	7	10
58	M	33^{+3}	1860	10	2.9	B	6	-
59	M	34^{+1}	2360	20	11.7	B	12	7

*C = Caucasian. B = Black. O = Other. # Unassisted ventilation.

of the infants, during which time the eyes were patched, and allowance was made for this in calculating the duration of light exposure prior to recording of each ERG. The mean age at recording of the first ERG was 16.8 ± 2.7 days, with a range from 7 hours to 87 days.

Thirty-six infants had one or more (up to a maximum of 7) follow-up ERGs at approximately weekly intervals until their discharge from the neonatal intensive care unit.

7.2. RESULTS

One hundred and thirty-eight ERG recording sessions were completed. The ERG was interpretable in 127 (92%) instances. One ERG recording session had to be abandoned because the infant vomited; all other sessions were completed without incident.

In two infants with technically satisfactory recordings the ERG was considered to be absent. One was an infant born at 28^{+6} weeks, aged 93 hours (duration of light exposure 24 hours) and the other was an infant born at 33^{+6} weeks, aged 14 hours. Both of these infants subsequently demonstrated one or more clearly defined ERG(s). An ERG was recorded as early as 7 and 9 hours after birth (gestational ages 35^{+2} and 32^{+3} weeks, respectively), and the least mature infant from whom an ERG was recorded was of 30^{+3} postconceptional weeks (gestational age 28^{+6} weeks).

To exclude the possibility of introducing statistical bias, only the first interpretable ERG recorded from each infant was included in the following analysis of data unless stated otherwise.

7.2.1. FACTORS INFLUENCING THE ELECTRORETINOGRAM

i) FUNDAL ABNORMALITY

ERGs were obtained consistently from the 2 infants with grade III retinopathy of prematurity (n = 8) and the 1 infant with grade I disease (n = 1). There was marked interindividual variability in the amplitude of the ERGs (Table IX). However, they were all of normal waveform and when compared to ERGs recorded from infants of similar age and maturity with normal fundi did not differ significantly in any parameter.

Nevertheless, for the establishment of normative data, these 3 infants were excluded from subsequent analyses.

ii) RACE

The mean a- and b-wave latencies were longer in black infants (n = 3) than in Caucasian infants (n = 51) but the differences were not significant (Table X). The black infants tended to be of greater postnatal age.

When data from term (Table V) and preterm (Table X) infants were considered together, the differences in both the

TABLE IX - THE ELECTRORETINOGRAM[†] IN PRETERM INFANTS WITH ABNORMAL FUNDI.

Patient number	Sex	Gestational age (weeks)	Postnatal age (days)	Postconceptional age (weeks)	Race[*]	Fundi[Δ]	Degree of eye opening[#]	a-wave latency (msec)	b-wave latency (msec)	Amplitude (μV)
2	F	27^{+0}	87	39^{+2}	C	I	1	15.6	53.1	39.5
10	M	30^{+3}	35	35^{+2}	C	III	1	20.0	53.1	11.0
54	F	26^{+0}	71	36^{+0}	B	III	0	20.6	50.6	4.7

[*]C = Caucasian. B = Black.

[Δ]I = Grade I retinopathy of prematurity (ROP). III = Grade III ROP.

[#]0 = Continuous eyelid closure. 1 = Intermittent eye opening.

[†]First recorded electroretinogram from each infant.

TABLE X - PRETERM INFANTS: RACIAL DIFFERENCES IN THE ELECTRORETINOGRAM.

	Caucasian (n=51)	Black (n=3)
a-wave latency (msec)	23.0 ± 0.6	25.7 ± 0.1
b-wave latency (msec)	53.1 ± 0.7	59.3 ± 4.3
Amplitude (μV)	13.5 ± 1.0	11.7 ± 3.1
Gestational age (weeks)	33.1 ± 0.3	32.3 ± 1.5
Birthweight (grams)	1879 ± 75	1703 ± 432
Postnatal age (days)	13.9 ± 2.4	26.3 ± 11.7
Exposure to light (days)	10.4 ± 2.1	18.3 ± 11.3
Postconceptional age (weeks)	34.9 ± 0.3	35.9 ± 0.6

Values are expressed as mean ± SEM.

None of the differences between the racial groups are significant.

a-wave ($p < 0.05$) and b-wave ($p = 0.06$) latencies were more marked (Table XI). The combined racial groups did not differ with regard to postnatal age, maturity or degree of eye opening.

Accordingly, only data from Caucasian infants are included in further statistical analyses.

iii) EYELID CLOSURE

Preterm infants with eyes mostly open during recording of the ERG were of greater postnatal age ($p < 0.05$) but were less mature ($p < 0.01$) than those with continuous eyelid closure (Table XII). None of the ERG parameters was influenced independently by eyelid closure in preterm infants.

iv) SEX

There were no differences in either the latencies or the amplitude of the ERG between male and female preterm infants.

v) INTRAUTERINE GROWTH

Small for dates preterm infants did not differ significantly with regard to any of the ERG parameters from appropriately grown infants of comparable gestational and postnatal age.

vi) TWIN STUDIES

Genetic influences upon the ERG could not be evaluated

TABLE XI - RACIAL DIFFERENCES IN THE ELECTRORETINOGRAM[Δ].

	Caucasian (n=96)	Black (n=8)	p*
a-wave latency (msec)	23.3 ± 0.4	26.4 ± 1.2	<0.05
b-wave latency (msec)	53.8 ± 0.5	57.9 ± 1.9	=0.06
Amplitude (μV)	18.0 ± 1.5	14.2 ± 2.3	NS
Postnatal age (days)	8.7 ± 1.4	11.1 ± 5.9	NS
Exposure to light (days)	6.3 ± 1.2	7.4 ± 4.9	NS
Postconceptional age (weeks)	37.2 ± 1.1	37.9 ± 0.7	NS
Number with eyes open	9(9%)	1(13%)	NS

Values are expressed as mean ± SEM.

*p values obtained by Mann-Whitney U test.

ΔCombined data from term and preterm infants.

TABLE XII - CAUCASIAN PRETERM INFANTS: THE ELECTRORETINOGRAM IN RELATION TO EYE OPENING.

