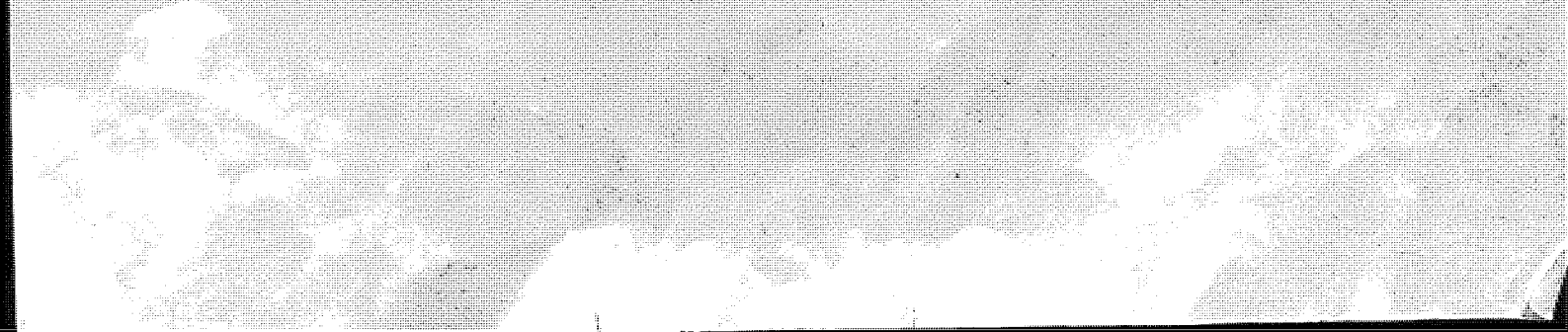

THE RETINOPATHY OF PREMATURITY

A STUDY OF INCIDENCE AND THE IDENTIFICATION OF THOSE BABIES AT RISK

MYOPIA ASSOCIATED WITH PREMATURITY

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Doctor of Philosophy

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March 1989

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SUMMARY

The ocular problems associated with premature birth have been with us ever since it was discovered that the application of high levels of inspired oxygen provided a reduction in mortality. The consequence of this reduction in mortality has been a rise in morbidity; these mortality and morbidity rates have oscillated during the attempt to find a reasonable balance. The use of contemporary technology during the attempt both to understand the premature baby's delicate physiology and to maintain life to younger and lighter babies has not yet produced stability.

The incidence of typical retinal maldevelopment, retinopathy of prematurity (ROP), was analysed by serial weekly ophthalmoscopy examinations in a regional special care baby unit, 579 examinations being made on 138 babies. The best instrument for this examination was found to be a compact indirect ophthalmoscope incorporating an inverting eyepiece - the Reichert Jung monocular indirect ophthalmoscope.

The optimum time for ocular examination to discover potential ocular morbidity was at 33 weeks post-conceptual age (PCA) with continued examinations to the age of 37 weeks PCA.

The babies that were found to be at risk of a significant grade of ROP were found to be of a birth weight of less than 1251 grams or had an estimated gestational age at birth of 30 weeks or less.

A refractive state of myopia was found to be the norm. The myopia reduced as life progressed to attain emmetropia around the age of 50 weeks PCA or 22 weeks survival. The reduction of the myopic state was found to be dependent on birth weight and gestational age at birth, the youngest and therefore the lightest being more predictable in attaining emmetropia.

Refractive variations were found to be coincident with the timings of certain medical treatment regimes and a hypothesis is postulated as to the mechanism of this association.

KEY WORDS: retinal development, prematurity, retinopathy of prematurity, myopia of prematurity, retrolental fibroplasia.

to my own children

ANDREW CAROLINE JACQUELINE BENJAMIN RACHAEL

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CHAPTER 1

INTRODUCTION

The retinopathy of prematurity is an unfortunate companion of ever-improving premature baby care. Never before have premature babies so small and gestationally immature survived and these are the infants that are most at risk of blindness due to ocular lesions as a result of the retinopathy of prematurity, (ROP).

ROP is a disease of the infants' retinal vasculature where growth of vessels is arrested and vessels already patent altered to the detriment of the developing retina. If the retinopathy proceeds, disorganised and chaotic vascularisation occurs to produce cicatricial changes (a state which until recently was known as retrolental fibroplasia (RLF)).

ROP is not a new complaint. There is a history of the disease for about 45 years, and in that time its status has somewhat changed. Initially it was a curiosity, then an 'epidemic' which was cured and almost disappeared, only to reappear again to stay as a problem yet to be solved. The premature baby is very vulnerable; it is susceptible to illness and malformation associated with its early stage of development. The maturing retina in particular appears to be sensitive to changes in the baby's metabolism. Inspired oxygen levels were, for a long time, blamed for the presence of the disease but the precise effect of

oxygen is unknown and in this lies the key to understanding the problem of ROP. Oxygen's relation to some of the homeostatic components of the body does however go some way to providing important information.

The examination of the preterm infants' retina is clinically difficult and demanding. Those babies that are considered to be 'at risk' are very small, mostly weighing 1500g. or less. Premature babies are placed in a controlled environment known as an incubator which standardises heat and humidity and contains essential monitoring equipment. One must therefore examine in as short a time as is possible and without disturbing equipment. Typical equipment includes:

1. An apnoea mattress.
2. Umbilical or other arterial catheters.
3. Intubation tubes and ventilator lines or head boxes used to control the inspired gases.
4. Equipment for monitoring blood gases either by means of: (a) an indwelling arterial catheter; or (b) by heated surface transcutaneous electrode.
5. Monitors to record essential functions.
6. Intravenous lines for feeding and maintaining the infant's physiological norms.

At birth these infants, often less than 32 weeks gestation and as little as 25 weeks gestation (8-15 weeks premature), not only have very small eyes and a poorly developed retinal vasculature, but they often have a significant amount of receeding hyaloid

tissue in the vitrious chamber and the posterior crystalline lens surface which, although fascinating to observe, precludes any attempt at ophthalmoscopy. The palpebral apertures of these infants are also small and initially tight to the extent that they are occasionally fused. Their pupils do not respond readily to drug induced mydriasis.

It has therefore been my experience that it took several months to gain confidence, understand individual problems and devise a technique for the examination of the preterm infants' retina. Only when a successful technique has been established can one begin to monitor development with some level of confidence. The results obtained from the first 6 months of attendance at the Special Care Baby Unit at Coventry Maternity Hospital have therefore not been included in this report.

The protocol for this study was agreed by both the hospital and the university's ethical committees. (see appendix 1).

Clinical notes were recorded as shown in appendix 2. This record sheet was initially developed to indicate the retinal change in terms of the "Kieth classification" of ROP, but the final grading was changed, so as to conform with the "International Classification". Both classifications may be seen in appendix 2.

CHAPTER 2

LITERATURE SURVEY

2.1 History of the Disease

The first clinical report of the retinopathy of prematurity was made by M. Terry (1942), who described a 'fibroplastic overgrowth of a persistent vascular sheath behind the crystalline lens' suggesting a maintenance and extension of components of the hyaloid system. The only factors that he associated with this condition were prematurity and low birth weight. He termed the condition 'retrolential fibroplasia' (RLF).

Owens and Owens (1949) showed that RLF was not an abnormal development of the hyaloid system. They reported that the eyes of an infant developing RLF were essentially normal at birth, and that the condition was a retinopathy with neovascularisation and associated complications.

In the few years leading to the 1950s , the incidence of the disease increased dramatically, becoming the largest single cause of child blindness (Patz, 1979). Paediatricians began to search for a cause of the disease, and studies of many variables in relation to incidents were made. Much of the controversy revolved around the role that oxygen had to play. There were

conflicting theories on the effect of oxygen on the eye, ranging from the exposure of added oxygen as a cause of RLF to a lack of oxygen causing RLF.

Campbell (1953) implicated increased oxygen as a causative factor after a comparative study in three Australian hospitals. He suggested that as high inspired oxygen concentrations (FiO_2 70%-80%) had been shown to be toxic to adult tissues, one would therefore assume that lower concentrations might be toxic to newborn tissues, particularly in the premature baby. Patz Horck and De la Crux (1952) performed the first clinical study to demonstrate a link between high levels of oxygen concentration and the pathogenesis of RLF. The report recommended that "in view of the bizarre manner in which the incidence of the disease fluctuates, additional rigidly controlled observations were necessary to establish this concept". This report led to the work of the co-operative study headed by Kinsey (1956). Low birth weight babies were divided into two groups: the 'routine oxygen group', who received inspired oxygen at a concentration greater than 50% (FiO_2) for approximately 28 days and the 'curtailed oxygen group' receiving a minimum concentration of oxygen compatible with the clinical condition of the infant. The data collected showed that the incidence of both active and the cicatricial (severe) changes was significantly higher in the routine oxygen group than in the curtailed group.

Ashton, Ward and Serpell (1953) assumed that they had proved

oxygen could form a lesion, or vascular retinal developmental anomaly, similar to that of RLF in their work on experimental pathology performed on kittens. Because of this evidence there followed a rigid curtailment of the use of oxygen throughout the world in the maintenance of life of premature babies, and this was followed by a drop in the occurrence of RLF. This decline was taken as further proof of the dangers of oxygen supplementation.

By the late 1950s and early 1960s reports suggested that the reduction in oxygen provision in hospitals was having serious side-effects. In particular there was an increasing mortality of premature babies from hyaline membrane disease producing respiratory distress syndrome (RDS) (Avery and Oppenheimer, 1960). Cross (l973) showed, following a study of the *Registrar General's Statistical Review* in Great Britain and the *Vital Statistics* of of the USA, that "the fall in death rate in the first day (of life) shows an abrupt hold-up from about 1950". He went on to further state that for each sighted baby that had survived, the regimen may have cost some 16 other lives. Understandably, the reusage of oxygen was somewhat liberalised from this time.

Statistical reports have shown that in recent years the incidence of ROP is once more increasing (Phelps, 1981; Gunn, Aranda and Little, 1978). In these studies it has been recognised that the increase may be due to the increased survival of very small premature babies at risk of ROP, to increased recognition or

diagnosis of the disease and to an increase in the capacity to improved oxygenation by blood-gas monitoring. Phelps (1981) stated that:

"The incidence of blindness from RLF is highest amongst the infants weighing less than 1.0 Kg."

This was clearly a reminder that this disorder is caused first by prematurity and only secondarily by the administration of oxygen or other factors. Present skills in maintaining physiological norms are not sufficient to prevent RLF in the most premature and these are the infants who are now surviving in greater numbers.

The number of affected infants and the severity of their lesions varies greatly from one study to another. This can be partly explained by each examiner using their own techniques and times of examination. Also there are different treatment regimens, and many grading systems are used.

The most recent reports on the incidence and aetiological factors of ROP all show a percentage of children developing lesions which strongly correlate to the degree of prematurity: degree of prematurity is associated with the appearance and severity of the lesion (Keith, Smith and Lansdell, 1981; Yu, Hookham and Nave, 1982; Keith and Kitchen, 1983; Kalina and Karr, 1982; Bauer, 1978; Gunn, Easdown, Outerbridge and Aranda, 1980). All of the above reports also correlate some aspect of oxygen

therapy with ROP with the exception of that by Yu *et al.* (1982).

Recent developments in the monitoring of the arterial oxygen partial pressure and carbon dioxide partial pressures means that the medical staff have more information about the infant's condition and can therefore exercise more control over the infants environment and physiological state.

2.2 The Illness of Prematurity; Factors Associated with ROP

When a child is born their environment changes dramatically. The fetus is used to an aquatic environment where it is provided with oxygen and nutrients, its body continuously flushed of metabolites, a constant temperature maintained, movement is easy and it is protected from external stimuli. The new environment *exutero* is very dry, oxygen must be worked for, food comes intermittently and via a new route, the temperature fluctuates, movement is achieved less easily and external stimuli act directly. In the full term infant, most of the organ systems are subject to change during the perinatal and early post-natal periods, adapting to the new environment. The premature infant is ill equipped to succeed in this hostile new world. The cardiovascular system in particular is ill-suited to a life of breathing air. In the fetus the foramen ovale and the ductus arteriosus provide a change by which the pulmonary system is essentially bypassed . The ductus venosus allows most of the blood returning from the placenta to pass directly into the inferior vena cava thus bypassing the liver sinusoids.

In a full term baby, the ductus arteriosus and the foramen ovale start to close when the infant first starts to breathe air. In the premature baby, however, they do not always close or close completley leading to a significant reduction in efficiency. The blood flow in the ductus is reversed to form a left-to-right shunt so that some blood completely bypasses the lungs. This

condition has sometimes been treated using the prostaglandin inhibitor indomethacin which may have some effect on the incidence of ROP (Gersony *et al.* 1983). Alternatively, the ductus may be 'tied' surgically.

The premature babies' immature lungs lack lecithin-rich pulmonary surfactants thus part of the lungs are unable to expand and other alveoli collapse after each inspiratory inflation. The infant develops respiratory distress syndrome (RDS) which was formally termed hyaline membrane disease and has the characteristics of general cyanosis with trachypnoea and expiratory grunting. As a result of the anoxaemia there is metabolic acidosis exacerbated by a high carbon dioxide tension ($PaCO_2$). Treatment includes careful maintenance of temperature, acid-base balance and humidity. Oxygen administration has not only been linked with ROP but has also been linked with bronchopulmonary dysplasia. If the infant survives, fibrin exudes into the aveoli and forms a fibrous matrix which enmeshes protein materials and forms a hyaline membrane that lines the alveoli.

If the maternal and fetal rhesus types differ and there is a leakage across the placenta a mother may produce antibodies against the fetal circulation. This can produce fetal red cell agglutination leading to congestive heart failure and death. However, the fetus may be able to adjust by increasing the red cell production and this can only occur if the bilirubin produced by red cell destruction can be excreted across the placenta. At

birth the baby appears normal but is not able to cope with the rising tide of serum bilirubin and after 12-24 hours becomes jaundiced. Severe cases are treated with exchange transfusions and these have been implicated as part of the causative factor of ROP. As adult red cells have a different oxygen dissociation curve they increase the oxygen available at the retinal level (Shohat, Reisner,Krikler, Missenkom, Yassur and Ben-Sira, 1983).

The premature baby is particularly susceptible to infectious diseases and the problems are increased due to the vague nature of early signs of infection.

The aspects of prematurity which have been linked with ROP are numerous. Many reports have been published and analysed statistically and it can therefore be confidently stated that ROP can be strongly associated with infant ill-health (Gunn *et al.* 1980). Factors other than those mentioned include septicaemia, complications of pregnancy, hypothermia, spastic diplegia, and recurrent apnea (Gunn *et al.* 1980; Weiter 1981; Keith *et al.* 1981; Francois, 1983; Shahinian and Malachowski, 1978).

2.3 ROP, Appearance and Progress

The time after birth at which ROP appears has been the subject of some confusion which is understandable when the problems of examining the premature infant are considered. There may be a period when it is difficult or even impossible to perform any form of ocular examination - for example, very soon after birth. It is not possible at this time to perform ophthalmoscopy and visualise the fundus owing to hazy media. Palmer (1981) recommended an examination at the 7-9 weeks stage. The more usual criteria for an ocular examination have been: sick infants, small infants or infants who have received oxygen supplementation. The pupils of such infants to be examined are dilated and normally examined by indirect ophthalmoscopy with or without scleral depression.

The normal human fetus has no retinal blood supply until the fourth month of gestation (Cogan, 1963). At that time, retinal vessel growth proceeds centrifrugally and is complete on the nasal side at about 8 months of gestation and on the temporal side at about 9 months gestation or shortly before birth. Thus premature infants show incomplete retinal vascularisation especially on the temporal side. In the normal infant vessel size decreases as the periphery is approached and the branching angles of the fourth - and fifth - order vessels is between 20 and 40 degrees with the outside possibility of 60-70 degrees (Kingham, 1977). The peripheral retina may have a greyish opaque silky appearance, more noticeable in poorly pigmented

individuals. The transition between vascularised retina and asvascular retina is gradual rather than abrupt.

The earliest stage seen in ROP is an abnormal terminal arborisation of peripheral vessels rather than the uniform 20-40 degree branching angle of normal vessels.These peripheral vessels terminate in a group of dilated vessels with 'tufts' which may vary in size and these vessels may follow a path parallel to the ora serrata . These retinal vessels terminate at the existing junction between the vascular retina and the avascular retina and, as the condition progresses, the vessels become more dilated and the junction between the normally vascularised and the neovascular retinal region develops into a visible network of yellowish-pink tissue. This junction becomes highly elevated to form a 'shelf' or a 'shunt'. Anterior to this structure and posterior to the ora serrata there is a zone of non-vascularised retina that is grey and opaque.

Kushner *et al.* (1977) took sections through the retina and found large vessels in the nerve fibre layer of the vascularised retina leading up to the anomalous retina which consisted of a dense mass of mesenchymal cells. Within this mass they found irregular vascular spaces containing red blood cells but beyond this, in the avascular region, there was no blood vessels or erythrocytes. Garoon *et al.* (1980) described the vascular tufts to the shunt that appear as reddish-pink globules and it is postulated that their appearance, posterior to the shunt, suggests a poorly developed retinal circulation and poor

prognosis for vision. Kushner *et al.* (1977) described sac-like dilations and hypothesized that they occur on the capilliaries normally connecting the superficial and the capillary network.

At a later stage this shunt becomes markedly thickened to form an inter-retinal ridge, some neovascularisation may become evident. Kingham (1977) identified two forms of neovascularisation. In the first, fine capillary strands are formed on the anterior or posterior surface of the shunt and these capillaries may expand in networks into the vitreous progressing towards the posterior lens surface.

The second form of neovascularisation are extra retinal orange-red terminal blood channels termed sinusoids which occur just behind the ridge and communicate with the retinal circulation. Kushner *et al.* (1977) sectioned the sinusoids and found them to be above the vascularised retina and consisting of PAS-positive cells intermixed with large vascular channels and lined by mature endothelial cells. There may also be some surface haemorrhages on the posterior surface of the shunt.

Owing to the presence of the shunt, the posterior vessels are affected by the need to supply an adequate volume of blood through it. This causes the veins to become dilated and the arteries to be tortuous. This change in blood vessels is the first sign of the retinopathy to be apparent near the posterior pole of the eye, and it is at this stage that cicatricial scarring and fibrous tissue first appear. This fibrous tissue accompanies the

extra retinal vessels growing into the vitreous and as the capillary growth progresses so the fibrous tissue may also grow, sometimes to the extent of producing pressure onto the posterior capsule of the lens. Kingham (1977) described the proliferation from the surface of the optic disc into the vitreous as an opaque non-vascular structure present in advanced cases of ROP and stated that it may represent condensation of the vitreous and intravitreal debris.

As a shunt increases in protrusion and the blood vessels become engorged it may cause the retina to become detached from the retinal pigment epithelium by traction posterior to the ridge. The vessels, which are fat at the posterior pole, detach about halfway to two-thirds of the way to the periphery and insert into the crest of the shelf. This detachment may be localised in one quadrant. Haemorrhages may be visible on the surface of the retina and in the vitreous, caused by leakage of small neovascularised vessels.

The retinal lesions may progress ultimately to a point where the retina is completely detached to the optic nerve head and accompanied by fibrovascular growth. This growth extends interiorly to the posterior lens surface, where there may be fibrous tissue emanating from the optic disc towards the posterior surface of the lens which arcs anteriorly towards the ora serrata. This situation can be associated with a circumcapillary traction retinal detachment (Kingham, 1977). At this stage the media is hazy due to the vitreous condensate and

haemorrhages.

For ease of recognition and diagnosis by the paediatrician several grading systems have been devised thus allowing the stage of progression of a disease to be noted and compared with other variables to facilitate research, but it is only recently that there has been an acceptable international classification (1985). Keith (1979) provided one such popular table. This classification is of the fundus as seen with indirect ophthalmoscopy and scleral indentation. An older favoured classification of Reese, King and Owen (1953) concentrates on the posterior pole so the earliest changes which undoubtedly take place in the periphery would be missed.

Before the formation of cicatricial tissue within the eye there is a possibility of complete regression of the condition so that the eye can develop more normally. Resolution occurs by the extension of small terminal vessels into the zone of avascular retina. There is a lot of abnormal aborisation and a decrease of the intra-retinal line. Kushner *et al.* (1977) indicated that as the vascularisation proceeds in regressing ROP, it is via pathways that differ from the normal. There is the formation of unserved vascular tissue at the anterior edge of the shunt and at a later stage they noticed a "faceted nubbin of tissue" where the arteries and veins join up and this forms due to the lack of a capillary network.

If spontaneous regression begins early the final visual outcome

may be normal, the only lesion being mild scarring at the upper temporal periphery; but if the peripheral scarring is more severe the retina may be displaced towards the scar and an ectopic macula produced (Wise, Dolly and Henkind. 1971). Fortunately, the macula does not fully mature until the sixth postnatal month. The displaced macula may therefore be normal in appearance and have good foveal reflexes and function. Still more severe scarring may produce retinal traction lines from the disk temporally. In this case the disc will be slightly filled and deformed with a pale nasal side and an arc of proliferated pigment epithelium at the nasal border and retinal vessels drawn across the disc temporally as a brush, some of which may pass through the macula area although at some point the nasal vessels turn back in a more normal direction. If the retinopathy is more severe than this, vision is substantially reduced by retinal detachment although a small part of the nasal retina may remain attached, allowing light perception. In cases of progressed total retinal detachment and scarring behind the lens, no perception of light will be achieved.

ROP has been linked with susceptibility to retinal detachment in later life, particularly in adolescence (Tasman, 1979), infant myopia (Nissenkorm *et al.* 1983) and secondary glaucoma (Pollard, 1980).

Treatment of ROP has taken place, though on a very experimental basis. The treatment usually entails cryotherapy or photocoagulation of proliferitive or neovascularised tissue

(L'Esperance, 1983) or scleral buckling in cases of retinal detatchment. There is some controversy about when and in what cases treatment should be undertaken as a possibility of regression always exists (except for extreme cicatricial changes). There is also some argument over the efficiency of treatments. Keith (1982) found the benefits to be negligible in a comparative study, whereas McPherson and Hittner (1982) and Grunwald (1980) are advocates of surgical intervention.

2.4 Development of the Retinal Vasculature

Before we can study the physiological abnormalities occurring in ROP we must first understand the structure and progressive growth of the normal developing retina. It is also beneficial to define any difference between the developing and mature retina.

Initially, the developing retina receives its nutrient supply from the choroidal circulation (Wise *et al.* 1971). As development proceeds, the retina thickens (Kawabara and Weidman,1974) and its innermost layers become further removed from the choroidal circulation. The anterior part of the embryonic eye gets its nutrients from the hyaloid system; this system is starting to wane in the fifteenth to sixteenth week of development. The retina is starting also to show differentiation in two or three component nuclear layers and at this stage the retinal blood supply begins to develop at the disc. Previously this vessel growth was thought to be by budding from the existing hyaloid artery (Michaelson, 1948; Mann, 1928),but more recently Cogan (1963) and Ashton (1957) have shown that the developmental ingrowth of retinal vessels was formed by a preliminary invasion of mesenchymal cells. These cells (sometimes referred to as spindle cells) appear in the vicinity of the hyaloid artery, proliferate into the nerve head and subsequently invade the nerve fibre layer of the retina. Shortly after this, the cells differentiate into endothelial cells and solid endothelial cords sprout from the nerve head. Clefts appear between cells lying adjacently, intracelluar junctions develop and the cords

gradually canalise to form primitive capillaries in a polygonal (often pentagonal) network (Ashton, 1970). This network is referred to as a 'chicken wire' patterned by Cogan (1963). Its constituent endothelial cells have large, ellipsoid easily identifiable nuclei with inconspicuous or absent nucleoli; mitosis is frequent. Cross-sections of the retina show that the endothelial cells are associated through the abundant glycogen granules (Cogan, 1963) and intense alkaline phosphatose activity.

The vascular part of the chicken wire pattern is characterised by loops and free ending sprouts and, by the seventh or eighth month, the pattern extends to the ora serrata nasally and into the equator temporally. The advancing peripheral edge is a dense plexus of thin bore vessels enmeshed in a mass of hyaline PAS-positive material (Wise *et al.* 197l). Just anterior to this there are condensations of spindle shaped cells with nuclei orientated to the retinal surface. Blood intercedes primitive channels at an early stage but there is no differentiation into arteries or veins but it must soon develop its own inflow and outflow. At the same time capillaries begin to narrow, retract and atrophy. As this takes place on a considerable scale (Ashton, 1970), the blood must progressively be shunted into the remaining channels which, because of the increased flow, enlarge to become major vessels . Arteries and veins can be identified even within the chicken wire pattern the arterial capillaries form a wide meshwork and are narrower and less cellular than the venous capillaries. This suggests that some difference in the qualities of arterial and venous blood is influencing the

endothelial growth.

As the arterial and venous channels develop further, the capillaries close to these primitive channels which now become more susceptible to retraction and atrophy leading to a capillary-free zone which is considerably wider in the case of arteries than in the case of the veins. Obviously some branches must remain and their persistence may be due to the increased blood flow within them. The process of 'remodelling' continues until the normal number of pre-capillary arterioles and post-capillary venules remain. The difference in width of the perivascular capillary free zones raises an interesting point: Is the difference due to morphogenic influences or is it chemically determined, possibly by the variance in oxygen concentration of the blood? The latter possibility is supported by the fact that the peri-arterial capillary free zone can be widened by using high ambient oxygen concentrations, and according to Ashton (1970) this is due to acceleration of the fundamental processes of capillary retraction. Conversely, the width of the arterial capillary free zone may be narrowed by lowering the oxygen tension (Campbell, 1951).

The capillaries retract and atrophy by a mechanism whereby endothelial cells forming the retractile vessel migrate towards a persisting capillary (Ashton, 1970). This leaves fibrillary strands which stain with the PAS reagent (Cogan, 1963). It has been suggested by Ashton (1966) that these migrating endothelial cells align themselves along with their basement

membranes to the outside of the capillary which they are retracting toward. These cells thus form the intramural pericyte.

By the ninth month of gestation vascularisation is just short of the ora serrata on the nasal side and considerably shorter than the ora serrata on the temporal side. The advancing edge shows oversize buds from peripheral vessels and loops connecting with peripheral arterioles and venules. Karyorrhexis is prominent in the peripheral vessels. Cross sections of the retina in the terminal weeks of pregnancy show large groups of endothelial cells and accompanying glycogen granules but little else to distinguish them from adult vessels (Cogan, 1963).

2.5 The Effects of Oxygen on Premature Retinal Vessels

It has been known for many years that oxygen can act as a toxic substance, and it is this feature that is referred to when oxygen is linked to ROP.

Much work has been done on the effects of oxygen on the retinal vasculature, much of which is on animals rather than humans, perhaps because of the false idea of the similarity of the new-born animal retina to that of the human fetus.

The classically accepted theory states that the effect of oxygen obliterates the capillary endothelium (in the capillary meshwork). With this segment gone, the two tissues that remain (the mesenchym and the mature arteries and veins) unite via a few surviving capillary channels to form a shunt (Flynn *et al.* 1979). Because of the obliteration of the capillaries, parts of the retina become hypoxic and growth of new vessels occurs. This is generally termed vasoconstriction followed by vasoproliferation.

Ashton, Graymore and Pedler (1957) considered the vessels destroyed because of the development of intracelular retinal oedema. Experiments on young rabbits disproved this theory. The capillaries in rabbits initially lie on the surface of the retina surrounded only by vitreous, making damage from retinal swelling unlikely; but even so the response to ambient hypoxia is similar to that in the kitten whose vessels lie within the retina.

More advanced techniques of studying the retina show that later obliteration begins by capillary closure in the kitten (Ashton, 1967) and this is soon followed by degenerative changes in the endothelial nucleus and cytoplasm. There is, at this stage, a pronounced increase in the normal process of retraction with the migration of endothelial cells to ever-decreasing areas of surviving capillaries. At the same time endothelial disintegration with pronounced pyknosis and karyorrhexis proceeds until, in the final stages, only a fragile skeleton of the original network remains. This network is composed of threads of basement membrane and small clumps of dying endothelial cells that can migrate no further. An identical process may occur in babies exposed to hyperoxia (Ashton, 1967).

Ashton (1966) has described a method by which the capillaries may close as within the endothelial cells there are protein filaments which may be contractile. He showed this in retinal endothelial cells from a baby rabbit and said that these filaments were actin filaments. Their presence, along with some finer contractile filaments, suggest that primitive endothelial cells are well equipped to contract when perfectly stimulated. It would now seem reasonable to attribute the closure of the circulation of oxygen to contraction of the growing endothelial cells. Whether this final destruction of the endothelium is due to the toxic effects of oxygen or secondary to the failure of circulation and vessel closure is unknown.

Interestingly the destructive effect of oxygen on growing retinal

vessels is, in the intact organism, entirely confined to the immature retinal endothelium (Ashton,1957). This suggests that the endothelium is either unique or rendered vulnerable by its environment or anatomical location.

Vasoproliferation occurs as a sequel to the capillary closure, and has nothing to do with excessive oxygen directly. This has been illustrated by Phelps and Rosenbaum (1982) whose work on kittens showed that general hypoxia caused a more significant prolific lesion than normal air following hyperoxic injury to the retina. In the infant the proliferation can be seen to be closely comparable with normal growth, it involves both the advancing mesenchymal element and the endothelium within the retina and vitreous. The mesenchymal element is only associated with glycogen granules in the retina and not the vitreal proliferations.

In the vitreous, some of the anterior mesenchymal cells and primitive endothelial cells form a solid and differentiated band associated with many macrophages. At the rear of the growth pattern, the capillaries gradually differentiate into fully formed vessels. The mesenchymal element determines the formation of fibrous tissue and therefore detachment of the retina and formation of the retrolental fibrous membrane. Kittens and rabbits' retinae have been found not to vascularise by mesenchymal precursors but rather by endothelial budding, so the final fibrous stage of cicatricial ROP does not develop to the same extent. However recent work on the effect on the retinae of Beagle puppies has however showed circatricial retinopathy

production, strengthening the relationship between animal experiments and the clinical situation (Flower and Black, 1981).

One of the most useful and revealing animal studies was carried out by Ashton, Tripathi and Knight (1982 and 1984). Initially, the work was done *in-vivo* studying the effects of oxygen on the retinal vessels of rabbits using injection methods, whole mounts, histology, electron microscopy and direct viewing using a fundus window inserted in the anterior segment of the eye. The experiments showed that the growing vessels of the rabbit, like those of immature animals and of the premature infant, can be completely closed and finally destroyed by high concentrations of oxygen and that this activity is directly related in severity to the degree of immaturity and ceases when the vessels reach adult form.

These vascular changes occurred after a 12-14 hour exposure to high oxygen levels (FiO_2 80%-90%) starting with narrowing of the vessels lying on the surface of the retina. After 48-72 hours the lumina of most of the vessels are closed or occluded by degenerate leucocytes and desquamated endothelial cells. Ultrastructurally, the first indication of injury is the appearance of dense bodies (presumed by Ashton *et al.* to be lysosomes) and the formation of autophagic vacuoles. At this stage the mitochondria are usually unaffected, but the structures within the cytoplasm gradually degenerates to form a condensation of amorphous granular material. Finally, the cells are so disrupted that only the autophagic vacuoles remain, the only recognisable

feature, occasionally with complete nucleii still present.

Autophagic vacuoles occur as a common non-specific cellular reaction to physiological stimuli. As in embryological develoment, metamorphosis and involution occur with sublethal injury from various abnoxious agents (Ashton *et al.* 1972). They also occur in both hypoxia and hyperoxia. Interestingly, unaffected mitochondria are present until the final stages of cellular disintegration, mitochondria are affected in hypoxia. This implies that the endothelial injury is caused by the selective toxic action of oxygen rather than being due to an anoxic injury secondary to capillary closure. This idea is strengthened by the fact that in immature animals whose growing vessels are within the retina (e.g. kittens) the nervous elements immediately surrounding the capillaries destroyed by hyperoxia are unaffected. This suggests a selective injury to the endothelium rather than a failure of nutritional supply due to capillary closure.

Ashton and others also performed experiments on growing tissue cultures of retinal endothelium. The rabbit retina is suited to this task as for the first 2 weeks of life the vessels lie entirely on the surface of the retina. At this early stage, the growing vascular system can be lifted from the retina and transferred under sterile conditions to specially designed tissue culture chambers. The reaction of the growing culture to various gaseous concentrations was observed using phase and light microscopy and conventional and electron microscopy of

appropriately fixed preparations.

There was a very similar reaction to that described in the oxygen experiments *in-vivo*, although the vessels of the immature material always disintegrates to some extent. A large number of experiments were therefore carried out and it was concluded that the degeneration and disintegration of capillaries in pure oxygen occurs at an earlier stage (after 8-12 hours) than those exposed to air only. Oxygen toxicity was found to take effect with an ambient oxygen level of 30% with total destruction of cells at 40% which correlates to the *in-vivo* studies in the kitten (Ashton *et al.* 1953).

The facts as presented by Ashton and his associates imply that the capillary damage due to oxygen is caused by cytocidal action as opposed to capillary constriction. The actual amount of correlation between *in-vivo* and *in-vitro* studies requires clarification as does the action of hyperbaric oxygen upon the retinal vessel and endothelial cell.

These similarities and dissimilarities between animal model and the human ischaemic retinal disease were discussed by Kremer *et al* . (1987) following their work on hyper-oxygenated kittens. They suggested that there is a similarity between the other vasoproliferative retinopathies such as diabetic retinopathy, retinal vein occlusion etc. where there is also retinal capilliary obliteration. Although their work was not directed purely at the pathogenesis of ROP, they did obtain total obliteration of the

retinal vasculature following a 65-72 hour exposure to 80% oxygen.

In the following 18-21 days of living in normal air, florid preretinal vessels were found growing into the vitreous from the revascularised retina and from the optic disc itself. Neovascularisation of the iris was also observed.

2.6 Oxygen Toxicity

It is clear from the work of Ashton and his associates that oxygen in certain circumstances is highly toxic to growing retinal endothelium, apparently acting directly on the cells. Indeed, it has long been recognised that oxygen can be toxic to most living things. Unfortunately, the mechanism of the cytocidal action is not clear, although not for lack of suggested explanations.

Oxygen is capable of inactivating enzymes within cells. The essential sulphydryl groups are particularly susceptible. Coenzyme A and lipoic acid may also be oxidised (Haugaard, 1968). During biological oxidations, most oxygen is reduced by two electron transfers through the cytochrome carriers. There are other methods whereby a small part of the cellular molecular oxygen can be reduced through univalent pathways. Complete reduction of a molecule of oxygen to water requires four electrons and in a sequential univalent process several intermediates will be encountered. These are the superoxide and anion radical (0_2-), hydrogen peroxide (H_20_2) and the hydroxyl radical ($OH\cdot$) and these are too reactive to be well tolerated within living systems (Fridovich, 1978). Fluxes of superoxide anions generated enzymatically or photochemically have been shown to inactivate viruses, induce lipid peroxidation, damage membranes and kill cells.

Within the infant body the superoxide anion has been associated

with damage to the lungs (bronchopulmonary dysplasia) and other organs (Frank, Bucher and Roberts, 1978) as well as to the retinal circulation (Ashton *et al.*1974). According to Ashton lysosomal activity occurs initially simulating autolytic changes in the cells. This is dependent upon the breakdown of lysosomal membranes with the release of lysosomal enzymes.

It is known that all oxygen metabolising cells have developed protective mechanisms that either minimise the production of free radicals or, alternatively, destroy them as rapidly as they are formed (Feeney and Berman, 1976). Superoxide dismutase (SOD) is used in one such reaction where by dismutation it cathalyses the reaction:

$$20_2^- + 2H^+ = H_2 0_2 + O_2$$

(Fridovich, 1978)

It can be seen from the formula that in destroying the superoxide anion radical the SOD produces hydrogen peroxide. This, although more stable than the superoxide anion radical, is still highly toxic to the cell since it can by reacting with the 0_2^- or with Fe_2^+ produce the extremely reactive hydroxyl free radical ($OH\cdot$). There are further enzymes such as catalases and peroxidases which reduce this to water (Feeney and Berman,1976).

Many of the free radicals are capable of acting on biomembranes. They act specifically on the polyunsurated fatty acid forming

lipid free radicals which, in the presence of oxygen, become lipid peroxide radicals. Allison (1965) showed that the permeability of lipoprotein membranes of cells was increased due to the action of these lipid peroxide radicals . The radicals are highly reactive and start a chain reaction with an adjacent polyunsaturated fatty acid molecule. This is termed auto-oxidation and is self perpetuating in the presence of oxygen, being the leading cause of membrane damage.

A third line of defence is able to intercept and terminate the chain reaction protecting the membrane from further damage, this consists of free radical scavengers, notably alpha-tocopherol (vitamin E). Lipid peroxide free radicals may also decompose forming highly reactive fragments such as the malonaldehyde. This combines with cell contents to form high molecular weight fluorescent polymers, simultaneously immobilising functionally important enzymes in the cellular and subcellular membranes. The chemically damaged cell organelles are autophagocytosed by the cells lysosomal system but fragments known as lipofuscin or age pigments remain.

It can be seen that the antioxidant superoxide dismutase plays a large part in the prevention of damage by oxygen free radicals. SOD is found in all aerobic organisms (Fridovich, 1978). Specific types are localised in either mitochondria (copper-zinc SOD) or cytosol (manganese or iron SOD).

Bougle *et al.* (1982) studied the levels of SOD in kittens exposed to high inspired oxygen levels. The kittens were in three groups; one group was treated with a 72 hour exposure to an atmosphere containing 80% oxygen, the second similarly but with a pre-dose of lOO mg of alpha-tocopherol and a final group acted as a control. The retinae obtained from the litter mates who were not exposed to high oxygen levels exhibited greater SOD activity than those of the two hyperoxic groups. The pretreatment with vitamin E particularly prevented this drop in SOD levels.

In infants (and animals) with various premature disorders (e.g. broncho-pulmonary dysplasia), the level of SOD can be a possible guide to prognosis. According to Crappo and Tiernry (1974) if the SOD outcome is low, the prognosis is usually poor. They suggested that there is a relationship in the newborn retinae between a decreased SOD activity and hyperoxia induced lesions. The fall in SOD activity seems to reflect an inadequacy of tissue defence against hyperoxia and an increase in free radical concentrations related to it. The lower SOD activity may be related to alterations in the cell membrane through lipid peroxidation by the free oxygen radicals.

It is speculated that the partial prevention of drop in SOD activity by the use of vitamin E provides an explanation for the partial prevention vitamin E provides against ROP (Hittner *et al.* 1981).

Another drug which may be effective in a similar role to SOD is

D-penicillamine (Lakatos *et al.* 1982). In a clinical study they showed that D-penicillamine in large doses reduces the instance of severe ROP. It is suggested that its action, like SOD, protects biomembranes against oxygen toxicity. Further tests are necessary to clarify effectiveness and safety of the regimen although this initial report is interesting.