	Eyes open (n=4)	Intermittent eye opening (n=7)	Eyes closed (n=40)
a-wave latency (msec)	20.0 ± 3.4	23.5 ± 1.5	23.2 ± 0.6
b-wave latency (msec)	53.6 ± 0.8	51.3 ± 2.5	53.4 ± 0.7
Amplitude (μV)	19.7 ± 8.6	16.2 ± 3.2	12.5 ± 0.9
Gestational age (weeks)	29.4 ± 1.3	32.2 ± 1.0	33.6 ± 0.3#
Birthweight (grams)	1318 ± 257	1500 ± 181	2001 ± 78*
Postnatal age (days)	24.8 ± 10.6	24.6 ± 8.1°	11.0 ± 2.3*
Exposure to light (days)	19.7 ± 10.0	18.9 ± 6.7°	8.0 ± 2.1*
Postconceptional age (weeks)	32.8 ± 0.3	35.6 ± 0.7#	35.0 ± 0.3#

Values are expressed as mean ± SEM.

# $p<0.01$ versus eyes open.

* $p<0.05$ versus eyes open.

° $p<0.05$ versus eyes closed.

p values obtained by Mann-Whitney U test.

critically because of the small number of twins. One twin of a monozygous pair (patient number 10) developed grade III retinopathy of prematurity. There was no consistent similarity between age-matched pairs of ERGs (total of 6 pairs) recorded from any of the other sets of twins, regardless of zygosity.

7.2.2. MATURATION OF THE ELECTRORETINOGRAM

i) CROSS-SECTIONAL DATA

The mean ± SEM values of the ERG parameters by gestational and postnatal age for Caucasian preterm infants (n = 51) are given in Table XIII.

The a-wave latency during the first 5 days of life was greater in infants of gestational ages $31\text{-}33^{+6}$ weeks than in preterm infants born after 34 completed gestational weeks ($p < 0.05$).

For infants of gestational ages $31\text{-}33^{+6}$ weeks the amplitude of the ERG correlated with duration of exposure to light ($r = 0.49$; $p < 0.05$).

There was a reduction in the b-wave latency after 48 hours' exposure to light ($p < 0.01$) which was evident at all preterm gestational ages (Table XIV).

The b-wave latency correlated inversely with post-conceptional age after the second day of life ($r = -0.35$; $p < 0.05$). However, neither the a-wave latency nor the amplitude of the ERG was related significantly to

TABLE XIII - ELECTRORETINOGRAPHIC PARAMETERS IN 51 CAUCASIAN PRETERM INFANTS.

	Gestational age (weeks)	Day of life		
		1-5	6-14	15+
a-wave latency (msec)	25 - 30^{+6}		24.3±2.1	23.6±1.5
	31 - 33^{+6}	27.7±1.7#	20.9±2.1	22.8±1.7
	34 - 36^{+6}	23.1±0.7#	21.3±0.8	21.3
b-wave latency (msec)	25 - 30^{+6}		55.5±1.8	51.3±1.8
	31 - 33^{+6}	55.9±1.4	53.1±1.3	52.6±1.9
	34 - 36^{+6}	53.4±1.5	52.2±2.2	44.8
Amplitude (μV)	25 - 30^{+6}		10.8±1.2	14.9±3.3
	31 - 33^{+6}	8.9±1.8*	9.9±2.1	14.3±1.2*
	34 - 36^{+6}	12.2±1.3	13.8±2.1	31.3

Values are expressed as mean ± SEM.

Values bearing the name symbol (# or *) differ significantly ($p<0.05$).

TABLE XIV - CAUCASIAN PRETERM INFANTS: RELATIONSHIP OF b-WAVE LATENCY TO DURATION OF EXPOSURE TO LIGHT.

Gestational age (weeks)	Calculated total exposure to light (days)		
	0.1 - 2	2.1 - 10	>10
$25 - 30^{+6}$	58.8	53.9 ± 1.1	51.8 ± 1.8
$31 - 33^{+6}$	57.1 ± 0.9	52.9 ± 1.2	52.6 ± 1.9
$34 - 36^{+6}$	55.2 ± 1.5	51.4 ± 1.6	44.8

Values are expressed as mean ± SEM

For preterm infants of all gestational ages, the b-wave latency is longer during the first 48 hours of exposure to light than at later observations ($p<0.01$).

postconceptional age (Table XV).

ii) LONGITUDINAL DATA

A series of 7 weekly ERGs was performed in one infant of gestational age 28^{+6} weeks (patient number 5) (Table XVI). He had normal fundi and received phototherapy for a total of 116 hours, commenced on the first day of life. On day 5 (aged 93 hours; total duration of exposure to light 24 hours) the ERG was absent, but by day 12 there was a clearly defined response. Both the a-wave latency and the b-wave latency shortened, and the amplitude increased, over time.

Fifteen Caucasian infants with normal fundi had at least 3 interpretable ERGs recorded at weekly intervals. Analysis of variance revealed a significant decrease in the a-wave latency over two weeks ($p < 0.005$) and a tendency towards both a decrease in the b-wave latency ($0.05 < p < 0.1$) and an increase in the amplitude of the ERG ($0.05 < p < 0.1$) (Figure 7).

7.2.3. INTERRELATIONSHIP OF ERG PARAMETERS

The a-wave latency correlated inversely with the amplitude of the ERG in Caucasian preterm infants ($r = -0.43$; $p < 0.005$). The correlation was not significant in the 3 black infants. The b-wave latency did not correlate with either the a-wave latency or the amplitude in any group of preterm

TABLE XV - CAUCASIAN PRETERM INFANTS: RELATIONSHIP OF THE ELECTRORETINOGRAM TO POSTCONCEPTIONAL AGE.[#]

	Postconceptional age (weeks)			
	$30\text{-}32^{+6}$ (n=8)	$33\text{-}34^{+6}$ (n=17)	$35\text{-}36^{+6}$ (n=16)	$37\text{-}38^{+6}$ (n=2)
a-wave latency (msec)	23.6±2.1	23.9±1.1	21.7±0.8	23.2±1.8
b-wave latency (msec)	55.1±1.1*	54.1±1.0	50.9±1.3	44.9±1.0*
Amplitude (μV)	10.9±1.8	13.6±2.2	12.6±1.2	20.9±10.4

Values are expressed as mean ± SEM.