2.7 The Effect of Vitamin E in ROP

One role of vitamin E (alpha-tocopherol) has already been discussed in association with SOD. Its role is a dietary supplement acting prophylactically against ROP has also been the subject of clinical study. Premature infants have low levels of fat soluble vitamins (such as vitamin E) due to malabsorption of fats. This, combined with a need for more vitamin E when vitamin A and iron are being supplemented, was the initial reason for the addition of vitamin E to the infant diet.

Owens and Owens (1949) were the first to study the efficacy of vitamin E in the prevention of ROP. Their results were not statistically conclusive. They reported that "so far results in the prophylaxis of RLF have been encouraging". Phelps and Rosenbaum (1977) showed that vitamin E treatment was effective in reducing the extent of the retinopathy using a kitten model.

Recently there have been several clinical trials studying the use and effectiveness of vitamin E within the premature ward. Hittner and her associates (1981,1983) are the main advocates of vitamin E therapy. They administered vitamin E by mouth in pharmacological doses (100 mg/kg body weight), to a treatment group and a dose of 5 mg/kg/ day to a control group. The infants were divided between treated and control group on the basis of a random number table. When multivariate analysis was applied to both control and treatment groups, the severity of the ROP was

found to be significantly reduced in the treated groups. The study was continued, although its associates considered it unethical to have a control group as they were considered to be exposed to an unacceptable risk.

Finer *at al.* (1982) formed an independent double blind randomised control trial using intra-muscular injected vitamin E as opposed to Hittner's oral dosage which resulted in much higher plasma levels of vitamin E. The trial concluded that while not affecting the frequency of ROP, early administration of ROP significantly reduces the severity of subsequent ocular damage. A third study was in progress simultaneously (Puklin, Simon and Ehrenkraz, 1982) again using intramuscular injections of vitamin E. They found that initially the treatment group of infants appeared to fare no better than the placebo group of infants, but later, when the statistics were revised in 1982, efficacy was demonstrated. These data (and ultrastructural evidence) were examined by Hittner *et al* . (1983) which led them to conclude, "(the data) suggest the need for uninterrupted administration of the vitamin E to obtain plasma vitamin E levels greater than 1.2 mg/100 ml from the first day until the eighth week of life".

The opinion on the benefits derived from vitamin E therapy is not unanimous as highlighted by Phelps (1983) who studied the methods and statistics employed. Phelps combined the statistics on the incidence of blindness and found that the chances of disproving the effect of vitamin E is very low. Further studies may show that the vitamin is effective in reducing the incidence

of ROP but the strength of the statement is not great "because of the manoeuvres required to make it" (Phelps, 1982). This would be acceptable if the treatment is considered to be completely without unpleasant side effects, but several observers have indicated their concern over the safety of the procedures (Phelps, 1983; Kalina, 1982; Hillis, 1982).

Much of the concern is about the possible effects of the increased vitamin E levels on the incidence of necrotizing entercolitis (NEC), a disease of the small intestine and colon. Vitamin E may also affect the occurrence of haemorrhages in the central nervous system as it can induce and enhance bleeding in vitamin K deficient adults (Phelps, 1982). Both Hittner *et al.* (1983) and Puklin *et al.* (1982) studied the morbidity and mortality within the groups and found the difference between the groups insignificant. Unfortunately, it seems that different opinions can be formulated with the different forms of statistical analyses and we must wait further carefully planned clinical studies to give conclusive evidence. At present a large body of the medical profession, possibly remembering the changing views on oxygen treatment and ROP, withhold support of a universally prescribed vitamin E supplement.

Phelps (1979) studied the actual effects of vitamin E on the immature eye. They suggested two modes of action: firstly direct antioxidant effects during periods of hyperoxia and secondly a suppression effect on the formation of new vessels in response to retinal ischaemia (post-retinopathy). Phelps quoted

several sources of information. Vitamin E is known to impair new vessel growth throughout the body (Ehrilich, Tarber and Hunt, 1972) suppressing collagen synthesis and wound repair. Vitamin E also decreases platelet aggregation, reducing vascular thrombosis and thus possibly lessening the severity of ROP (Fong, 1976). Platelet aggregation is also reduced by an increasing regeneration of postacyclin (caused by vitamin E) which is antithrombic (Stuart, Grack and Clarke, 1981). The work on the clinical efficacy of vitamin E has been backed up by a study of the retinal ultrastructure at various stages of ROP in infants who died in the treatment and in the control groups by Kretzer *et al.* (1982) and by Hittner *et al.* (1983). Eyes from 58 infants were studied, 23 from the control and 35 from the treatment group.

The spindle cells and mesenchymal cells were observed ultrastructurally as they progress in the rearguard posterior to the differentiating endothelial cells. Between these spindle cells there are gap junctions which are low resistant pathways; a form of cellular interaction. (Hertzberg, Lawrence and Gillula, 1981). The hypothesis of Kletzer *et al.* stated that the spindle cells are the sight of initial injury in ROP and that this is seen infrastructurally as an oxidative induced increase in gap junctions. The spindle cells are minimally gap junction linked for up to the first 4 days of life despite the change from hypoxia *in utero* to the hyperoxia of oxygen therapy. After this the gap junctions increase and this is accentuated by oxygen and suppressed by vitamin E. These changes remove the spindle cells

from the normal vasoformative process and apparently potentiate further neovascularisation at the posterior of the shunt which is seen clinically approximately 8 weeks later.

From this study it was assumed that the gap junction is linked with the extent of hyperoxic treatment and the extent of ROP yet to develop. The protection afforded by oral administration of vitamin E is maximal in infants who have a gestational age greater than 26 weeks and is much less effective in infants younger than this. The results also suggest that this explains the constant incidence of ROP in the population.

2.8 Prostaglandins and the Retinal Vasculature

Prostaglandins are a group of naturally occurring fatty acids. There are four types - F, E, A, and B, - and these are subdivided accordingly by the side chains. They can stimulate contractility of smooth muscle and have the ability to lower blood pressure and to effect the action of certain hormones. They are of interest as they affect the blood flow and calibre of the vessels within the retina. Indomethicin is an inhibitor of prostaglandin synthesis and has been used for closure of patent ductus arteriosis in low birth weight infants. They help the ductus arteriosus to close as it complicates the course of prematurely born infants with respiratory distress syndrome (RDS). Prostaglandins affect the smooth muscle tone of the ductus arteriosis (Friedman *et al.* 1976), and indomethacin as a prostaglandin inhibitor causes constriction and closure.

There is a similarity between the retinal vessels and the ductus arteriosus in their unique sensitivity to oxygen. Exposure to high PaO_2 continuously results in a vasospasm that is eventually irreversible. Schrager (1978) postulated that as the ductus is affected by the indomethacin retinal vessels may also be constricted leading to an increase incidence of ROP. Gersony *et al.* (1983) and Procianoy *et al.* (1980) have studied the effects of indomethacin and both suggest that the use of indomethacin for patent ductus arteriosus closure does not increase the incidence of retinopathy in low birth weight infants. Garsony continues to

say that indomethacin's inhibition to prostaglandin E, as a vasodilator, may cause vasoconstrictive effects on the retinal vessels which are beneficial and reduce the risk of ROP. This view is directly opposed to that of Schrager who advocates the testing of prostaglandin E supplements in hyperoxic animal experiments to see if it will maintain the intact retinal circulation. This is obviously a controversial point.

Aspirin (salicylic acid) is also a prostaglandin inhibitor and Flower and Black (1981) studied its effect in therapeutic levels in beagle puppies subject to hyperoxia. This study goes some way to determining whether vasoconstriction or vasodilation is more beneficial in hyperoxia. Aspirin inhibits prostaglandin synthesis and irreversibly inhibits platelet thromboxane production. In the oxygen exposed puppies, those that were administered aspirin developed a significantly more severe retinopathy than their unmedicated litter mates. A control experiment was performed showing that aspirin alone produced no reversible untoward effect on the immature vessels.

Biomedical studies were performed (on the aorta, as the retinal vessels do not compose sufficient bulk) showing the marked inhibition of vascular prostacyclin and platelets from thromboxane production in the oxygen and aspirin treated puppies. Thromboxane is a vasoconstrictor and prostaglandin a vasodilator, the relative potencies of which are unknown. Techniques employing direct visualisation of the puppy fundus showed vasodilation in hyperoxia when treated with aspirin as

opposed to usual vasoconstriction. This suggests that the effect of aspirin is mediated predominantly by prostacyclin vasodilation. The puppies that were treated with oxygen and aspirin had no vasoconstrictive response and developed a more severe retinopathy suggesting that retinal vasoconstriction may be a normal physiological mechanism to protect the immature retina from high oxygen tensions. Thus vasoconstriction in the retina may be a protective rather than a pathological process in response to hyperoxia. As the retinal vasculature develops *in utero* there is a shift away from total dependence on the adjacent choroidal and hyaloid blood flow for tissue maintenance. The retinal blood flow must be modulated during this period and the vasoconstriction seen may only be an extreme of a normal physiological response. The modulation would protect the new developing vessels from too great a transmural pressure while they are still structurally incomplete, and controls tissue PO_2, PCO_2 or even pH during the transitional period.

At birth, the retinal blood flow changes markedly. The partial pressures of the blood gases change (from PaO_2; 25 to 70 torr; $PaCO_2$, from 45 to 35 torr) and the blood pressure rises (from 25-3O torr to 35-40 torr). Some degree of constriction would control these changes, resulting in a clinically normal level of retinal vasotonia. If this vasoconstriction did not occur sufficiently, there would be inadequate vasotonia leading to spontaneous retinopathy in supposedly normal levels of oxygen.

Flower and Black (1981) acknowledged the possibility that factors other than oxygen may contribute to the pathogenesis of ROP. If the vasoconstrictive process is functioning correctly, the contact between immature vessel walls and high oxygen content blood would be greatly reduced and the transmural pressures at near normal levels.

Flower's study not only elucidated the role of prostaglandins in the immature retina but also strengthened the case for continued confidence in the relevance of animal studies by producing a cicatricial retinopathy in several of the most severely affected aspirin treated oxygen exposed puppies. This answered the major criticism directed at animal models of ROP.

2.9 The Effect of Carbon Dioxide on Immature Retinal Vessels

In his reports of experimentation on beagles Flower and Black (1981) mentioned that the litter of puppies with the most severe retinopathy were those that experienced elevated carbon dioxide levels ($PaCO_2$) during oxygen administration. Bauer and Widmayer (1981) and Bauer (1982) interpreted the experiment as showing vasodilation rather than vasoconstriction as a pathological process in ROP and suggest that CO_2 levels may be a causative factor. The ROP and cicatricial changes are separated, the former being attributed to hyperoxic injury and the latter due to hypoxia (Phelps and Rosenbaum 1982) or hypercarbia. Bauer (1982) provides clinical support for the role of $PaC0_2$ in the pathogenesis of ROP finding the highest $PaC0_2$ and hypercarbia associated with simultaneous hyperoxia the most important variables in distinguishing those infants who develop cicatricial changes from those who do not.

In looking for a reason for the increase in $PaCO_2$ in the retina, the indirect effects of oxygen administration have been implicated (Wolbarst *at al.* 1983). It is suggested that as the blood oxygen tension increases the choroidal circulation is able to supply the whole of the retina's metabolic needs with dissolved oxygen (as compared with haemoglobin bound oxygen). This raises the inner retinal oxygen tension close to the

haemoglobin dissociation level thereby reducing its ability to bind with carbon dioxide which is normally carried from the retinal tissue as carbamino-haemoglobin. In addition, due to the hyperoxic conditions, the retinal vasculature constricts, blood flow decreases and regions of the retina become non-perfused further impairing carbon dioxide removal. This increases the $PaCO_2$ and thus decreases pH. This decrease in pH reduces the amount of bicarbonate function which in turn hinders another path of carbon dioxide removal and exacerbates the problem. Finally the pH may become low enough to induce vasodilation which Wolbarst *et al.* (1982) suggested causes proliferation, quoting Wolbarst, Landers and Steffansson (1981) as a reference from their work on diabetic retinopathy.

To counter this increase in carbon dioxide and its associated effects, it would possibly be beneficial to administer a carbon dioxide buffer such as tri-hydroxy-methyl aminoethane (THAM) (Wolbarst *et al.* 1982), although this has not as yet been clinically tested.

Gole and Gammon (1983) criticise the above speculation on the role of carbon dioxide in cicatricial changes, citing the following facts:

1. That Carbon dioxide has a high diffusion coefficient and would diffuse to the high flow choroidal system.
2. That any drop in pH would increase the Bohr effect, causing haemoglobin to unload oxygen and take up carbon dioxide.

Contrary to this a greater diffusion rate of carbon dioxide necessitates an increase in concentration gradient. Consequently the inner retinal PCO_2 must rise and the Bohr effect may not be exibited as it is mediated by the oxygen tension (which is high) as well as the pH (Wolbarst *et al.* 1983). The series of pH-dependent haemoglobin oxygen dissociation curves are all nearly coincident at these tensions so a drop in pH has a very small effect.

Possibly the most important suggestion of the pro-carbon dioxide causing cicatricial changes is that vasodilation can cause neovascularisation. The results of Flower and Black (1981) can be cited to strengthen this case and if correct this means a major turnabout in thought on the pathogenesis of ROP.

2.10 Anomalies in the Occurrence of ROP

Oxygen has been strongly implicated in many reports of ROP as a causative factor. Having said this, since the first evidence of oxygen's role in the pathogenesis of the lesion there have been reports of cases where cicatricial changes have developed in the absence of oxygen therapy (Schulman, 1980; Brockhurst 1975; Karlsburg, Green and Patz, 1973). One particularily interesting case is reported by Naiman *et al.* (1979) where an infant whose PaO_2 never exceeded 91 mm Hg due to a cardiac defect showed some stages of ROP on autopsy. These results point to other factors involved in the pathogenesis of ROP.

Interuterine anoxia has been suggested as having a role by making the retina "hypersensitive" to oxygen conditions *ex utero,* (Editorial, 1974). This editorial speculated whether other conditions have been mistaken for ROP. Retinal vasoproliferation is a non-specific secondary reaction so neovascularisation could occur in full term and non-oxygenated infants because of a variety of conditions such as Coats disease, Norrie's disease, hydrocephalus, calciform retinal detachment, intraocular haemorrhage and congenital retinal detachment.

Flower and Black (1981) suggested in their study that vasotonia varies in certain infants and those developing ROP without oxygen administration could have inadequate vasotonia for protection of structurally immature vessels at birth, suggesting that when the arterial blood oxygen tension and blood pressure rise suddenly, the

retinal vessels are damaged.

The variation in susceptibility to ROP is also of interest. Aranda and Sweet (1974) report cases where infants exposed to high levels of oxygen therapy do not develop any eye abnormalities and they concluded that these findings suggested that there may be one or more factors other than oxygen operative in the aetiology of cicatricial ROP .

2.11 Discussion

The formation of the vascular system of the retina is unusual in that a primary blood supply is created only to be remodelled and partly destroyed in favour of the adult pattern. This change of form occurs owing to endothelial cell contraction, using retractile protein filaments, eradicating some capillaries and enlarging others into major retinal vessels. The precise pattern of the final blood supply is dictated by this reaction which is thought to be mediated by the concentration of oxygen in the surrounding vessels. This mediatory effect becomes somewhat chaotic if the oxygen concentration is not at normal physiological levels. The threat posed by added oxygen after this interruption in normal development can be studied in the two following ways.

The older school of thought studies the effect of raised oxygen levels directly on the cells composing the vascular system. If the vascular system is compromised by damage due to oxygen toxicity, once the excess has passed there will be a lack of oxygen. The retina will thus become ischaemic and vasoproliferation will follow (in a similar fashion to diabetes mellitus only with a more rapid onset and worse prognosis). High oxygen concentrations damage cells in several ways; particular attention has been given in the cause of ROP and to the action of the free oxygen radical and the ways that it can be combatted. Superoxide dismutase appears to have some efficacy here. Penicillamine and vitamin E also act as free oxygen radical scavangers, as well as reducing the extent of proliferation (though little is known of the effects of

penicillamine). Clinical trials appear to be needed to establish whether SOD and penicillamine can be used prophylactically. Vitamin E has been the the subject of quite extensive study and there is still doubt as to whether the benefits derived (which appear to be slight) outweigh the possible adverse effects.

More recent thoughts on the subject centre on the role played by vasotonia. The vasoconstriction exibited by the eye is considered to be a protective function rather than part of a pathological process. Thus what was thought to be the initial phase of ROP is actually a defensive process. The constriction aims to protect the vessels from increased PaO_2 and increased transmural pressures. If the vasoconstriction was insuffficient the toxic effects due to oxygen would be greater and the vessels could be effected by increased pressure within them, causing damage and possible proliferation. As the oxygen concentration rises, the carbon dioxide levels increase, lowering pH values of the blood; this has been been shown to cause vasodilation and thus proliferative retinopathy. An agent (such as a prostaglandin) which controls the amount of vasoconstriction/vasodilation could be used to avoid the high PaO_2/low pH situation.

Thus it might be possible to reduce the risk of ROP in two ways. Unfortunately neither method is clear in its action or practical and effective at present. The answer may now be beyond the scope of clinicians, the real progress being made at a cellular level. Perhaps the answer is more likely to be attained by cytological and

biochemical researchers.

Perhaps the best solution to the problem is the prevention of the factors associated with premature birth therefore minimising the numbers of vulnerable infants.

CHAPTER 3

REASON FOR THE STUDY

3.1 The Incidence of the Retinopathy of Prematurity

Despite advances in neonatal care, ROP remains a problem in infants born prematurely. According to Phelps (1981) its incidence would appear to be rising In the USA. In the United Kingdom there appears to be little relevant information since the 1950s until the recent study by Fielder, Ng and Levene (1986) who studied the age of onset of the disease in a retrospective study. Also in 1979, Keith looked at the incidence of ROP in his work that preceded his classification of ROP.

Many early surveys were carried out into the incidence of ROP in the hope that a common factor might be found which could lead to identification of the cause or a treatment for the disease. Many of these early studies are not reliable or indeed comparable with each other as the diagnostic criteria had not been clearly defined. Some reports therefore included cases with severe secondary damage while others included all cases with both early and late changes.

Zacharias (1952) reported the incidence in general to be rising but commented on quite large fluctuations in numbers. He gave a table of incidence of visually handicaped premature babies as

found in the 'Boston Lying-in Hospital' for the years 1938-1951 reproduced below:

Year	No of infants	No of cases diagnosed	% Incidence
1938	22	4	18 %
1939	25	1	4 %
1940	32	1	3 %
1941	34	0	0 %
1942	37	4	11 %
1943	35	8	23 %
1944	34	9	26 %
1945	37	12	32 %
1946	55	8	14 %
1947	40	6	15 %
1948	41	11	27 %
1949	65	9	14 %
1950	65	4	6 %
1951	66	9	14 %

This immediately highlights the incompatability of results as from 1938 to 1948 the diagnosis of cicatricial disease or retrolential fibroplasia (RLF), as it was then known, was made on cases that showed partial or complete occlusion of the pupil. For

the last 3 years of the study, 1949-1951 it was made on cases which reached stages 5 or 6 of the Kinsey and Chisholms classification (1951).