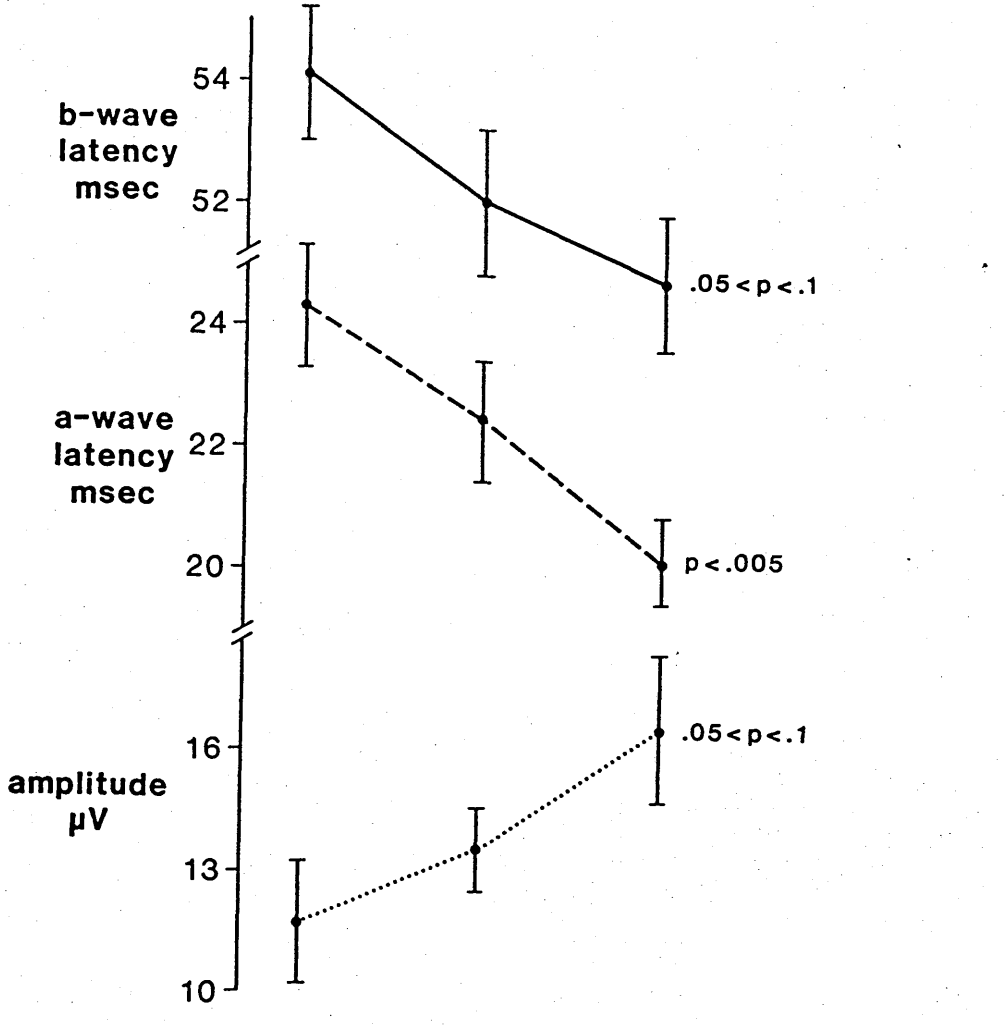
*$p<0.05$ by Mann-Whitney U test.

[#]includes only infants aged >2 days.

TABLE XVI - SERIAL ELECTRORETINOGRAMS IN A CAUCASIAN MALE INFANT (GESTATIONAL AGE 28^{+6} WEEKS).

ERG number	Postnatal age (days)	Duration of exposure to light (days)	Postconceptional age (weeks)	a-wave latency (msec)	b-wave latency (msec)	Amplitude (μV)
1	5	1.0	29^{+3}	electroretinogram absent		
2	12	6.2	30^{+3}	28.4	52.8	8.4
3	19	13.2	31^{+3}	27.6	54.8	12.7
4	25	19.2	32^{+2}	21.3	53.8	18.0
5	32	26.2	33^{+2}	21.6	49.2	18.1
6	39	33.2	34^{+2}	21.6	48.4	24.9
7	47	41.2	35^{+3}	18.0	42.5	23.7

FIGURE 7. SERIAL WEEKLY ELECTRORETINOGRAMS IN A GROUP OF 15 PRETERM INFANTS.



Values are expressed as mean ± SEM.

The mean postconceptional ages were 34.9, 35.0 and 36.0 weeks.

The significance of the slopes was estimated by Friedman's two step non-parametric ANOVA.

infants.

7.2.4. COMPARISON WITH TERM INFANTS

The amplitude of the ERG was less ($p < 0.005$) and the a-wave latency was longer ($p < 0.05$) during the first 5 days of life in infants of gestational ages $34\text{-}36^{+6}$ weeks (mean age 3.0 ± 1.3 days) than in newborn term infants with eyes open (mean age 3.2 ± 0.7 days) (Table XVII).

7.3. DISCUSSION

The technique described for studying the ERG in term infants was 92% successful in recording an interpretable ERG from preterm infants, even although the amplitude of the response was less than 5 μV in five preterm infants. Furthermore, no problems of heat loss, apnoea, bradycardia or corneal irritation were encountered.

Consistently reduced or absent ERGs have been described in blind children with retinopathy of prematurity (117). There were no changes in the ERG associated with retinopathy of prematurity grades I and III in three infants and this is probably a reflection of the relatively mild degree of retinal involvement.

Racial differences in the ERG were similar to those observed in term infants and were unexplained by the effects

TABLE XVII - COMPARISON OF THE ELECTRORETINOGRAM IN TERM AND PRETERM INFANTS.

	Gestational age (weeks)		
	$34\text{-}36^{+6}$ (n=14)	$\geq 37^{\#}$ (n=5)	p*
a-wave latency (msec)	23.1 ± 0.7	19.0 ± 0.8	<0.05
b-wave latency (msec)	53.4 ± 1.5	55.3 ± 0.4	NS
Amplitude (μV)	12.2 ± 1.3	57.8 ± 7.5	<0.005
Postnatal age (days)	3.0 ± 1.3	3.2 ± 0.7	NS

Values are expressed as mean ± SEM.