Zacharias, in the same report, outlined figures found by others. For example, Krause (1951) found an incidence of 7% from 1937 to 1946 and it then rose sharply to 40% in 1949. Ryan (1952) stated that there were no cases in Australia until 1948 and that for the years 1948-1950 the incidence of RLF in prematures weighing 1588 g or less was 17%. Rees and Blodi (1951) stated that in the proceeding 10 years RLF had become the most common cause of infant blindness in the USA and Rees estimated that one third of all blindness in pre-school children was due to RLF. Bembridge *et al.* (1952), at that time, regarded RLF as the largest single cause of blindness.

These few examples of the many available at that time illustrate the difficulty in ascertaining reliable figures for the actual incidence. Interestingly all the researchers so far mentioned appear to have recorded a high incidence in 1948.

In 1951 Boyd and Hurst (1955) carried out a survey into the incidence of RLF in England and Wales for the preceding year. They investigated the incidence with respect to several factors; weight, place of birth, sex, multiple births and region. Of the 6926 infants, 1.83% were diagnosed as having RLF. Only infants with a birth weight of 2000 g or less were included in the survey. This figure is noticeably lower than the incidence obtained by

Zacharias for the same year, even bearing in mind that her survey included a lower birth weight cut off (1800 g)

Silverman, Blodi and Locke (1952) published the results of a survey covering the period December 1949 to December 1951 looking at the incidence of RLF in a New York nursery. In this 2 year period 437 infants were admitted and 280 discharged alive. The incidence of the disease was found to increase in the second year, 27% as opposed to 11% in the first year. The nursery admitted all babies who were born with a birth weight of 2000 g or less. Over the 2 years 75 of the surviving babies developed RLF and 19 of these were left with serious desease in one or both eyes. The criteria used by Silverman for the diagnosis were: "significant and progressive dilation and tortuosity of the retinal vessels with or without oedema in the periphery of the fundus, hemorrages or neovascularisation". This equates to an equivalent of a grade 3 ROP.

Not all results of the incidence of RLF were based on the results of examination of premature babies soon after birth, as indirect ophthalmoscopy of the preterm's retina appears not to have commenced as a possible routine in the intensive care unit until 1969. Some results such as those of Gunn Aranda and Little (1978) were based on follow up examinations of infants born within the two periods 1962-1971 and 1972-1976. They found the incidence of RLF to be 11% for the first period and an astonishing 43% for the second period although most of these cases were of the less severe form.

The aetiology of the disease still remains obscure in so far as the original concept, describing it as purely oxygen related, has recently been dismissed. One of the original researchers into the disease, Ashton (1984), has recanted on his original thoughts on the role of oxygen and admitted that adding oxygen to the inhaled gas mixture given to premature infants is only part of an overall physiological problem that can influence their normal retinal development.

Most recent research has concerned itself with the disease in its most acute form and has therefore been concentrated within the areas of surgical intervention when the retinopathy is entering the cicatricial phase. Recent research has also concerned itself with prophylactic treatment in the form of vitamin therapy.

3.2 Myopia and its Association with Prematurity

The preterm infant is, during its first weeks of life, in an abnormal situation. It cannot therefore be assumed that its development following preterm delivery parallels that which occurs *in utero*.

Ever since ROP was first described, myopia has been an occasionally reported sequel. Some of the earlier papers reporting this association with myopia are those of Fletcher and Brandon (1955), and Graham and Grey (1963)

Some reports on the incidence of myopia have supported the association with prematurity. A rigorous study undertaken by Nissenkorn *et al.* (1983) followed by studies by Shapero *et al.* (1980) and Nathan *et al.* (1985), have supported this association. Some authors such as Fledelius (1976) and Scharf *et al.* (1975), have stated that that the myopia present in early infancy may not continue into childhood.

Fledelius screened 137 18 year-old-Danes, half of whom weren of low birth weight (<2000 g). He concluded that the corneas of the myopes and hypermetropes in the low birth weight group were steeper than those of the controls; the low birth weight group also had shorter ocular axial lengths than those of the controls. Gross and Cox (1985) concluded that the steeper than normal corneas and the reduced axial lengths in eyes of premature babies reflected an 'under developed eye'.

The study by Scharre (1984) showed no statistical difference between refractive errors of matched populations of post-neonatal infants, one group being born premature and the other group full term infants. Opposed to this report, however, Nathan *et al.* reported that the myopia associated with prematurity comprised a significant group of vision-impaired children, caused by refractive errors (19 out of 496 subjects). Most studies have concentrated on the refraction of children who were born premature and examined outside the neonatal period. This does have the advantage that statistical comparisons can be made using the normal full term children as a control.

One study that was undertaken using premature babies while they were still in their preterm period was carried out by Dobson *et al.* (1981). However, this study does not relate to the findings of the present study as Dobson did not find such high levels of myopia and found less refractive variations than were found within the population studied in this report.

The literature has shown that until relatively recently it was thought that all normal full term babies were hypermetropic at birth and also that this hypermetropia decreased in amount during the early years of life. Hirsh (1964) dispelled this notion by pointing out that the majority of the data reported between 1880 and the early years of this century originated from a study reported by Herrmheiser, who used an ophthalmoscope to refract a large number of newborn infants and found that all but two had

between 1.00D and 6.00D of hypermetropia.

Hirsh then analysed a more recent study reported by Cook and Glasscock (1951). They had used retinoscopy under atropine cyclopegia and had shown a wide distribution of refraction at birth. Hirsh plotted the frequency distribution for the 375 Caucasian infants included and noted that the refractive state ranged from -7.00D to +10.00D, with 24% of the children having myopic refractions.

In a later study, Goldschmidt (1969) reported on a study of 356 full-term infants that had a birth weight in excess of 2500 g and were aged between 2 and 10 days old. These babies were refracted by retinoscopy under atropine sulphate cyclopegia; and like Cook and Glasscock, Goldschmit found a wide distribution of refraction, extending from -7.00D myopia to +8.00D hypermetropia. Of these infants, 86 (24%) had myopic refractive errors. In discussing these results Goldschmit made the point that 'development age' of babies can vary considerably, and that birth weight is only one criterion of full-term birth. He stated that during the last few months of pregnancy and the first few months of life the eyes undergo a considerable amount of development, with the result that refraction can be assumed to change during this period. Gleiss and Pau (1952) followed the refraction of premature infants and found that hypermetropia increased and myopia decreased during what would have been the final months of pregnancy.

As mentioned in the introduction, the tendency for premature infants to be myopic was first noted by Fletcher and Brandon (1955). They used an ophthalmoscope to refract 462 premature infants and found all the infants to be myopic. For infants weighing less than 1250 g at birth, myopia in the order of 10.00D. to 20.00D. was reported with wide fluctuations from week to week. For those babies weighing more than 1700 g they found that the myopia varied up to 6.00D with less fluctuation.

Banks (1980) combined the data of 10 studies on full-term babies and five studies on premature babies and came to the conclusion that the mean refraction for a 'normal' baby at the end of the neonatal period was +2.00D whereas it was +0.20D for those that had been born premature.

Other non-refractive factors that have been reported as affecting vision of the premature baby are neurological disorders and strabismus. A good synopsis of these other sequelae is to be found in a paper by Scharre *et al.* (1984).

Since this study is recent it has little in the way of follow-up but those babies that have been seen after discharge have either been emmetropic or low hypermetropes. The long-term refractive effects are discussed later, with examples of older children that have been examined because of their premature birth and their refractive disorders.

3.3 Aims

The aim of this reported research was initially based on a mistaken idea that many questions could be answered, and all at the same time. Many of of these questions were impossible to research into as babies came and went, died or had severe medical complications.

The initial proposal was to record:

1. Birth weight.
2. Age since birth.
3. Birth process (e.g. elective section, etc.).
4. Sex.
5. Ophthalmoscopic examination.
6. Retinoscopic examination.
7. Tonometry.
8. Method of controlling blood gas (e.g. head box 60% 0_2), control of blood gases and level attained.
9. Relevant medical state (disease, etc.).
10. Any other relevant information.

Factors 2,5 and 6 were monitored.

Factor seven, tonometry, was found to be almost always impossible. The procedure was abandoned for the following reasons:

(a) Very small palpebral apertures.

(b) Size of the corneas.

(c) Natural eye elevation (Bells phenomenon) that attention to the eye and adexia produces.

(d) The appreciation that the normal intra-ocular pressure was low and the eyes very plastic thus rendering it very difficult to recognise the normal from the abnormal especially in the presence of factors a-c above.

Factor eight was not difficult in its simplest form. The use of ventilation and supplementary oxygen were recorded. The time during which this study was conducted saw great advances both in technology that was available on the market and the amount and type of equipment available to the unit. Other general medical conditions also had their effect. The problems are discussed later in the results chapter 6, especially factors nine and ten and their association.

CHAPTER 4

METHODOLOGY

4.1 Ophthalmoscopy

All babies that weighed less than or equal to 1500 g at birth and a limited number of babies that weighed between 1501 g and 2000 g that were possibly at risk as they were receiving supplementary oxygen and were either born at, or transferred to the Special Care Baby Unit at Coventry Maternity Hospital between August 1982 and January 1985 had routine weekly ophthalmoscopic screening from admission to the unit until discharge, medical circumstances permitting. Those babies that were considered to be "at risk" at discharge were seen after discharge as required.

The babies' pupils were dilated using 1% tropicamide HCl administered topically from 'minims' 20 minutes prior to the examination.

There have been a few papers discussing the problems of obtaining a sufficient pupil size to accomplish indirect ophthalmoscopy on these premature infants (Merritt and Kreybill 1981). Problems arise because the sphincter muscle of the iris is poorly developed in both premature and newborn full term babies and hense antimuscarinic drugs do not produce the same degree of dilation as found with older babies and young children. Some authers recommend the use of a supplementery sympathomimetic

topically administered drug to boost pupil dilation by its effect on the pupil dilator muscle. This was dismissed after observing the reaction of a few preterm babies to a topically administered sympathomimetic, phenylephrine HCl 2.5%. It was noted that the cardiac rate always increased sometimes almost to the extent of fibrillation. The effect of lid retraction was also pronounced owing to the drug's effect on the levator muscle.

The choice of ophthalmoscope also had a significant bearing on the requirement to produce various amounts of pupil dilation.

Initially several types of ophthalmoscope were tried. All of these but one was of the binocular indirect type. Although conventional indirect ophthalmoscopes gave a reasonably wide field of view of the retinal fundus, the detail required to observe the minute changes in the area of the vascularising retina were difficult to observe. Also the light levels required at the retina were necessarily large and there was a feeling of anxiety in subjecting babies to these high light levels for routine screening. A conventional binocular indirect ophthalmoscope was however, occasionally used as required.

The final instrument of choice for screening was the American Optical Monocular Indirect Ophthalmoscope, now marketed by Reichert Jung. This instrument has a magnification of approximatley 5.5x and a field of about 15 deg. The view of the peripheral retina is extremely distortion free, light levels at the retina are considerably lower than that with the conventional

indirect ophthalmoscope and the instrument, being compact, is far easier to handle in the confined spaces of an incubator. Having one hand free I was able to both hold the subjects head steady and at the same time hold open the lids using the index finger and thumb of the left hand. Occasional additional help was given by the nursing staff when required.

A ray diagram and a further details of the monocular indirect ophthalmoscope may be found in appendix 3.

Records of the relevant clinical details of all the babies examined on each day were recorded at the time of the examination onto a portable tape recording machine and the information transferred to separate record sheets at the end of the examinations. No reference was made to the existing records prior to the examination. However many of the very young that were at risk and had had many previous examinations were automatically remembered.

4.2 Retinoscopy

The same population of premature babies that underwent ophthalmoscopy were also subjected to a routine weekly retinoscopic examination, unless other medical factors dictated otherwise.

As time was of the essence at most of these examinations retinoscopy was carried out using a limited number of trial spherical lenses: -6.00, -3.00, +1.00, +4.00. As most of the observations were of myopic eyes little use of auxiliary lenses was required. The distance from the eye that reflex movement reversal occurred was measured using a metric tape measure, the refractive error being the reciprocal of the distance measured in metres and recorded in dioptres. This procedure has the disadvantage in that a dioptric scale does not transform to a linear far point distance scale. This results in a descending level of accuracy when attempting to measure the higher levels of myopia in units of dioptres. This effect was minimised by using the auxilliary lenses previously mentioned.

The instrument of choice was a Keeler spot retinoscope. This instrument was chosen as it was familiar to myself.

Many of the eyes refracted had astigmatism. This astigmatism was in general 'against the rule', i.e. positive cylindrical lenses axis horizontal required for retinoscopic reversal and was ignored

in all cases (although noted when abnormally high, (highest 4D)). The refraction was recorded as the reciprocal of the mean linear distance between reflex reversal positions obtained from the two principal meridians and therefore recorded in dioptres. It must therefore be noted that the results are not the exact equivalent of the generally accepted definition of the 'mean sphere'. The classical understanding of the mean sphere is the dioptral centre (rather than the linear centre) of the two focal lines found in astigmatism.

As all babies examined had an antimuscarinic instilled to dilate the pupil, it also counteracted any accommodation. As stated in the section on ophthalmoscopy, the drug of choice was tropicamide HCl 1.0% and was instilled topically approximately 20 minutes before the examination. Tropicamide was chosen as stronger acting antimuscarinics are of no advantage when examining premature babies; their accommodation ability is almost non-existent.

4.3 Measurement Criteria

In this study the post conceptional age (PCA) is used as a frequent measure of age. PCA was assessed either from the mother's reported last menstural period, ultrasound scans or both. For those mothers that presented with no or unreliable information to formulate the PCA, the criteria used by the paediatrician was the Dubowitz score (Dubowietz *et al.* 1970) which is a mathematical model based on 10 neurological criteria. It must be noted that even the 'best method' is only reliable to within 1 week.

4.4 Conduct of the research

As mentioned in Chapter 2 the protocol for this study was agreed by both the relevant ethical committees the Coventry Maternity Hospital, Walsgrave and the University of Aston - and forms Appendix 1 of this report.

A total of 138 babies were examined and form the basis of this report. They were born between 18 April 1982 and 19 May 1985.

A pilot study during which examination procedures were established was carried out, the period required was of 6 months and the criteria was at that time to examine all babies that had a birth weight of less than 2000 g. All observations recorded during this period have been ignored in this report. One of the babies seen during this period acquired cicatricial changes and he, unfortunately, now has perception of light in one eye only.

This report is primarily confined to those babies that had a birth weight of less than 1500 g when examining the analysis of refractive change. Other babies weighing up to 2000 g were occasionaly seen for a variety of reasons but primarily because they had required respiratory support and oxygen. None of these other babies showed any signs of either high myopia or ROP but are included in the analysis as appropiate.

The total number of babies reported on is 138.
The total number of observations reported on is 579.

A sample of the record sheet used may be seen in Appendix 2.

A graphical analysis of the birth weights and the number of examinations carried out on babies of each birth weight group is shown in Fig 4.1.

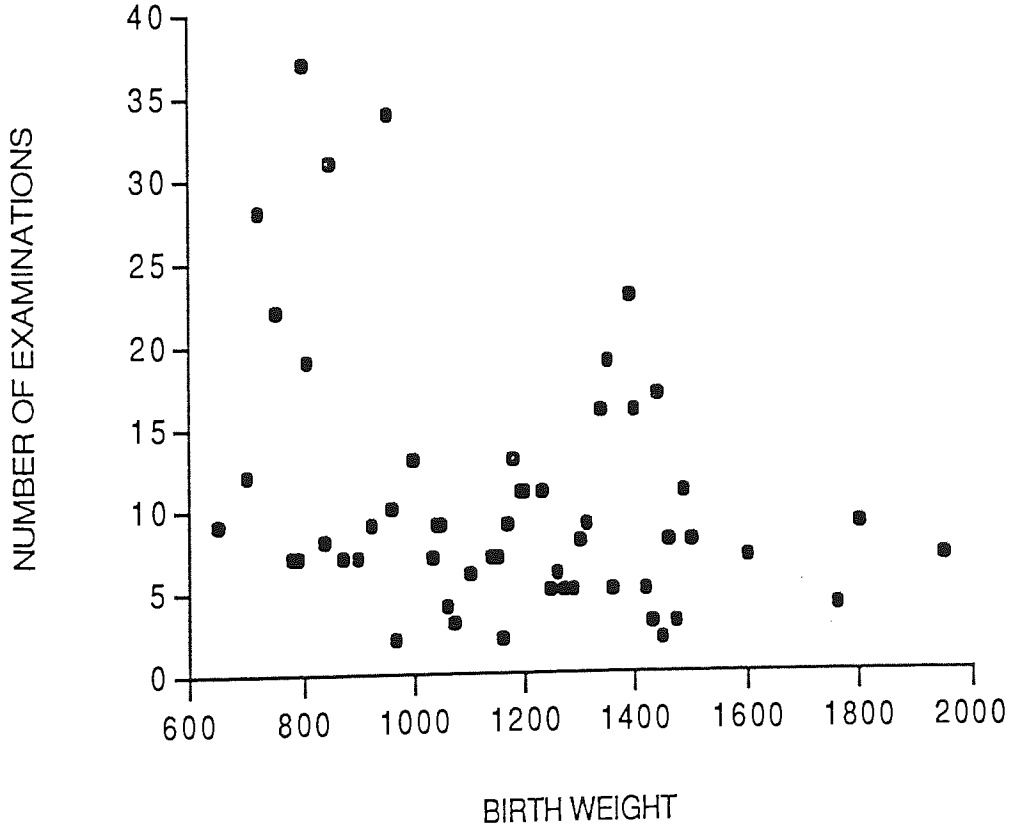


FIG 4.1 Birth weights and the number of examinations carried out on each birth weight group. The birth weight is measured in grams.

The mean of this birth weight grouping is 1099.8 g.

As the heavier babies were also had the highest PCAs, the number of examinations on each individual decreased with birth weight and PCA as they did not have as long a stay in the Unit.

CHAPTER 5

RESULTS - RETINOPATHY

5.1 Analysis of birth weigh and estimated post conceptional age (PCA)

Fig 5.1 shows the relationship between the birth weights of the total number of babies seen plotted against their estimated PCAs

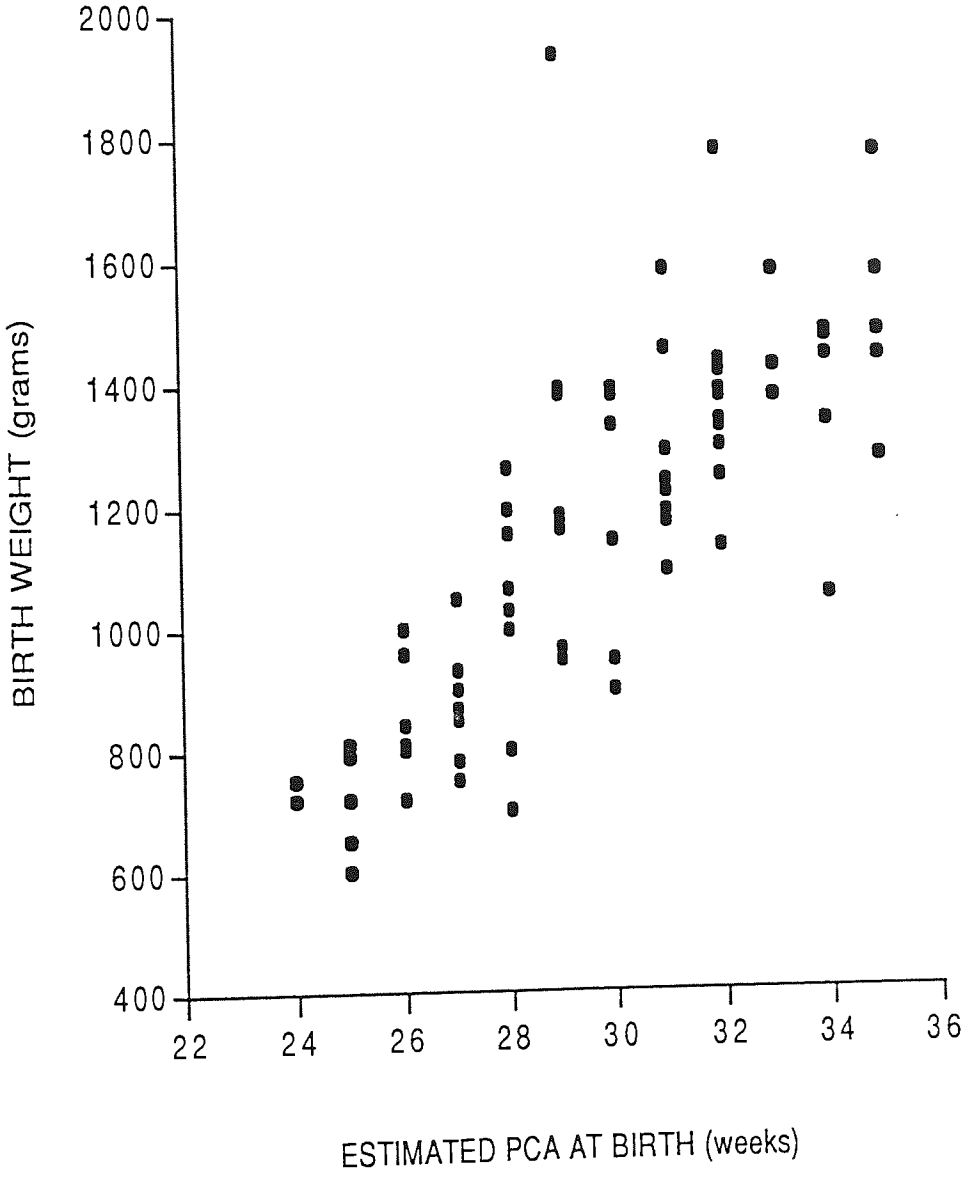


Fig 5.1 The analysis of babies birth weight plotted against their estimated gestational age at birth (PCA)

Analysis of the regression shows a best fit line that is a second-order polynomial, that is that the birth weight slightly reduces against an increase in estimated gestational age. The relevant statistics of the regression are:

$y=11.411 + 0.024x -7.092E - 6x^2$

Coefficient of Determination 0.84

Adjusted Coefficient 0.706

Coefficient of Correlation 0.84

"F" Ratio 693.0, $p = 0.0001$

5.2 Age weight and retinopathy

As stated before there were several constraints on the ability to examine babies; primarily their medical state at the time and also their age at discharge.

Fig 5.2 is a scattergram showing their PCAs at the time of the examination plotted against their PCAs at birth. The general age at discharge was between 36 weeks PCA and 46 weeks PCA.

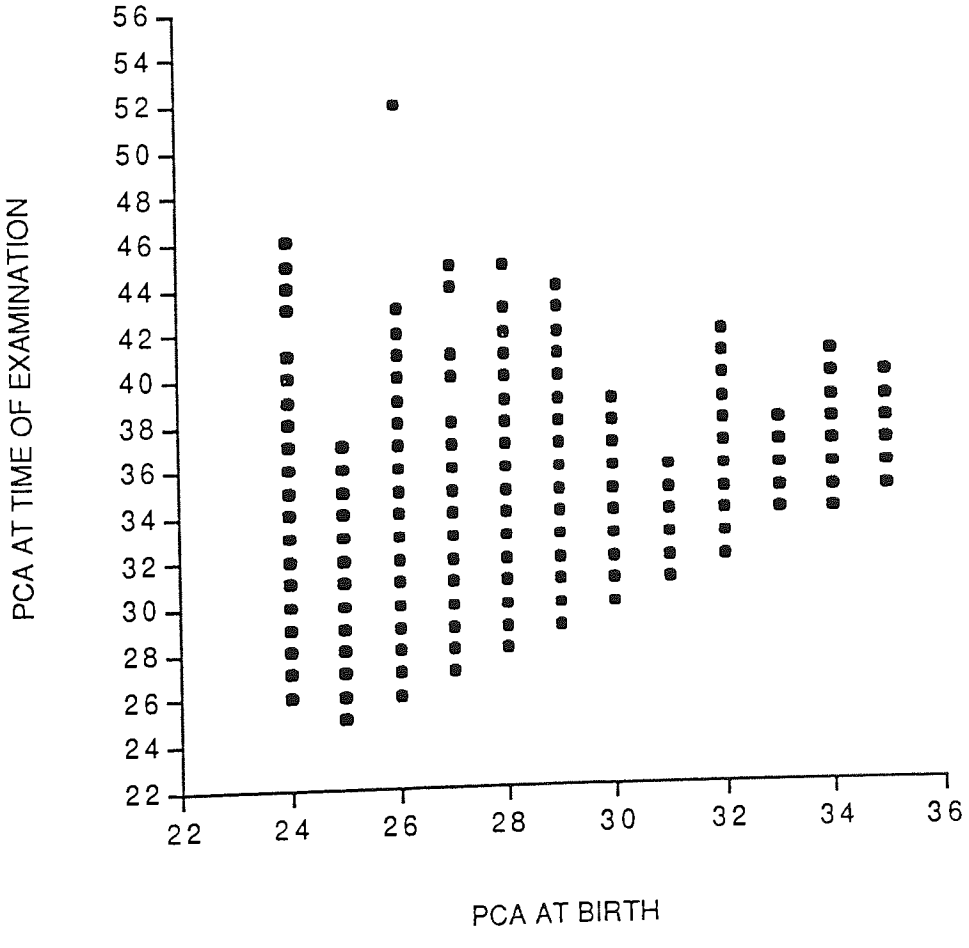
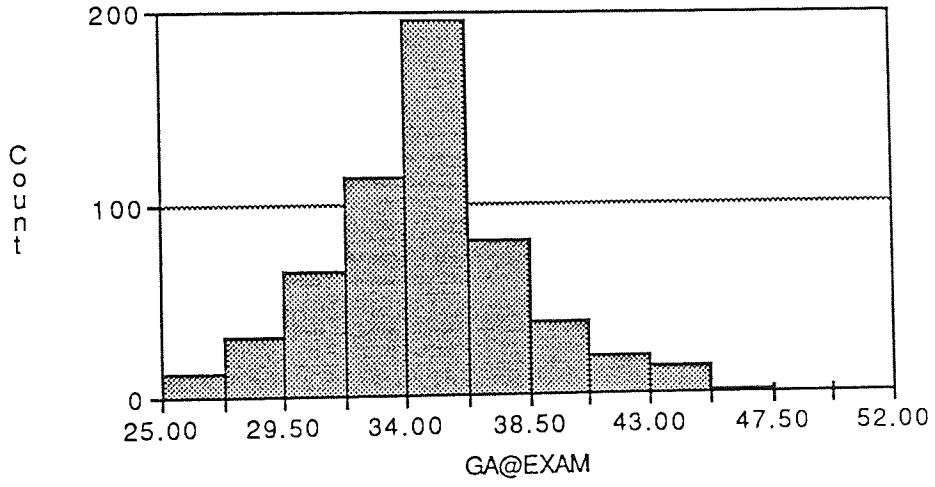


FIG 5.2 PCA at birth plotted against the PCA at the time of the examination

An alternative method of presentation to illustrate the age range at the time of the examination is to show a histogram of their PCAs when they were seen (Fig 5.3). Interestingly the mode of the age range is 32-36 weeks: It is shown later that it is within this age group that the ROP is normally at its most severe.



MEAN =34.644 SD = 3.745

FIG 5.3 Number of examinations and PCA at the time of the examination.
(Count = number of examinations and GA@EXAM = PCA at the time of the examination).

The grade of any retinopathy plotted against their PCA at the time of the examination (or gestational age) also shows the

upward trend of retinopathy starting at a PCA of 33 weeks (for a grade 2 retinopathy) and rising to the more severe grades at about 37 weeks. The plot is shown as Fig 5.4. It must, however be understood that this figure contains many overlapping points especially in the nil or lower retinopathy categories. It does not therefore represent the total number of examinations. As the "International Classification" has three different recognisable stages of Grade 3 retinopathy, recorded as 3, 3+ and 3++, these are included in the illustration. The distribution of categories of retinopathy are therefore as follows:

No retinopathy found in	397 examinations
Grade 1 found in	118 examinations
Grade 2 found in	35 examinations
Grade 3 found in	23 examinations
Grade 4 found in	6 examinations

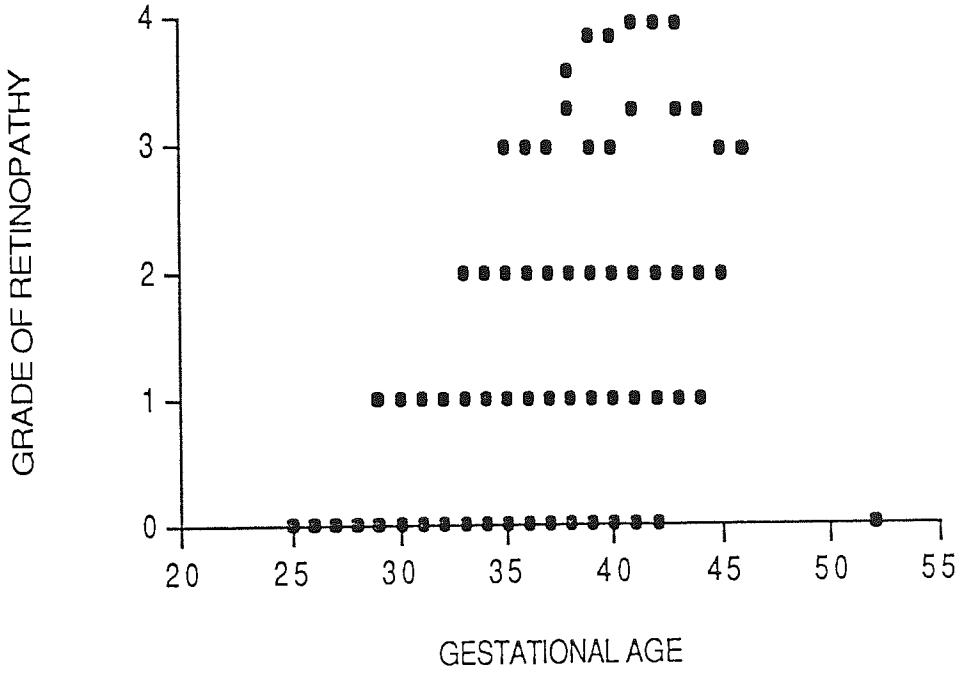


FIG 5.4 Grade of retinopathy found plotted against the PCA (Gestational age in weeks) at the time of the examination.

As was expected, there was an association between the maximum grade of retinopathy observed and birth weight. The regression line obtained when calculating this reduction of retinopathies with increased birth weight is shown in Fig 5.5. The statistics of this line are as follows:

linear regression $y = -0.001x + 1.571$

$R = 0.407$ $R^2 = 0.166$ $F=18.7$ $p= 0.0001$

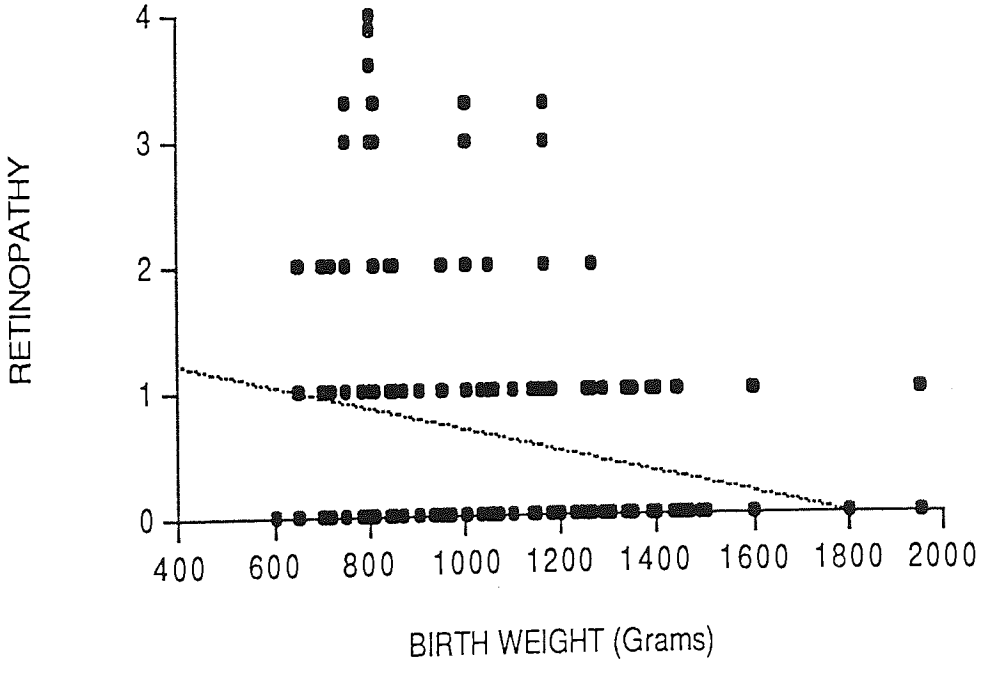


FIG 5.5 Grade of retinopathy observed plotted against the birth weights of the total population. One dot represents one or several observations.

If all those babies that did not show a retinopathy of any grade are eliminated and the maximum grade observed is plotted against their birth weight we obtain the following for the remaining 96 babies:

Grade of retinopathy	Mean weight(g)	SD	SE
1	1180	229.6	53
2	883	184	58
3	932.5	190.8	95
4	862.5	88.4	62

The linear regression line is described as:

$y = -127.834x + 1269.7$

These results are shown graphically in Fig. 5.6.

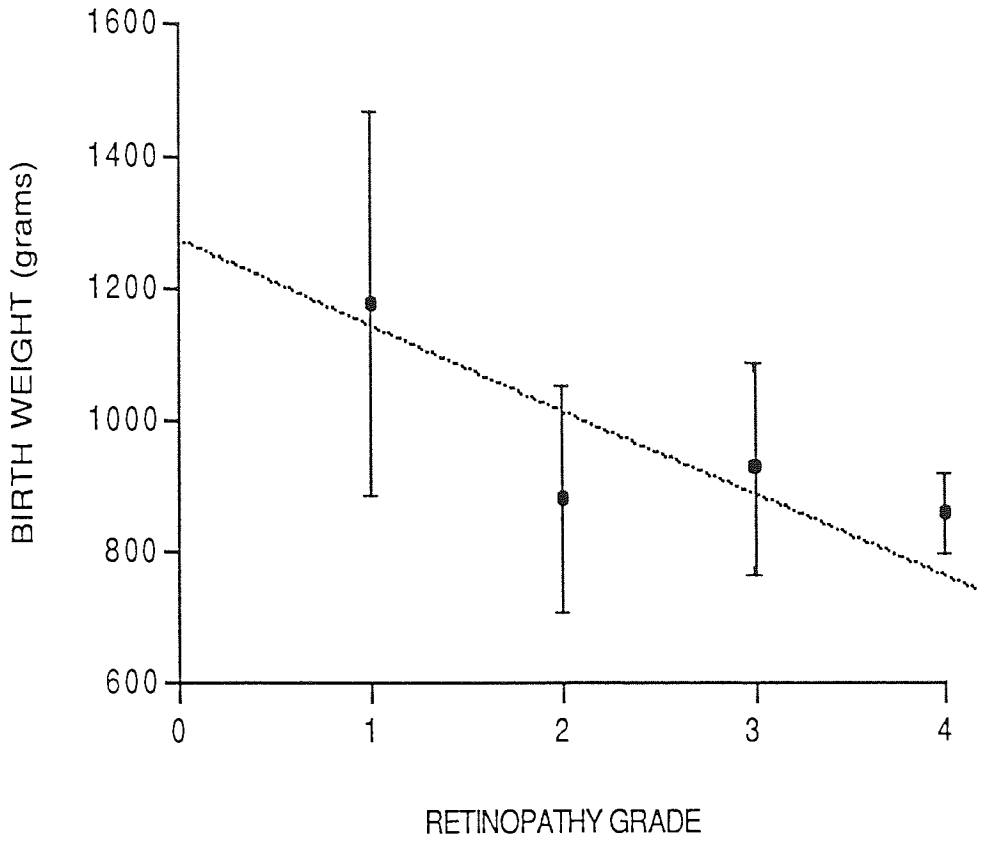


FIG 5.6 Means and standard deviations of the birth weights of those groups of babies seen with a maximum retinopathy of grades 1, 2, 3 or 4 ROP

If the same procedure is repeated using the 96 PCAs to replace birth weight, the results are very much the same and are shown in Figs 5.7 and 5.8.

The statistics of the regression line in this case are as follows:

linear regression $y = -0.085x + 3.029$

$R = 0.269$ $R^2 = 0.072$ $F = 41.3$ $p = 0.0001$

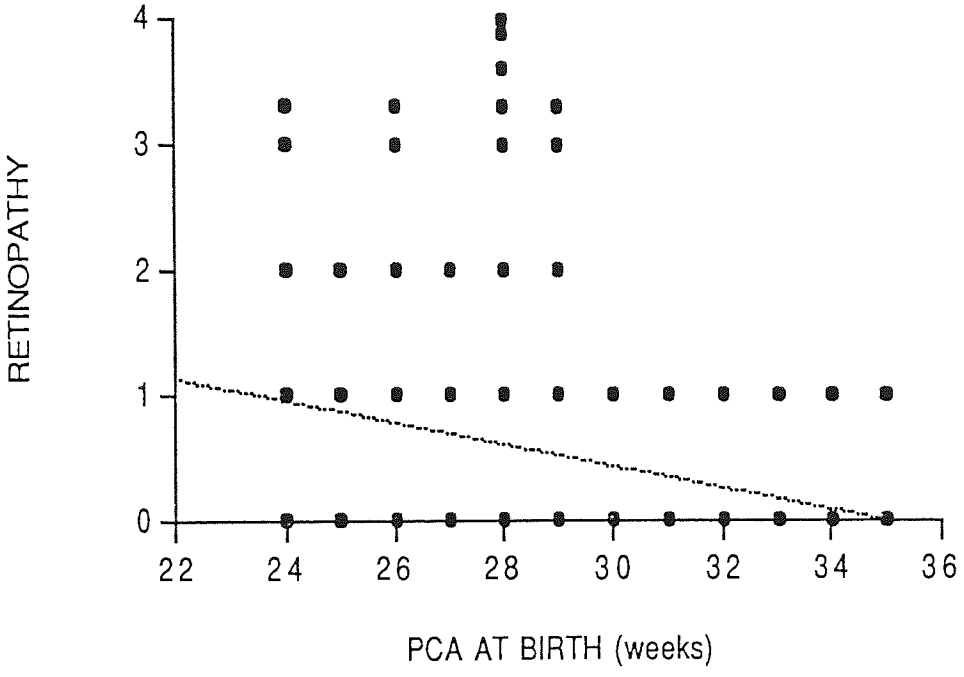


FIG 5.7 Grade of retinopathy observed plotted against the PCAs of the total population. One dot represents one or several observations.