* p values obtained by Mann-Whitney U test.

only term infants with eyes open are included.

of postnatal age or maturity. Indeed, the tendency for the black infants to be older would have been predicted to decrease both a- and b-wave latencies.

Eyelid closure did not have a significant influence upon any of the ERG parameters in preterm infants, suggesting that the eyelids are not sufficiently opaque to exclude enough light to affect the ERG.

In two very young, immature infants the ERG appeared to be absent. In both of these recordings the background noise was low, and the infant quiet. Under such recording conditions one would have expected to be able to detect an electro-retinographic response of even 2-3 μV in amplitude. The subsequent demonstration of clearly defined ERG(s) in both infants leads to the conclusion that the ERG may normally be absent soon after birth in extremely preterm infants.

The amplitude of the ERG was less and the a-wave latency was longer in preterm infants than in term infants of comparable postnatal age. This is in keeping with observations in 73 mature and premature infants on the first day of life (40). In our study cross-sectional and longitudinal data confirmed a decrease in both a- and b-wave latencies over time and a concomitant increase in the amplitude of the ERG (40).

The a-wave latency was significantly longer during the first 5 days of life in infants of less than 34 weeks' gestation. However, there was subsequently no relationship between

a-wave latency and either gestational or postconceptional age. This may reflect anatomical or physiological immaturity of the photoreceptors prior to completion of the seventh intrauterine month (17,118).

As in newborn term infants, the a-wave latency did not correlate with the b-wave latency. The suggestion that this could be due to independent maturation of photochemical and neural processes in the retina has already been discussed in relation to data obtained from term infants. Furthermore, in preterm infants there was an inverse correlation of the b-wave latency with postconceptional age. This is consistent with maturing of synapses between photoreceptors and post-synaptic bipolar and horizontal cells, since these synapses influence the rate at which the b-wave is generated subsequent to excitation of the photoreceptors (26,119).

The amplitude of the ERG was influenced more significantly by duration of exposure to light than by postnatal age. Moreover, a marked decrease in the b-wave latency after 48 hours' exposure to light was observed at all preterm gestational ages, but was not seen at any time during the first week of life in term infants. The activity of dopamine in the human retina is reduced at birth (21), and in animal studies dopamine depletion of the retina results in a lengthening of the b-wave latency and a decrease in the amplitude of the ERG (24). Since dopamine activity is increased by exposure to light (22,23), these data on the amplitude and b-wave latency

of the ERG in preterm infants support the hypothesis that exposure to light triggers enzyme pathways in the retina necessary for the generation of the ERG (41).

7.4. SUMMARY AND CONCLUSIONS

1. The ERG could be recorded soon after birth in all but the most immature preterm infants.

2. The ERG in preterm infants was of lower amplitude and longer a-wave latency than in term infants of comparable postnatal age.

3. Eyelid closure during recording of the ERG in preterm infants did not have a significant effect upon any of the ERG parameters.

4. The a- and b-wave latencies of the ERG were longer in black infants than in Caucasian infants.

5. The latencies of the ERG shortened and the amplitude increased over time.

6. The b-wave latency correlated inversely with post-conceptional age after the second day of life.

7. Maturation of the ERG in preterm infants is mediated, at least in part, by exposure to light.

PART III

VITAMIN A AND THE ELECTRORETINOGRAM IN PRETERM INFANTS

CHAPTER 8. VITAMIN A STATUS OF PRETERM INFANTS

8.1. PATIENTS

The infants studied were 58 of the 59 preterm infants described in Chapter 7. One Indian infant was withdrawn from the study at the request of her parents prior to collection of blood.

Parenteral feeding was administered to 27 (47%) of the infants for a mean of 15.1 ± 2.7 (SEM) days (range 1-69 days), using a solution of amino acids and dextrose (2.1 grams amino acids and 12.5 grams dextrose/100ml). Eighteen infants requiring prolonged parenteral nutrition also received 10% lipid solution (approximately 10 ml/Kg daily) for a mean of 15.5 ± 3.2 days. Vitamin supplements added to the parenteral nutrition fluid provided 2000 IU/day vitamin A, 200 IU/day vitamin D and 1.0 IU/day vitamin E. Milk feeding was achieved with own mother's milk when available; otherwise most low birthweight infants were fed preterm milk formulae (80 Kcal/100ml) until shortly before discharge. Oral vitamin supplements (Poly-Vi-Sol(R)(Mead Johnson) 0.5 ml twice daily) were given at the discretion of the medical staff.

For comparison with preterm infants, 39 healthy term

infants were studied shortly before discharge from the postnatal wards (mean age 3.4 ± 0.2 days). Each had been previously the subject of an ERG recording session. Nineteen of the term infants were breastfed and 20 were receiving standard infant milk formulae (65 Kcal/100ml).

8.2. METHODS

Within 48 hours of recording each ERG from preterm infants, 0.8 ml of capillary blood was collected into a heparinised container. To minimise heel pricks, this was timed as far as possible to coincide with routine blood sampling. Capillary blood from term infants was obtained concurrently with the mandatory phenylketonuria test. The plasma was separated and stored at -70°C for later analysis. Retinol and α-tocopherol were assayed by high pressure liquid chromatography (120), and retinol-binding protein and prealbumin were measured by radial immunodiffusion (121).

Following the final ERG in 40 preterm infants, a retinol dose response test was performed (96,122). Blood was collected as above, and then a dose of 5000 IU aqueous dispersion of retinol (Aquasol$^{(R)}$A (Armour Pharmaceutical Co.)) was given orally with a feed. A second sample of blood was obtained 5 hours later, and the retinol dose response was calculated from the rise in plasma retinol after 5 hours, divided by the 5 hour plasma concentration, and expressed as a percentage.

The molar ratios of retinol to retinol-binding protein and retinol-binding protein to prealbumin were calculated assuming molecular weights of 286 for retinol, 21,000 for retinol-binding protein and 55,000 for prealbumin.

8.3. RESULTS

8.3.1. CROSS-SECTIONAL DATA

The mean ± SEM values for retinol, α-tocopherol, retinol-binding protein and prealbumin at the time of recording of the first interpretable ERG in 58 preterm infants are given in Table XVIII.