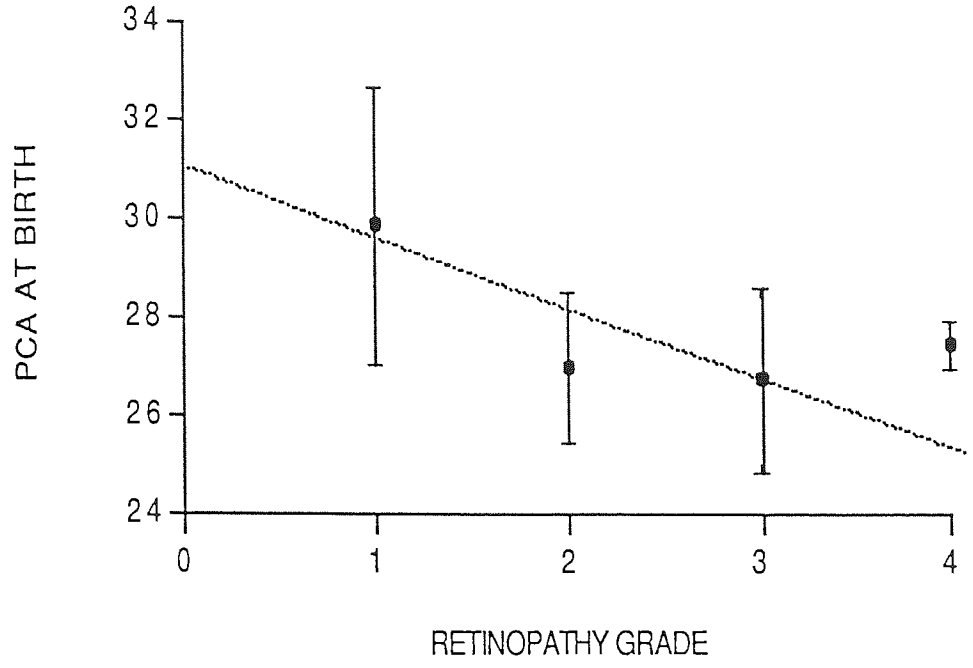


FIG 5.8 Means and standard deviations of the PCAs of those groups of babies seen with a maximum retinopathy of grades 1, 2, 3 or 4 ROP

Details of the means and standard deviations are as follows:

Grade of retinopathy	Mean PCA(weeks)	SD	SE
1	29.87	2.88	0.51
2	27	1.63	0.52
3	26.75	2.22	1.11
4	27.5	0.7	0.5

The regression line is described as:

linear regression $y = -1.406x + 31.03$

$R = 0.405$ $R^2 = 0.164$ $F = 9.03$ $p=0.0043$

Figs 5.6 and 5.8 clearly show that there is a cut-off line for babies that can be considered to be "at risk". The definition of this group is taken as those that show at some time a retinopathy that is regarded as a grade 2 change. In order to do this we have to assume that grade 1 changes are possibly not a real change but may possibly be only a natural feature of the developing retinal vasculature.

5.3 Identifying those babies that are at risk

If, from an initial total of 138 surviving patients, a criteria is set that they have to have had at least three observations extending to, or beyond the equivalent of 36 weeks PCA, and the upper limit to birth weight is set at 1500 g, the number of babies reduces to 87 in number. Of these, 29 babies weighed less than 1000 g at birth.

The make-up of this reduced population is as follows:

<=1000 g, N=29 (246 observations). Mean 8 obs (SD 4)

1001 g to1249 g, N=22 (135 observetions). Mean 6 obs (SD 2)

1250 g to1500 g, N=36(173 observations). Mean 5 obs (SD 2)

If the maximum grade observed is plotted against both the birth weight and the gestational age at birth, Fig 5.9 is the result.

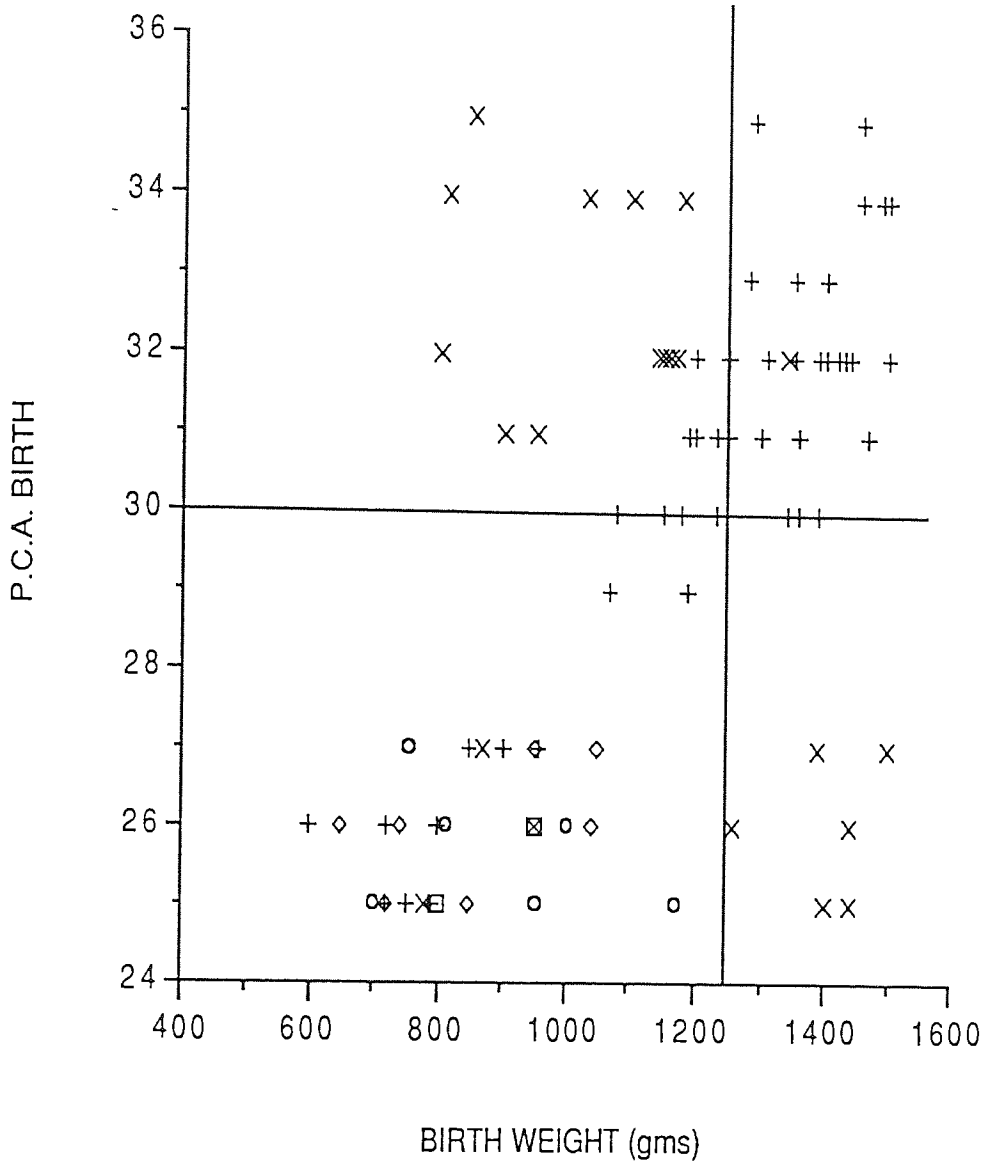


FIG 5.9 Grade of retinopathy plotted against both birth weight and PCA at birth

KEY

+ = No retinopathy

x = Grade 1 retinopathy

Diamond = Grade 2 retinopathy

0 = Grade 3 retinopathy

Square = Grade 4 retinopathy

Fig. 5.9 shows that the distribution of maximum grade of ROP observed relative to birth weight and gestational age indicater that no significant grades of ROP were found in the upper right hand quadrant, i.e. those babies that weighed more than 1250 g at birth or had a PCA of greater than 30 weeks gestation.

The two patients who developed RLF both presented initially with a grade 2 retinopathy and progressed to severe cicatricial changes within 3 weeks. It can be seen that they both weighed less than 1000 g at birth. They had both required intensive respiratory support with continuous arterial oxygen monitoring and they had also received prophylactic antioxidant (Vitamine E and Vitamine C).

These babies therefore that are identified as being "at risk" are found to have had a birth weight of less than 1250 g or a gestational age at birth of less than or equal to 30weeks PCA.

In this group of 51 subjects the overall incidence of ROP was found to be 60%, 12% had grades 3 and 2% had cicatricial changes, grade 4.

In those other patients that fell outside these parameters the overall incidence of ROP was 24% confined exclusively to minor changes (grade 1 and early grade 2 changes).

Irrespective of birth weight or gestational age, ROP if present consistently presented at 35 weeks. PCA (SD 2.25).

Except for the two subjects who developed cicatricial changes and presented initially with grade 2 disease, the initial observation was grade 1 and 14 patients progressed to grades 2 or 3 ROP. However, seven of these showed regression by at least one grade on discharge.

5.4 Age of onset of first detectable change

Table 5.1 below shows the age of onset when the first detectable retinal change was observed according to birth weight group.

Group	Mean birth weight	Mean onset
<=1000 g	812 (SD 99)	34 weeks (SD 2) n= 20 / 29
1001 g to 1249 g	1140 (SD 68)	35 weeks (SD 2) n=11 / 22
1250 g to 1500 g	1.39 (SD 77)	36 weeks (SD 1) n=7 / 36

For all patients who presented with any grade of ROP mean age of onset was 35 weeks (S.D.2.25)

Table 5.1 Birth weight and age of onset of first detectable change

Table 5.2 shows the grading of ROP by the subjects PCA at delivery. In all subjects who had a retinopathy the maximum grade observed occurred within 5 weeks of the first detectable change.

No onset was detected before the age of 32 weeks PCA (1 case only), or after 38 weeks (1 case only).

Grade	<= 30 weeks	>30 weeks	
0	17 = 37%	32 = 80%	(p<0.01)
1	15 = 32%	7 = 18%	(p <0.01)
2	7 = 15%	1 = 2%	(p<0.01)
3	6 = 13%	0	(p<0.01)
4	2 = 4%	0	(N.S.)

Chi^2 with Yates correction for small numbers.

Table 5.2 Grades of ROP by PCA at delivery

To illustrate the reduction of retinopathies seen when the babies mature, Table 5.3 shows the ROP gradings at 40 weeks PCA.

As previously mentioned the two patients who first presented as grade 2 disease both progressed rapidly to grade 4 (cicatricial disease) within 4 weeks. Of those presenting with grade 1 disease, 23% increased to higher grades but all had regressed by one grade at discharge.

Grade	<=30 weeks	>30 weeks
1	16 = 33%	6 = 15%
2	4 = 8%	0
3	0	0
4	2 = 4%	0
	(4 patients lost)	(2 patients lost)
	(to follow up)	(to follow up)

Table 5.3 Grades of ROP at 40 weeks PCA

5.5 Discussion on the relevance of type of baby screened to detect ROP

The results of this prospective study complement the conclusions of the retrospective study recently published by Fielder, Ng and Levene (1986).

The importance of low birth weight is stressed in the light of paediatricians' inability to determine gestational age at birth accurately, but agree with Fielder *et al.* (1986) that post-conceptional age is the probable key to the development of ROP.

To this end an analysis of variance was carried out to establish the relative significance both of birth weight and of estimated gestational age as a predictor of ROP. (Table 5.4).

F test weight	4.077	p = 0.122
F test PCA	4.395	p= 0.0086

Significant at the 95% level Fisher PLSD: Grades 1 and 2 (weight)

Significant at the 95% level Fisher PLSD: Grades 1 and 2 (PCA)

Significant at the 95% level Fisher PLSD: Grades1 and 3 (PCA)

Table 5.4 Analysis of variance, weight versus PCA and grade of retinopathy

The data therefore support a recommendation that all infants

equal to or less than 30 weeks gestation or less than 1251 g birth weigh require routine ophthalmoscopic examinations from 33 to 37 weeks PCA and if significant changes occur (grades 2, 3 or 4) continued surveillance as indicated.

5.6 ROP - Other Factors

The analysis of the other factors that were recorded at the time of the examination (listed on page 71) were all found to be not significant or were recorded in such a manner as to be not be compatable with statistical analysis.

The apparent prime factor at the beginning of the study was the effects of the changes in blood gases. Initially blood gas sampling was carried out via the hospital's pathology department. They required what was, to a tiny infant, relatively large amounts of arterial blood and there was a time delay in receiving the results. There was some limited blood oxygen monitoring by means of subcutaneous sensors ($TcPaO_2$) limited for use with either the sickest baby or transferred as required around several babies.

Indwelling blood oxygen monitoring catheters (PaO_2) were inserted via the umbilical artery into the arterial system whenever possible in the very premature. The life of these catheters was varied.

Towards the end of the study the technology within the unit was very different. Not only were there more blood oxygen sensors of both types but there was also a unit blood gas sampling machine that required very small samples. The unit was also attempting to measure blood CO_2 levels via subcutaneous sensors.

Maintenance of a stable blood gas status was also affected by the amount of adult blood that had been given to the infants. Because of the difference in the haemoglobin dissociation curves in the fetal and adult blood. There being, therefore a different requirement of blood oxygen level between the two types of blood.

Many of the infants had a variety of moderate or severe medical problems. The most common were: patent ductus arteriosus, pneumothorax, cytomegalovirus infection, central nervous system incidents and catastrophies and renal failure.

An attempt was made to try to measure the individual extra handicap with a mathematical model of the many medical problems that may be a barrier to normal development. This was abandoned as it was obvious that the younger and lighter the baby the more problems they generally had. Also the problems that these infants had were not only very varied but also multifactorial.

5.7 Case Examples

Two case examples are reported as the histories and other information provide a deeper understanding of the problems of the ROP.

(1) Baby "Len"

PCA at birth	24 weeks
Birth weight	750 g
Birth	Spontaneous delivery
General medical factors	Cytomegalovirus infection, slow to put on weight. Ventilated 3/4 weeks. Head-box and oxygen for 5/6 weeks

PCA at exam	ROP grade	Other Ophthalmic factors	Myopia
26	0	Vit Haze	8
27	0	Vit Haze	7
28	0	Vit Haze	-
29	0	Vit Haze	3
30	0	Nil	4
31	0	Nil	1
32	0	Nil	3
33	0	Nil	0
34	0	Nil	-2
37	1	Nil	-2
39	2	Nil	-2
40	2	Nil	-1

41	3+	Nil	-1
43	3+	Nil	0
45	3+	Nil	0
46	3+	Nil	0
47	3+	Nil	0

Unfortunately this baby died suddenly at a PCA of 54 weeks. The post mortem stated cause of death as "Cot death". Subsequentially permission was given to remove one eye which was sent to the Birmingham and Midland Eye Hospital for histological analysis. Figs 5.9, 5.10 and 5.11 show the pre-retinal anomalous vascular development that had taken place and also the neovascularisation changes that had taken place in the iris.

The pathologist's report appears in Appendix 3.

FIG 5.10 The pre-retinal neovascularisation forming a pre retinal network of blood vessels and sinusoids. The retinal blood vessels themselves are more numerous than normal. This section was taken in the region of the the original demarcation shelf and site of neovascularisation noted at the thirty ninth week PCA and possibly shows an early retinitis proliferans.

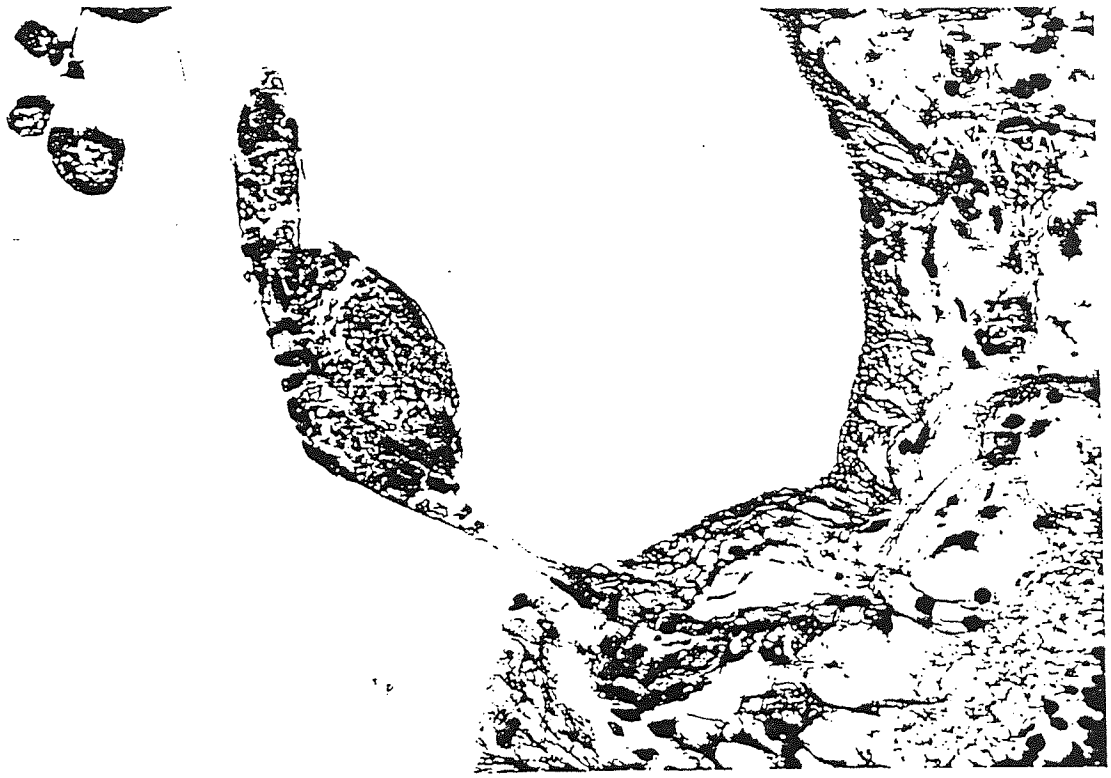


FIG 5.11 A higher magnification section of the same detail seen in Fig 5.10 to show the pre-retinal vascular development.

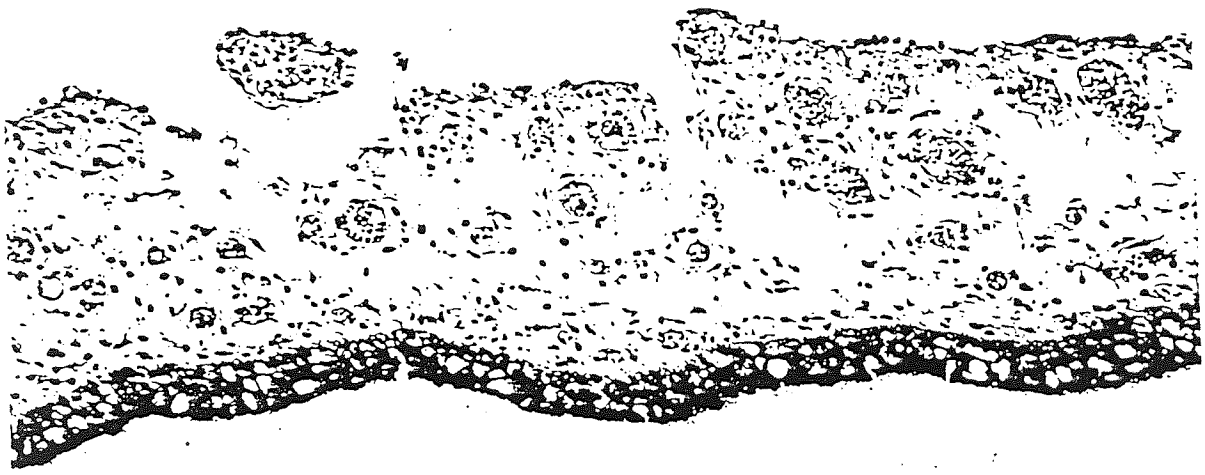


FIG 5.12 Neovascularisation of the iris.

(2) Baby "Katie"

PCA at birth	28 weeks
Birth weight	800 g
Birth	Spontaneous delivery
General medical factors	There were no special problems with this baby. She was ventilated , intubated and used a head-box was used to increase her blood oxygen level to a satisfactory level for a while.

PCA 29 weks Ophthalmoscopy not possible due to a large amount of hyaloid remnants.

PCA 30weeks Hyaloid remnants now less; Ophthalmoscopy N.A.D. Retinoscopy -4.50D.

PCA 31weeks Ophthalmoscopy: Blonde looking fundus, not fully vascularised, N.A.D. Retinoscopy -5.00D

PCA 32weeks Hyaloid remnants are still present: Ophthalmoscopy N.A.D. Retinoscopy -4.00D

PCA 33weeks Hyaloid remnants are still present: Ophthalmoscopy N.A.D. Retinoscopy -4.00D

PCA 34weeks Ophthalmoscopy:

RIGHT: Mesenchymal area with non-distinct margin from

7o'c to 1o'c outer area 2 and first signs of a ridge formation 10.30o'c.. Neovascularisation seen at the termination of the upper temporal blood vessels at the junction of vascularised retina and the avascular tissue. There was also a small flame shaped haemorrhage associated with this area of neovascularisation. Other retinal vessels terminated at the junction in what I describe as 'extra vascularisation'. Early signs of 'plus' disease were noted at the posterior pole.

LEFT: A similar symmetrical retinopathy was observed but without any observation of haemorrhage.

Retinoscopy -6.00D R+L.

RETINOPATHY GRADED AS ROP Grade1 to Grade 2

PCA 36weeks Ophthalmoscopy:

RIGHT: A complete annular ridge observed mid area 2. There were no haemorrhages seen. Neovascularisation at the ridge junction and 'plus' disease was noted.

LEFT: A similar clinical picture was seen to that seen in the right eye except the ridge was some 10 degrees further out towards the periphery and extended from 11 o'c to 7 o'c.

Retinoscopy: Emmetropic.

RETINOPATHY GRADED AS GRADE 3 WITH MILD TO MODERATE 'PLUS' CHANGES BOTH EYES.

PCA 37weeks Ophthalmoscopy:

RIGHT: No change from the last observation except that the plus disease was recorded as moderate.

LEFT: As seen before except the plus disease now recorded as moderate. There was however a small flame shaped

haemorrhage noted at the ridge margin in the 3 o'c position.

RETINOPATHY RECORDED AS ROP GGADE 3. WITH MODERATE PLUS DISEASE BOTH EYES.

Retinoscopy: R.Emmetropic L. -1.50

PCA 38weeks Ophthalmoscopy:

BOTH EYES: The clinical picture remains the same except that no haemorrhaging was observed but the plus disease continues to increase.

RETINOPATHY RECORDED AS ROP GRADE 3. WITH MODERATE TO SEVERE PLUS DISEASE IN BOTH EYES.

Retinoscopy: R.-1.75 L. -2.00

PCA 39weeks Ophthalmoscopy:

RIGHT: White fibrous tissue was observed in the far periphery between 10 o'c and 11 o'c. Other factors remained the same.

LEFT: A 'sheet' or sub-hyaloid type haemorrhage was observed in the area of mesenchymal tissue anterior to the ridge, this haemorrhage extended over an area of approx. 10 to 15 disc diameters.

PCA 40weeks Ophthalmoscopy:

BOTH EYES: The clinical picture exhibited an exaggerated retinopathy from the previous examination. Both eyes now show sub hyaloid haemorrhages and blood vessels are growing into the vitrius from the area of the ridge and neovascularisation.

Unfortunately, despite attempts that were made by an ophthalmologist, who was called in when Katie was at a gestational age of 38 weeks to dispel the intra-vitrious neovascularisation using cryotherapy at 40 weeks PCA, Katie is now bilaterally blind.

As a result of possible litigation, all the blood gas records were analysed by the consultant paediatrition. The results are shown in Appendix 4.

Fig 3.13 shows the results of an attempt to photograph the 'sheet haemorrhaging observed at week 39. A Docustar retinal camera which is a polaroid recording adapted monocular indirect ophthalmoscope was used.

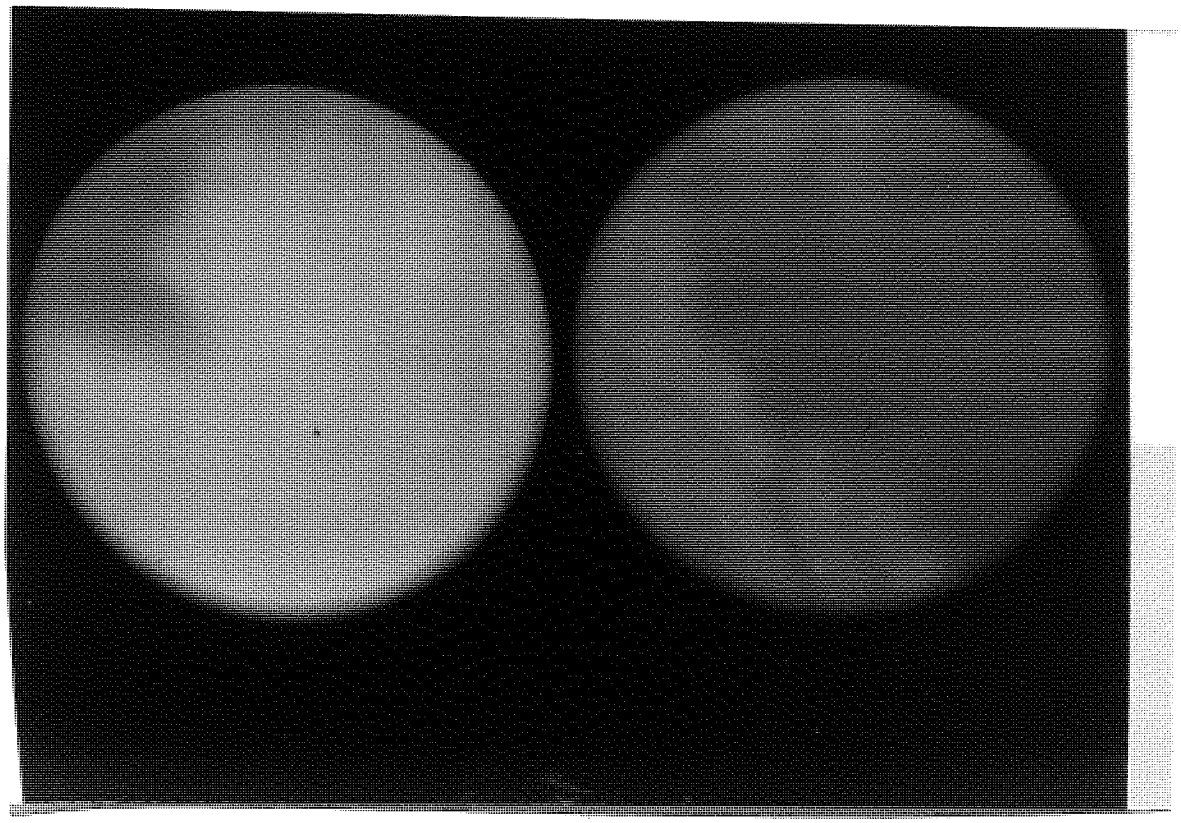


FIG 5.13 Polaroid photographs of the haemorrhages at Katie's retina seen at a PCA of 39 weeks.

CHAPTER 6

RESULTS - REFRACTION / MYOPIA

6.1 Refractive findings in children of premature birth

Fig 6.1 shows the distribution of refractive errors found in the total population of 96 surviving babies that had a birth weight of less than 1501 g. There is a large variance at all PCAs, but this variance reduces as the age increases thus providing some data on regression towards emmetropia. The full implications and statistical analysis of this regression are the subject of this chapter.

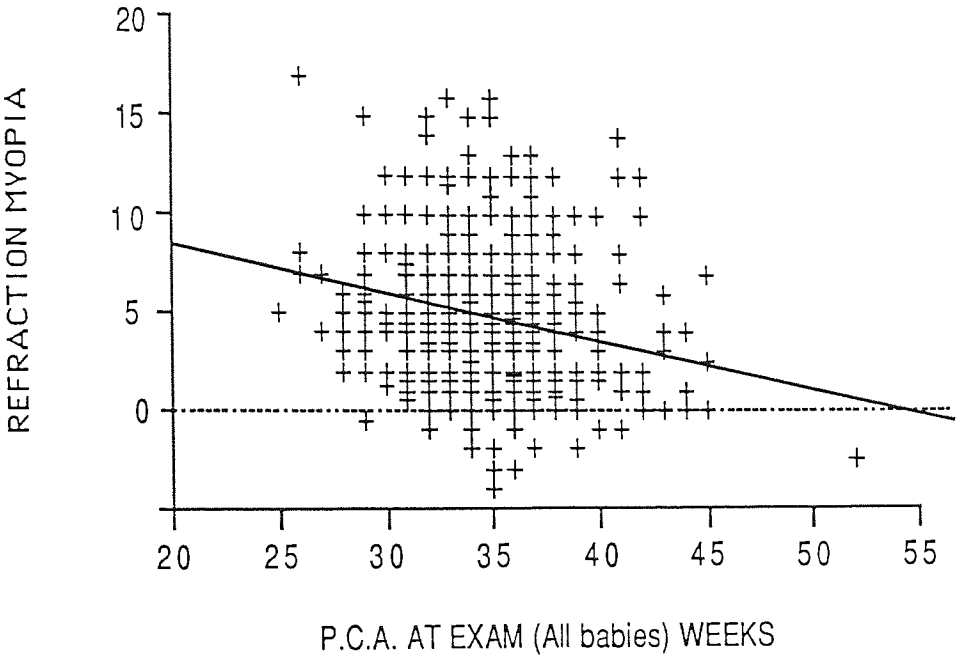


FIG 6.1 PCA and refractive error, all infants examined. N=515 observations on 96 babies.

The refraction is measured in dioptres of myopia, the hypermetropic results are therefore shown as negative. This scattergram represents 515 observations on the 96 babies.

Statistical analysis of the regression gives:
Standard error of the regression 3.5 dioptres.
Coefficient of correlation 0.2 due to the wide varience.
F' ratio of 33.3 on the analysis of varience. This however does give a probability on the 't' tables of 0.001 for the linear correlation.

The regression line has the equation of:

13.712 D - 0.254 X weeks PCA, where D= refractive error in dioptres of myopia. This indicates a regression to emmetropia at a PCA of 54 weeks.

Fig 6.2 shows the range of refractive errors measured. This histogram covers the whole period of the investigation, i.e. there are 515 separate observations on a total population of 96 babies covering PCAs of 26 to 54 weeks. Fig 6.3 shows the number of observations at each PCA.

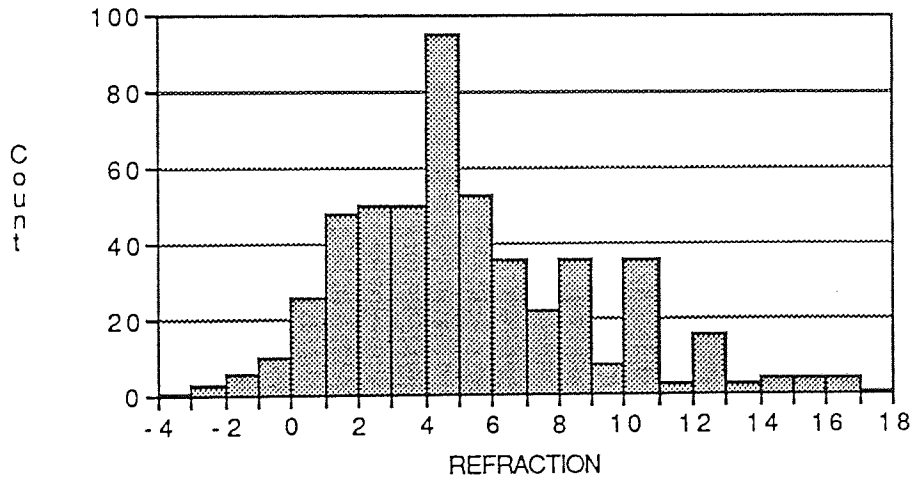


FIG 6.2 Distribution of the myopic refractive errors of the whole group during their stay in special care.

MEAN REFRACTIVE ERROR: 4.840 DIOPTRES MYOPIC.

STANDARD DEVIATION: 3.646

SKEW: 0.699 KURTOSIS: 0.425

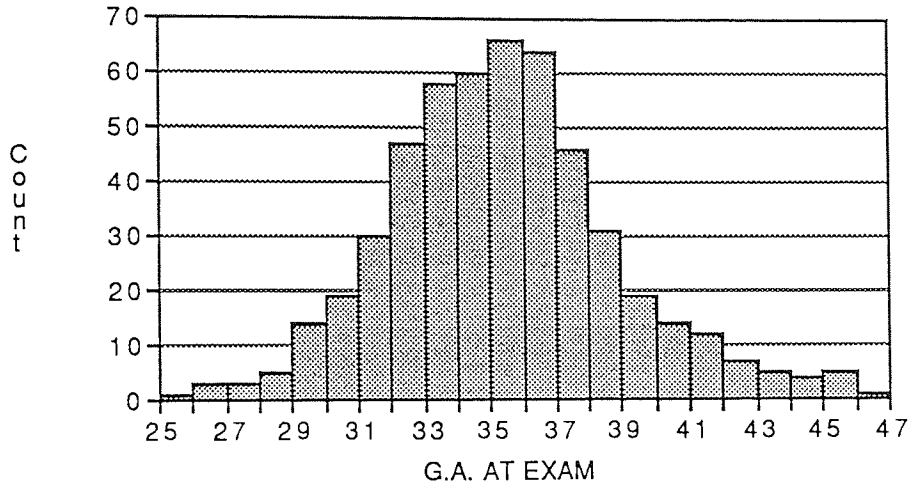


Fig 6.3 Distribution of gestational ages (GA) at the time of refractive assessment. N=515 observations on 96 babies.

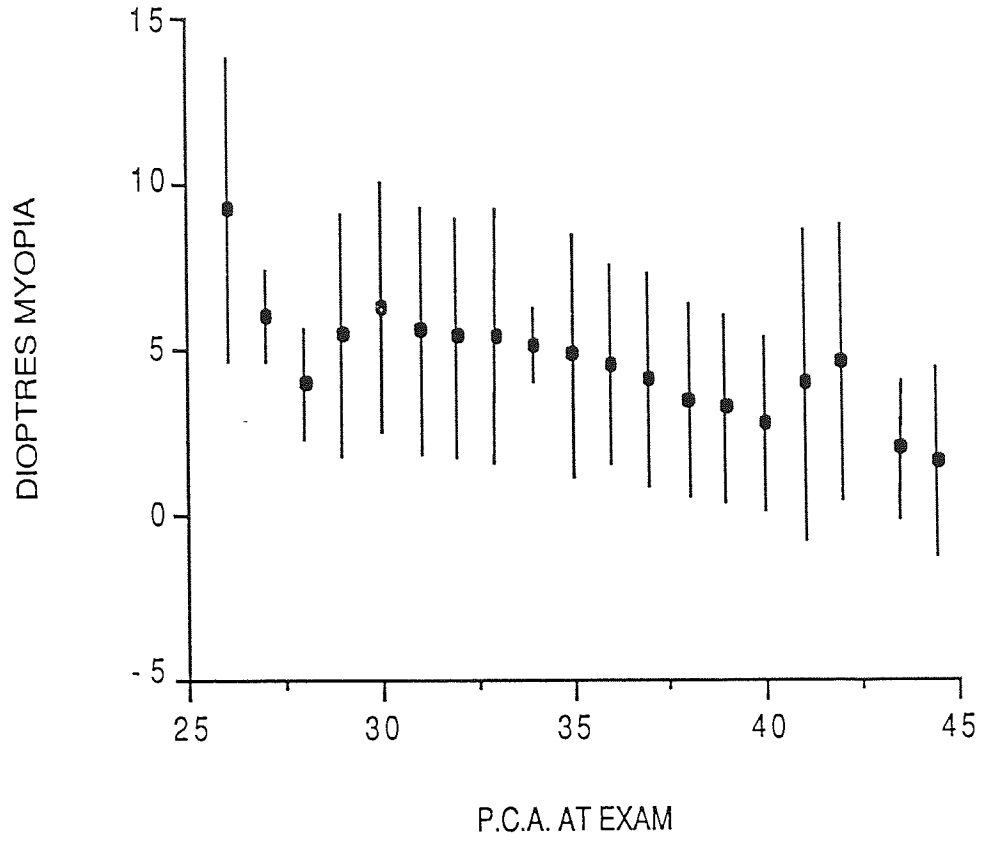


FIG 6.4 Refractive error of the total population of babies in terms of means and standard deviations, plotted against their PCA at the time of the examination.

Fig 6.4 shows the trend exhibited by the whole group may be observed. Subsequent statistical analysis is more readily understood when the graphical analysis is shown using standard error bars as opposed to standard deviation bars, although the effect of the uneven numbers at different ages is apparent. The results of such an analysis are shown in Fig 6.5.

These figures show general trends of the group as a whole and indicate that it is a rather interesting and varied group. There are wide variations from individual to individual at all PCAs and

further analysis is required in an attempt to show trends.

Of particular interest is the apparent levelling off of the trend towards emmetropia from the age of 41 weeks and the variations found in the earliest eight (25-32) weeks.

It must however always be remembered that this population of premature babies includes a wide variation both of individuals and of conditions. There is a gradual build up of babies to be examined during the first 8 weeks (25-32 weeks). Factors that influence discharge from the Special Care Baby Unit also play a role in the numbers of examinations recorded at the higher PCAs. Both fitness and the home environment are conditions that are taken into consideration for discharge from the Unit. For this reason reduced numbers of subjects were seen at both ends of the scattergrams (see Fig 6.1) producing poor standard errors. There are also the unfortunate few who do not survive, and there were those transferred to other units and those transferred to the Unit on a short-term basis from other local maternity hospitals.