There were no sex differences and no racial differences in any of the biochemical parameters.

Plasma retinol values were consistently low and correlated with both retinol-binding protein ($r = 0.85$; $p < 0.0001$) and prealbumin ($r = 0.80$; $p < 0.0001$). In 37 (64%) infants the plasma retinol concentration was less than 10 μg/dl, and only 3 (5%) infants had plasma retinol values ≥ 20 μg/dl. The highest plasma retinol concentrations (40.4 μg/dl and 26.5 μg/dl) were seen in two infants aged 85 and 71 days, respectively.

For infants of all preterm gestational ages, plasma α-tocopherol concentrations correlated with postnatal age ($r = 0.77$; $p < 0.0001$), and with the plasma retinol

TABLE XVIII - BIOCHEMICAL VALUES AT RECORDING OF THE FIRST INTERPRETABLE ELECTRORETINOGRAM IN 58 PRETERM INFANTS.

	Gestational age (weeks)	Postnatal age (days)		
		1-5	6-14	15+
Retinol (µg/dl)	$25\text{-}30^{+6}$		5.7±0.0*	14.4±3.0
	$31\text{-}33^{+6}$	7.3±1.1	11.8±1.3	9.3±1.5
	$34\text{-}36^{+6}$	6.0±0.5	9.1±1.0	7.9±2.2
α-Tocopherol (µg/dl)	$25\text{-}30^{+6}$		1496±63	2105±318
	$31\text{-}33^{+6}$	526±78	1290±183#	1716±263#
	$34\text{-}36^{+6}$	452±51	725±156*	735±356
Retinol-binding protein (mg/dl)	$25\text{-}30^{+6}$		1.2	1.8±0.3
	$31\text{-}33^{+6}$	1.2±0.2	1.8±0.2	1.4±0.2
	$34\text{-}36^{+6}$	1.2±0.1	1.6±0.1	1.2±0.6
Prealbumin mg/dl)	$25\text{-}30^{+6}$		6.5	14.9±1.5
	$31\text{-}33^{+6}$	6.5±0.7	12.7±1.1#	9.9±0.9
	$34\text{-}36^{+6}$	8.7±0.7	10.4±1.7	10.4±1.8

Values are expressed as mean ± SEM.

#p <0.05 versus days 1-5.

*p <0.05 versus gestational ages $31\text{-}33^{+6}$.

p values obtained by Mann-Whitney U test.

concentration ($r = 0.40$; $p < 0.005$). Tocopherol levels on days 6-14 were significantly lower in preterm infants born after 34 weeks' gestation than in infants born between 31 and 33^{+6} gestational weeks.

8.3.2. INFLUENCE OF FEEDING AND VITAMIN SUPPLEMENTS

In the 5 days prior to collection of the first sample of blood, 3 infants had received only parenteral nutrition (amino acids plus lipid solutions), 41 infants had been fed solely milk, and 10 infants had been given both milk and parenteral nutritional fluids. Four unfed infants (mean postnatal age 2.8 $\pm$ 0.9 days) had been given only intravenous dextrose and electrolyte solutions. Of the infants fully established on milk feeding, 17 (41%) were receiving preterm infant milk formulae, 15 (37%) were receiving standard infant formulae, 2 (5%) were being fed exclusively own mother's milk and 7 (17%) were being fed with own mother's milk plus fortifier or formula supplements. Nine (24%) of the milk-fed infants were receiving oral vitamin supplements.

Plasma concentrations of retinol, retinol-binding protein, prealbumin and tocopherol did not differ between enterally and parenterally fed infants. Neither were there any differences between unfed and fed infants of comparable postnatal age.

For infants established on milk feeding, tocopherol levels

were higher in infants receiving own mother's milk than in infants fed with standard infant milk formulae. However, for postnatal age-matched groups, this difference was not significant. Retinol and plasma protein levels tended to be lower in breast milk-fed infants (Table XIX).

Oral vitamin supplementation in preterm infants did not significantly affect plasma concentrations of either retinol or tocopherol.

In term infants the mean plasma α-tocopherol concentration was higher in breastfed infants than in formula-fed infants ($p = 0.05$) (Table XX). None of the other biochemical parameters measured was influenced by the type of feeding.

8.3.3. LONGITUDINAL DATA

Three or more consecutive weekly blood samples were obtained from 22 preterm infants, most of whom were established on enteral feeding (Table XXI). The first sample was collected concurrently with recording of the first ERG (mean postnatal age 14.2 $\pm$ 2.1 days). Retinol values were consistently low and did not change over time. An initial rise in plasma tocopherol was not significant. Biochemical measurements from the subgroup of infants followed for 5 or more weeks did not differ initially from those of infants from whom only 3 samples were assayed.

TABLE XIX - PRETERM INFANTS: INFLUENCE OF DIFFERENT TYPES OF MILK FEEDING.

	Preterm formulae* (n=9)	Standard formulae* (n=9)	Own mother's milk (n=9)
Retinol (µg/dl)	11.9 ± 3.8	10.5 ± 1.8	7.3 ± 0.8
α-Tocopherol (µg/dl)	1219 ± 390	948 ± 247	1308 ± 216
Retinol-binding protein (mg/dl)	1.7 ± 0.4	1.6 ± 0.2	1.1 ± 0.1
Prealbumin (mg/dl)	11.8 ± 2.4	12.1 ± 1.8	9.4 ± 0.7
Postnatal age (days)	19.9 ± 8.7	19.1 ± 9.1	16.9 ± 4.9
Postconceptional age (weeks)	34.9 ± 0.8	35.7 ± 0.5	35.1 ± 0.5

Values are expressed as mean ± SEM.

Preterm formulae = 80 kcal/100ml. Standard formulae = 65 kcal/100ml.

*includes only postnatal age-matched infants for comparison with own mother's milk.

None of the differences between formulae and own mother's milk was significant.

TABLE XX - TERM INFANTS: COMPARISON BETWEEN BREASTFED AND FORMULA-FED[#] INFANTS.

	Breastfed (n=19)	Formula-fed (n=20)	p*
Retinol (µg/dl)	11.0 ± 1.3	10.2 ± 0.9	NS
α-Tocopherol (µg/dl)	779 ± 91	572 ± 49	=0.05
Retinol-binding protein (mg/dl)	2.2 ± 0.2	1.8 ± 0.1	NS
Prealbumin (mg/dl)	11.0 ± 0.7	10.6 ± 0.5	NS
Postnatal age (days)	3.6 ± 0.3	3.3 ± 0.2	NS
Gestational age (weeks)	39.7 ± 0.4	39.3 ± 0.3	NS
Birthweight (grams)	3405 ± 135	3247 ± 94	NS

Values are expressed as mean ± SEM.

[#]65 kcal/100ml infant milk formulae.

*p values obtained by Mann-Whitney U test.

TABLE XXI - SERIAL WEEKLY BIOCHEMICAL PARAMETERS IN 22 PRETERM INFANTS.

	1	2	3	4	5	6
Retinol (µg/dl)	9.6±0.8	10.9±1.2	11.0±1.3	11.8±1.5	9.0±0.8	7.6±0.3
α-Tocopherol (µg/dl)	1301±154	1669±147	1859±183	2222±189	1963±233	1610±415
Retinol-binding protein (mg/dl)	1.5±0.1	1.6±0.1	1.3±0.1	1.4±0.1	1.2±0.1	1.5±0.2
Prealbumin (mg/dl)	10.6±0.8	11.8±0.7	11.2±0.7	11.7±1.0	11.2±0.8	12.8±0.8
	n=22	n=22	n=22	n=10	n=5	n=2

Values are expressed as mean ± SEM.

There were no significant trends in any of the biochemical parameters. (Friedman's non-parametric ANOVA)

8.3.4. COMPARISON WITH TERM INFANTS

Plasma retinol ($p < 0.001$) and retinol-binding protein ($p < 0.001$) concentrations were lower in preterm infants during the first five days of life than in term infants of comparable postnatal age (Table XXII). Preterm infants also had significantly lower circulating levels of both α-tocopherol and prealbumin. The molar ratio of retinol to retinol-binding protein was similar in the two groups.

8.3.5. THE RETINOL DOSE RESPONSE IN PRETERM INFANTS

The retinol dose response in 40 preterm infants ranged from 10.6 to 59.8%, with a mean of 33.5 $\pm$ 1.9%. The infants were aged from 4 to 87 days (mean age 28.9 $\pm$ 3.6 days), and their mean postconceptional age was 36.7 $\pm$ 0.3 weeks. All infants were milk-fed and were ready for discharge.

The predose retinol ranged from 2.5 to 20.5 μg/dl with a mean of 10.1 $\pm$ 0.7 μg/dl; 5 hours following an oral dose of 5000 IU vitamin A the mean plasma retinol concentration was 14.7 $\pm$ 0.8 μg/dl (range 5.2 to 28.7). The difference was significant ($p < 0.001$). Retinol-binding protein similarly increased, but prealbumin and tocopherol levels did not change (Table XXIII). There were significant rises in the molar ratios of both retinol to retinol-binding protein and retinol-binding

TABLE XXII - COMPARISON OF PLASMA CONCENTRATIONS OF RETINOL AND α-TOCOPHEROL IN PRETERM[#] AND TERM INFANTS.

	Preterm infants (n=16)	Term infants (n=39)	p*
Retinol (μg/dl)	6.3±0.5	10.6±0.8	<0.001
α-Tocopherol (μg/dl)	466±43	676±53	=0.01
Retinol-binding protein (mg/dl)	1.2±0.1	2.0±0.1	<0.001
Prealbumin (mg/dl)	8.3±0.6	10.8±0.4	<0.005
Molar ratio of retinol to retinol-binding protein	0.43±0.04	0.38±0.02	NS
Postnatal age (days)	3.6±0.3	3.4±0.2	NS
Gestational age (weeks)	35.4±0.3	39.5±0.3	<0.001
Birthweight (grams)	2291±97	3313±83	<0.001

Values are expressed as mean ± SEM.

*p values obtained by Mann-Whitney U test.

#includes only preterm infants aged 1-5 days.

TABLE XXIII - THE RETINOL DOSE RESPONSE IN 40 PRETERM INFANTS.

	pre-dose values	post-dose values	p*
Retinol (µg/dl)	10.1 ± 0.7	14.7 ± 0.8	<0.001
Retinol-binding protein (mg/dl)	1.5 ± 0.1	1.7 ± 0.1	<0.001
Prealbumin (mg/dl)	11.8 ± 0.6	12.3 ± 0.6	NS
α-Tocopherol (µg/dl)	1463 ± 110	1509 ± 115	NS
Molar ratio of retinol to retinol-binding protein	0.52 ± 0.03	0.66 ± 0.02	<0.001
Molar ratio of retinol-binding protein to prealbumin	0.32 ± 0.01	0.38 ± 0.02	<0.005

Values are expressed as mean ± SEM.

*p values obtained by Student's paired t-test.

protein to prealbumin.

The retinol dose response correlated inversely with the predose retinol concentration ($r = -0.57$; $p < 0.0005$) and with the predose plasma values of both retinol-binding protein ($r = -0.38$; $p < 0.05$) and prealbumin ($r = -0.37$; $p < 0.05$). There was no difference in the retinol dose response between infants receiving standard oral vitamin supplements and unsupplemented infants.

Neither the predose plasma retinol concentration nor the retinol dose response was related to birthweight, postnatal or postconceptional age.

8.4. DISCUSSION

Plasma retinol concentrations were consistently low in this typical preterm infant population. Only three preterm infants had plasma retinol concentrations considered adequate in older children or adults (94), and two of these infants were aged over 70 days. During the first five days of life, plasma concentrations of both retinol and retinol-binding protein were significantly lower in preterm than in term infants. These data are consistent with previous reports of circulating levels of retinol and retinol-binding protein levels in preterm infants at birth (83-86,93) and during the first two months of life (91-93,96).

The plasma concentration of retinol did not correlate with

gestational (83,84,93), postnatal or postconceptional age (96), and there was no significant change in plasma retinol values over a period of up to five weeks in a group of 22 stable infants (Table XXI) (92,93).

No significant differences were observed in plasma retinol, retinol-binding protein, prealbumin or tocopherol levels between parenterally and enterally fed infants of comparable postnatal age and maturity; however, the number of parenterally fed infants was small. With similar feeding regimes, Woodruff et al (91) observed significantly lower concentrations of retinol, retinol-binding protein, prealbumin and α-tocopherol in parenterally fed infants.

Oral supplementation of 1500 IU vitamin A and 5 IU tocopherol per day meets current recommendations (123), but did not significantly affect plasma concentrations of either retinol or α-tocopherol or the retinol dose response. In formula-fed infants, doubling the vitamin supplement over a two week period did not result in a significant increase in either retinol or α-tocopherol levels (91).

Plasma α-tocopherol levels were lower in preterm infants than in term infants during the first five days of life (Table XXII) (124), and in preterm infants there was a correlation between plasma tocopherol values and postnatal age. The longitudinal data did not confirm a significant increase in plasma tocopherol concentrations over time. However, most of the serial observations were made after the second week of

life, and thus did not encompass the marked rise in tocopherol levels seen over the first two postnatal weeks in our cross-sectional data (124).

After the first six days of life plasma α-tocopherol levels were above 500 μg/dl in all but one preterm infant. This value is considered adequate since lower plasma concentrations of tocopherol are associated with increased peroxide-induced haemolysis of red cells (124). Human breast milk is a better source of tocopherol than infant formulae (91,125), and this was reflected in higher plasma levels of tocopherol in both preterm and term infants fed with own mother's milk.

The plasma concentration of retinol is a poor index of vitamin A status since protein-calorie malnutrition (126,127), liver disease and other illnesses (128) influence plasma levels of retinol independently of hepatic reserves. Indeed, low circulating levels of retinol in preterm infants have been attributed to both deficient protein-calorie intake (85,86,93) and liver immaturity (83,84).

Under vitamin A deficient conditions excess unbound retinol-binding protein accumulates in the liver (129). Newly ingested vitamin A can then be taken up rapidly by the liver cells, bound to retinol-binding protein, and released into the circulation (122,129,130). Hence, a high retinol dose response distinguishes between low circulating levels of retinol due to depletion of liver stores, and low circulating levels of retinol secondary to reduced carrier proteins (122). When plasma

retinol is measured rather than total vitamin A activity there is no interference from transported dietary retinyl esters.

Following an oral dose of vitamin A in preterm infants there were significant increases in the plasma concentrations of both retinol and retinol-binding protein, and a concomitant rise in the molar ratio of retinol-binding protein to prealbumin. These data suggest that availability of retinol-binding protein is not a limiting factor in the low plasma retinol levels found in preterm infants, and are compatible with autopsy studies showing very low hepatic reserves of vitamin A in preterm infants (89,95).

Thus, it is concluded that low plasma concentrations of retinol in apparently healthy preterm infants reflect reduced liver stores of vitamin A.

8.5. SUMMARY AND CONCLUSIONS

1. Plasma concentrations of both retinol and retinol-binding protein were consistently low in a typical preterm infant population.

2. Preterm infants had lower plasma levels of retinol and retinol-binding protein than term infants of comparable postnatal age.

3. Plasma α-tocopherol values were lower during the first five days of life in preterm infants than in infants born at term, but were adequate in the majority of preterm infants after the fifth postnatal day. Higher tocopherol levels were seen in infants of all gestational ages fed with own mother's milk.

4. Plasma retinol and α-tocopherol levels in preterm infants were not influenced significantly by standard oral vitamin supplementation.

5. The retinol dose response suggests that low circulating levels of retinol in preterm infants reflect reduced liver stores of vitamin A.

CHAPTER 9. THE ELECTRORETINOGRAM IN RELATION TO VITAMIN A STATUS IN PRETERM INFANTS

9.1. THE AVERAGED ELECTRORETINOGRAM IN RESPONSE TO HIGH INTENSITY LIGHT STIMULI

9.1.1. PATIENTS AND METHODS

Electroretinographic data from 51 Caucasian preterm infants with normal fundi are presented in Chapter 7. These data were compared with the plasma concentrations of retinol, retinol-binding protein, prealbumin and α-tocopherol at the time of recording of each ERG. Biochemical methods and results are described in Chapter 8. Comparisons were made between the first as well as, where appropriate, the final ERG obtained from each infant and the relevant biochemical data.

9.1.2. RESULTS

Allowing for the effects of increasing age and maturity, none of the ERG parameters correlated with the plasma concentrations of retinol, retinol-binding protein, prealbumin or α-tocopherol. Similarly, neither the amplitude nor the

latencies of the ERG were related to the retinol dose response.

9.1.3. DISCUSSION

Relative sparing of retinal stores of vitamin A is recognised in hypovitaminosis A (67,68). However, this does not prevent the onset of impaired dark adaptation once liver stores of vitamin A have been exhausted (49,69,72). Young children are most susceptible to nightblindness (49,73), a fact attributed to their low hepatic reserves of vitamin A, and almost 10% of the preschool population will demonstrate impaired dark adaptation in association with plasma retinol values less than 20 μg/dl (49). A reduction in the amplitude of the ERG in conjunction with nightblindness has been observed consistently (73-77,79,81), and in five to nine year old children with serum vitamin A values comparable to those of our preterm infants, the amplitude of the ERG correlated with the serum vitamin A concentration (80).

Depletion of stores of vitamin A in the retina is light dependent (70), and so it might be anticipated that premature exposure to light in preterm infants would jeopardise ocular reserves of vitamin A, particularly at a time when rapid growth in the pigment epithelium and retinal surface area is occurring (18).

However, despite evidence that the low circulating levels of

retinol observed in our preterm infants reflect reduced liver stores of vitamin A (89,95,96), no significant relationship was demonstrated between any of the ERG parameters and either the plasma concentration of retinol or the retinol dose response. Indeed, the ERG was recordable in preterm infants having plasma retinol values as low as 2 μg/dl.

Averaging of responses was necessary to obtain consistently interpretable ERGs from preterm infants. A high intensity light stimulus was used, and the retina was therefore in a light adapted state at commencement of recording the ERG. Under such conditions one would expect rod function to be suppressed, and the ERG to reflect mainly cone function (15). In adult controls the latencies and amplitude of the averaged ERGs were consistent with predominantly cone responses (15,75,76,106). The rods are more susceptible than the cones to hypovitaminosis A, and so it is possible that early ERG changes of vitamin A deficiency in preterm infants would not be detected by our technique. Fulton (82) has used electroretinography to study rod function in a five month old child with cystic fibrosis and low serum retinol. The sensitivity of the retina was reduced in comparison to that of age-matched controls, and there was a concomitant reduction in the amplitude of the ERG.

Electroretinographic measurement of rod function requires prolonged dark adaptation and would increase greatly the duration of the recording session. Accordingly, the recording

protocol was modified in order to study the sensitivity of the retina to light in preterm infants. This is described in the subsequent section.

9.2. THE ELECTRORETINOGRAPHIC THRESHOLD IN PRETERM INFANTS

9.2.1. PATIENTS AND METHODS

i) PATIENTS

Twelve Caucasian preterm infants (7 males and 5 females) were studied. Their gestational ages ranged from 25^{+4} to 35^{+2} weeks with a mean of 32.3 $\pm$ 0.9 (SEM) weeks. Postnatal age at recording of the first ERG ranged from 4 to 85 days (mean 21.3 $\pm$ 6.8 days), and the mean postconceptional age was 35.2 $\pm$ 0.6 weeks. Eight infants had 2 or more (maximum 4) ERG recordings at weekly intervals. All infants had normal fundi.

ii) METHODS

The equipment was the same as that described in Chapter 5; however, the recording protocol was modified.

Following 5 minutes' adaptation to the dim red background light, the infant was adapted to the flashing light stimulus at intensity 1 for 1 minute. The flash rate was 1.1 Hz. One hundred responses were averaged, and then the stimulus intensity was doubled and a second 100 responses was

collected for averaging after 1 minute of adaptation to the flashing light. This procedure was repeated a further three times until a stimulus intensity of 16 (maximum) was reached. The stimulus intensity at which the ERG was first discernible was considered the electroretinographic threshold. This was expressed in logarithmic (log) units with intensity 1 designated arbitrarily 1 log unit. The entire recording session took approximately 25 minutes to complete.

Coincident with each ERG recording, a sample of capillary blood was obtained for the measurement of plasma retinol, retinol-binding protein and prealbumin. Subsequent to the final ERG, 6 infants had a retinol dose response test performed. Biochemical methods are detailed in Chapter 8.

Statistical analyses were performed using data from only one recording session per infant unless stated otherwise.

9.2.2. RESULTS

At recording of the first ERG, the electroretinographic threshold ranged from 1.3 to 2.2 log units (intensity 2 to intensity 16). The lowest threshold was seen in one infant with eyes mostly open during recording of the ERG (gestational age 25^{+4} weeks, aged 57 days). One other infant (threshold 1.9 log units) had intermittent eye opening; in all other infants ERGs were recorded with the eyes closed. The mean amplitude of the ERG at threshold intensity was 13.6 $\pm$

1.3 (SEM) μV. There was no significant correlation between the electroretinographic threshold and either postnatal age, postconceptional age or the plasma retinol concentration.

In adult controls, even with eyes closed, the averaged ERG could be recorded consistently at a stimulus intensity of 1 log unit.

Measurement of the electroretinographic threshold was repeated after 1 week in 8 of the preterm infants. Six infants had continuous eyelid closure during both recording sessions, and in one infant there was intermittent eye opening on both occasions. In these 7 infants there was a significant decrease in the threshold over 1 week ($p < 0.05$). The mean plasma retinol concentration did not change. In the eighth infant the eyes were open during the first ERG recording session (threshold 1.3 log units) but remained closed on two subsequent occasions. At the second and third recording sessions the threshold was 1.9 and 1.6 log units, respectively.

The retinol dose response ranged from 11.6% to 41.3% and correlated with the log threshold at the final ERG recording session ($r = 0.81$; $p = 0.05$) ($n = 6$). There was no significant relationship between the electroretinographic threshold and the predose plasma retinol concentration.

9.2.3. DISCUSSION

The electroretinographic threshold was demonstrated in all

12 preterm infants tested over a relatively small range of light intensities.

The lowest threshold was measured in the one infant with eyes open. In the subsequent recording session her eyes remained closed, and the electroretinographic threshold was 0.6 log units higher. This suggests that eyelid closure in preterm infants may increase the minimum intensity of light required to elicit an ERG. In earlier studies (Chapter 7) the stimulus was kept constant at maximum intensity (2.2 log units), and eyelid closure did not affect either the latencies or the amplitude of the averaged ERG in preterm infants.

A significant decrease in the electroretinographic threshold over time was seen when allowance was made for eye opening. This is consistent with data obtained in term infants showing a steady reduction in the electroretinographic threshold from six weeks of age, until the attainment of adult retinal sensitivity in the sixth month of life (33). Assessment of retinal sensitivity by means of electroretinography correlates with psychophysical measures (32,131), and is the only method applicable in the neonate.

The retinol dose response correlated with the logarithm of the electroretinographic threshold in 6 preterm infants. In alcoholic cirrhotics, the retinol dose response was higher in subjects with impaired dark adaptation than in those with normal visual function, even although the plasma levels of retinol were similar in the two groups (130). The retinol dose

response reflects liver stores of vitamin A and is thus a more sensitive index of vitamin A status than the plasma concentration of retinol (122,126-129). In vitamin A deficient rats the logarithm of the electroretinographic threshold is related linearly to the concentration of rhodopsin in the eye (69,70).

Thus, it is concluded that in apparently healthy preterm infants retinal stores of vitamin A may be reduced in association with depletion of liver reserves.

Further study of vitamin A function in preterm infants is required to investigate whether current recommendations for vitamin A supplementation in preterm infants are appropriate.

9.3. SUMMARY AND CONCLUSIONS

1. There was no relationship between vitamin A status and either the latencies or the amplitude of the averaged ERG in a group of 51 preterm infants.

2. The minimum intensity of light required to elicit an ERG in preterm infants was consistently higher than in adult controls.

3. In preterm infants the electroretinographic threshold decreased over time and appeared to be increased by eyelid closure.

4. The electroretinographic threshold correlated with the retinol dose response in preterm infants.

5. Retinal stores of vitamin A in preterm infants may be depleted in association with reduced liver reserves.

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