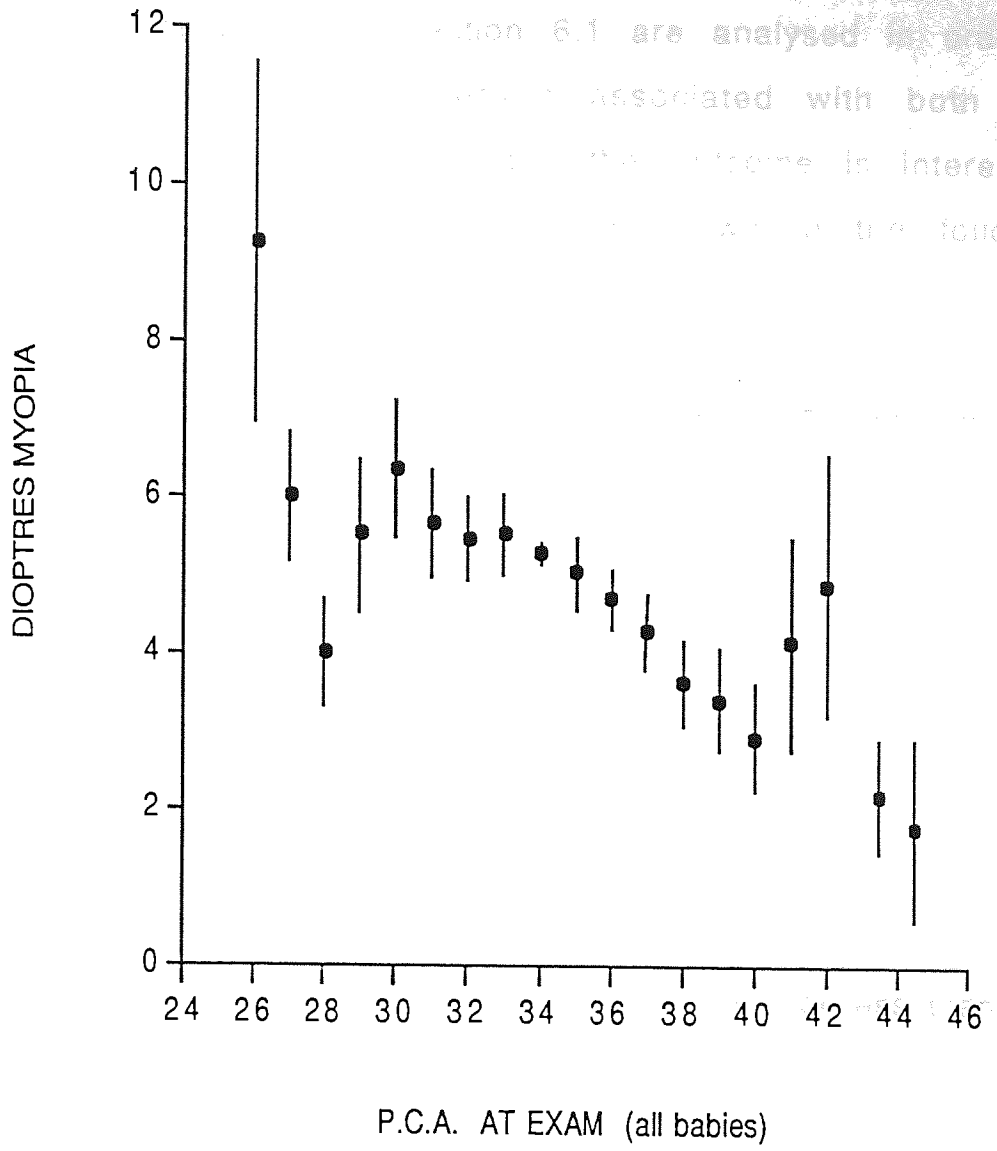


FIG 6.5 The total population of babies in terms of their PCA at the time of the examination, plotted against their refractive error. Bars are equal to +/- one standard error. N=515 observations on 96 babies.

6.2 The trend towards emmetropia.

If the results shown in section 6.1 are analysed in order to understand the refractive errors associated with both birth weight groups and PCA groups, the outcome is interesting. Accordingly the results were broken down in the following manner:

(a) Babies who weighed less than or equal to 1250 g at birth.

(b) Babies who weighed more than 1250 g at birth.

(c) Babies who weighed less than or equal to 1000 g at birth.

(d) Analysis of each PCA group and number of weeks of age fom birth.

(e) Babies who had an estimated gestational age of less than or equal to 30 weeks PCA at birth.

(f) Babies who had an estimated gestational age of more than 30 weeks PCA at birth.

(g) Analysis of the estimated PCA groups by using the number of weeks of age from birth that they were examined.

6.2 (a) Babies who weighed less than or equal to 1250 g at birth

Fig 6.a.1 shows the scatter and linear regression line of this population:

linear regression 15.767 Dioptres myopia X (-0.32weeks) indicating a regression to emmetropia at a PCA of 49.27 weeks.

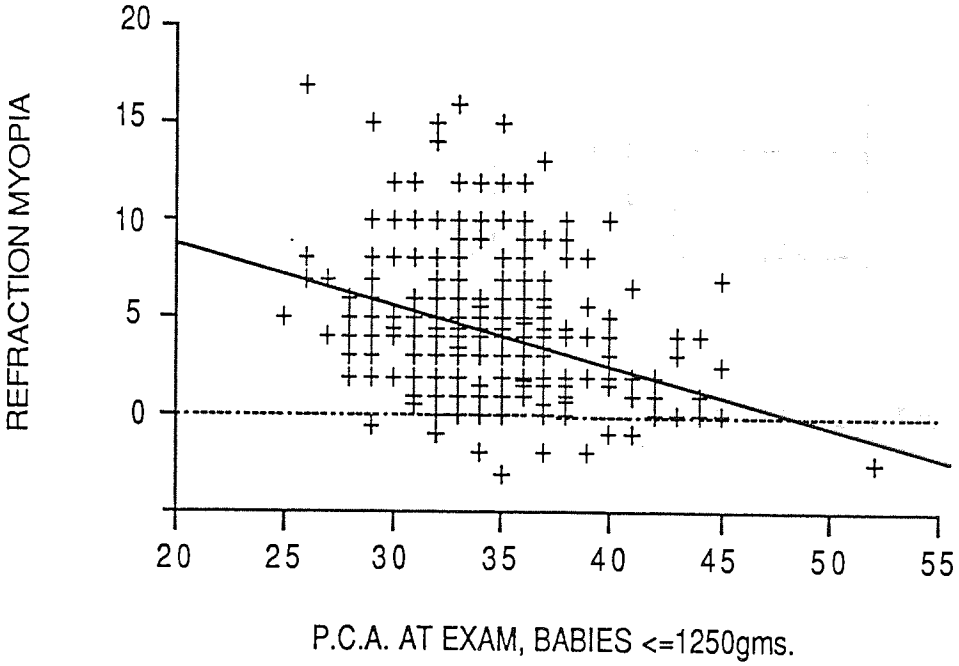


FIG 6.a.1 PCA and refractive error in infants who weighed less than or equal to 1250 g at birth. N=323 observations on a population of 54 babies.

Owing to the wide variation at all PCAs, there is a poor correlation of 0.355 and a standard error of estimate of 3.367 dioptres. However, because of the size of the sample there is a 0.001 statistic for the 't' test. Analysis of variance gives an 'F' ratio of 47.4 showing a strong association (0.001).

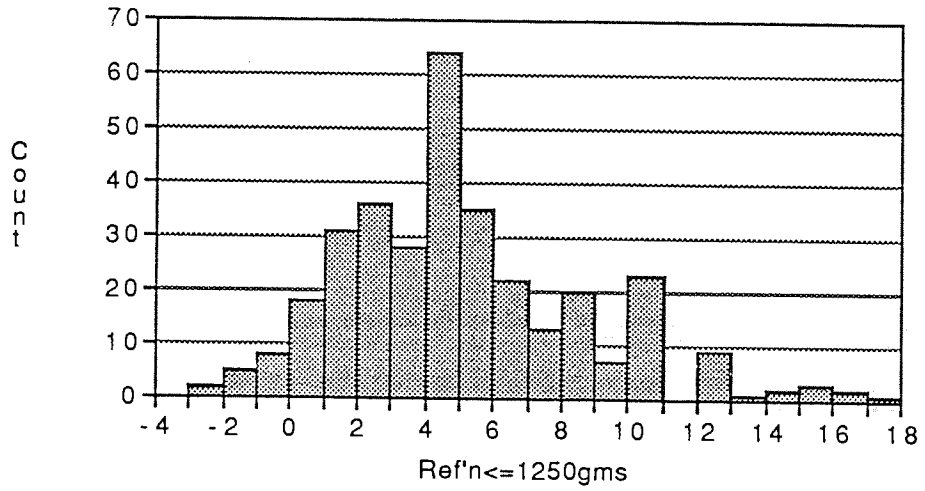


FIG 6.a.2. Distribution of myopic refractive errors in the group that weighed less than or equal to 1250 g at birth.

MEAN REFRACTIVE ERROR: 4.654 DIOPTRES MYOPIC.

STANDARD DEVIATION: 3.591

SKEW: 0.7 KURTOSIS: 0.6

The distribution is somewhat similar to the total group

distribution (see Fig 6.1).

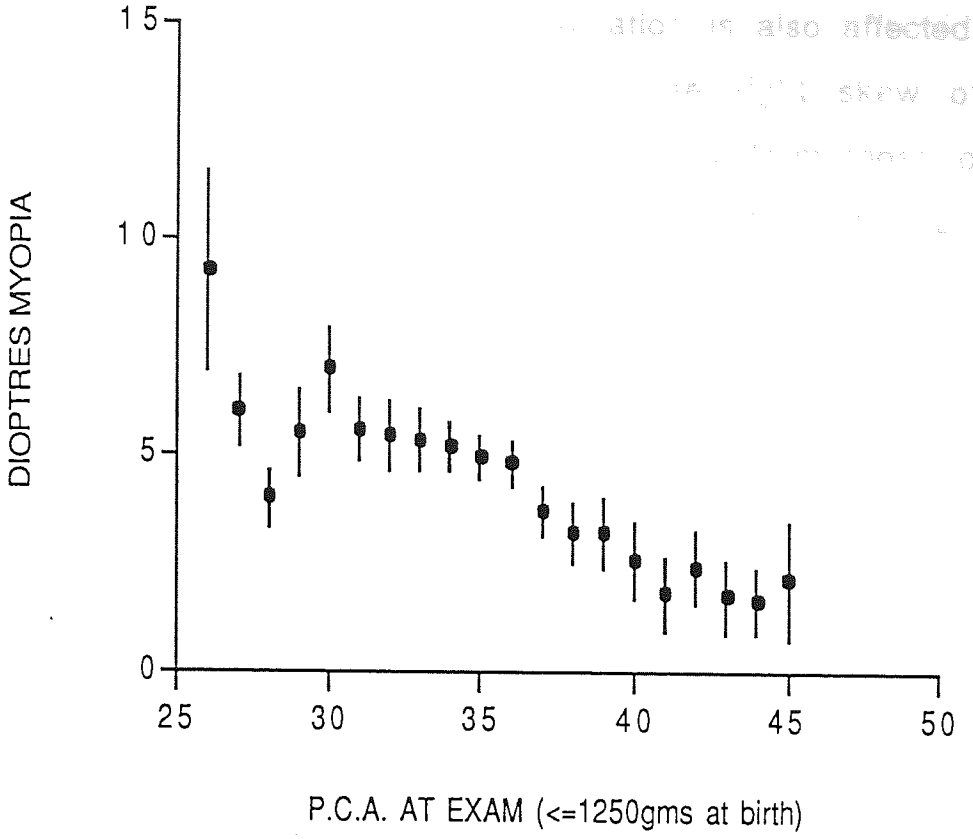


FIG 6.a.3. Refractive error of all babies weighing less than or equal to 1250 g at birth plotted against PCA at the time of the examination (lines show +/- 1 SE)

Fig (6.a.3) shows the means at each examination week, the bars on this occasion showing the standard errors (SE). In common with the total population (see Fig 6.4) it can be seen that the error bars are somewhat lengthened towards both ends of the graph. This is primarily caused by small sample numbers rather than a real increase in variation of refractive errors especially when the PCAs are greater than 40 weeks as by this age most of the babies had been discharged. The number of observations at these ages is less than 10 for each age group.

Because the healthier were discharged at an earlier PCA, the normality of the histogram of the population is also affected. The refraction findings that are shown in the right skew of the histogram (Fig 6.a.2) were obtained primarily from those babies that had a longer than normal stay in the unit owing to prolonged ill health or poor home conditions. This observation can also be seen in the scattergram Fig(6.a.1) where those babies that had both low myopic or hypermetropic refractive errors appear to gradually disappear from a PCA of 38 weeks.

6.2 (b) Babies who weighed more than 1250 g at birth

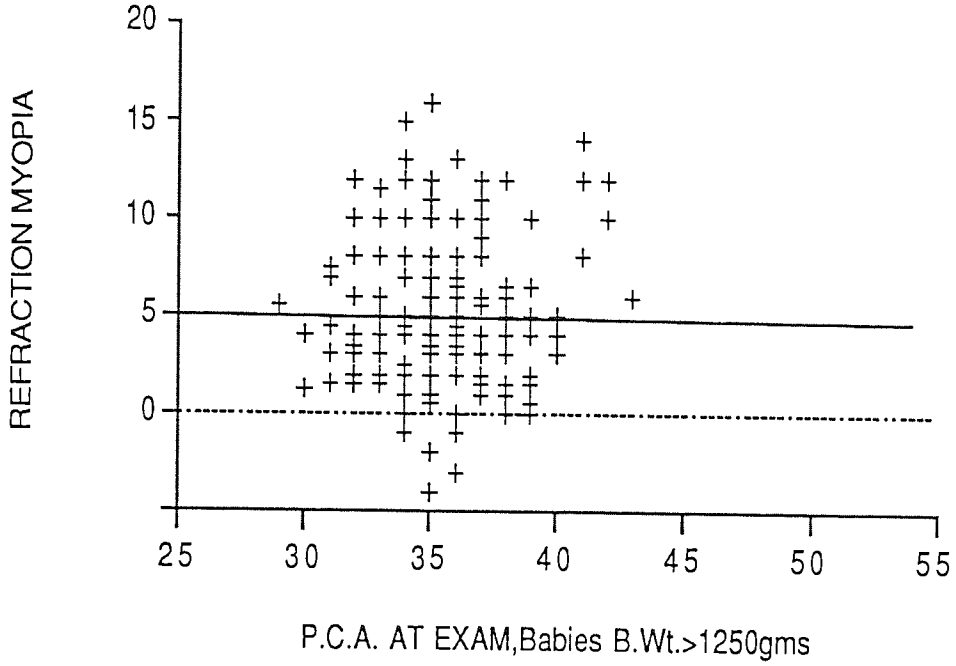


FIG 6.b.1 PCA and refractive error in infants who weighed more than 1250 g at birth. N=186 observations on 42 babies.

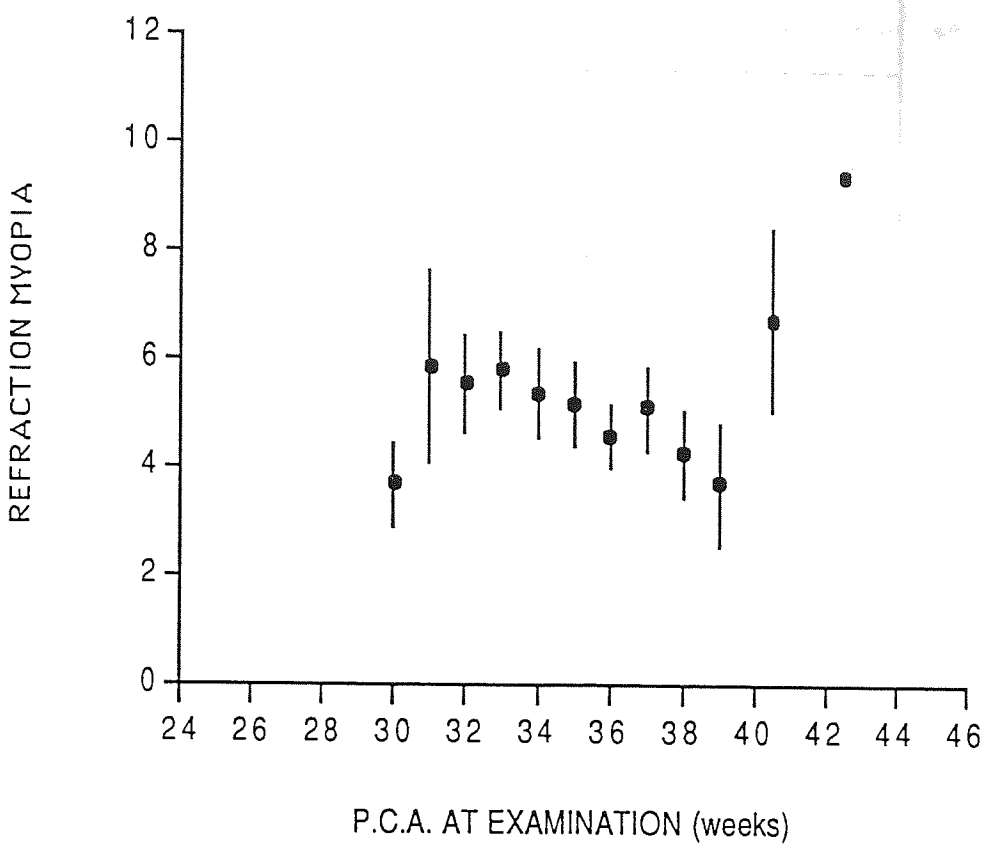


FIG 6.b.2 Refractive error in babies who weighed more than 1250 g at birth plotted against PCA at the time of the examination. Bars show +/- 1 SE.

There appears to be an almost linear trend, however statistical analysis does not support this with any degree of confidence.

Fig 6.b.3. shows a 'flatter' distribution than that of the less than or equal to 1250 g group. The skew to the right is enlarged and of interest in attempting to understand this group.

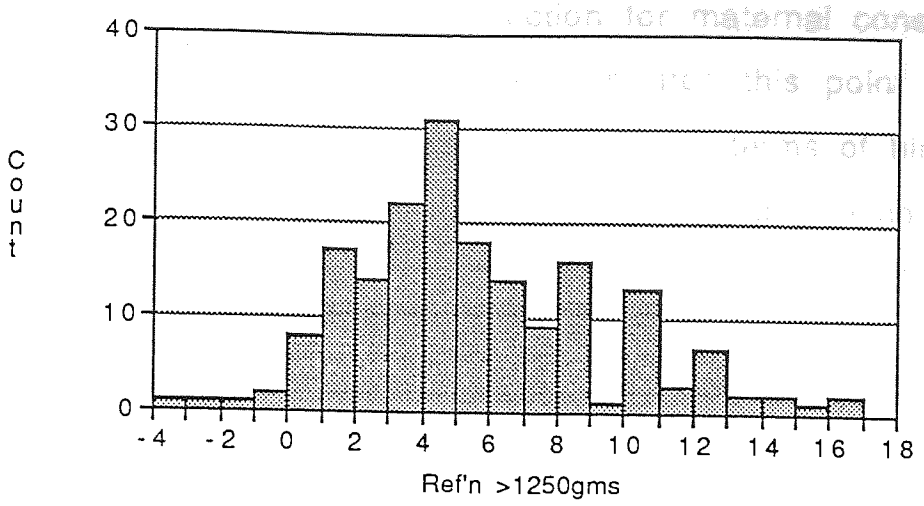


FIG 6.b.3 Distribution of myopic refractive errors in babies whose birth weight was greater than 1250 g The number of babies in this group is 42.

N = 165 OBSERVATIONS.

MEAN REFRACTIVE ERROR: 5.188 DIOPTRES MYOPIC.

STANDARD DEVIATION: 3.716

SKEWN: 0.6 KURTOSIS: 0.1

This higher weight group comprises of those whose birth weights bear a looser correlation to their gestational age than the lighter group. There are many reasons for this state of affairs: first, the variation in gestational age at birth varies to a greater extent; and secondly the reasons for being born prematurely, and/or being of severely reduced weight are numerous.

Many of these babies are "small for dates". They have not survived easily *in uterus* or have stopped growing. They are therefore often born by elective caesarian section. Others in this group are twins

and babies born by caesarian section for maternal considerations such as pre-eclampsia. Fig 6.b.4 illustrates this point and is a plot of the whole population of 96 babies in terms of birth weight and estimated PCA at birth, the dependent variable being the PCA. As discussed previously in Chapter 5, the relationship is not linear but is a second order polynomial regression.

Correlation 0.835

Analysis of varience, 'F' ratio 109.382 p=0.001.

The spread of ages for different weight groups enlarges to the right as the birth weight increases.

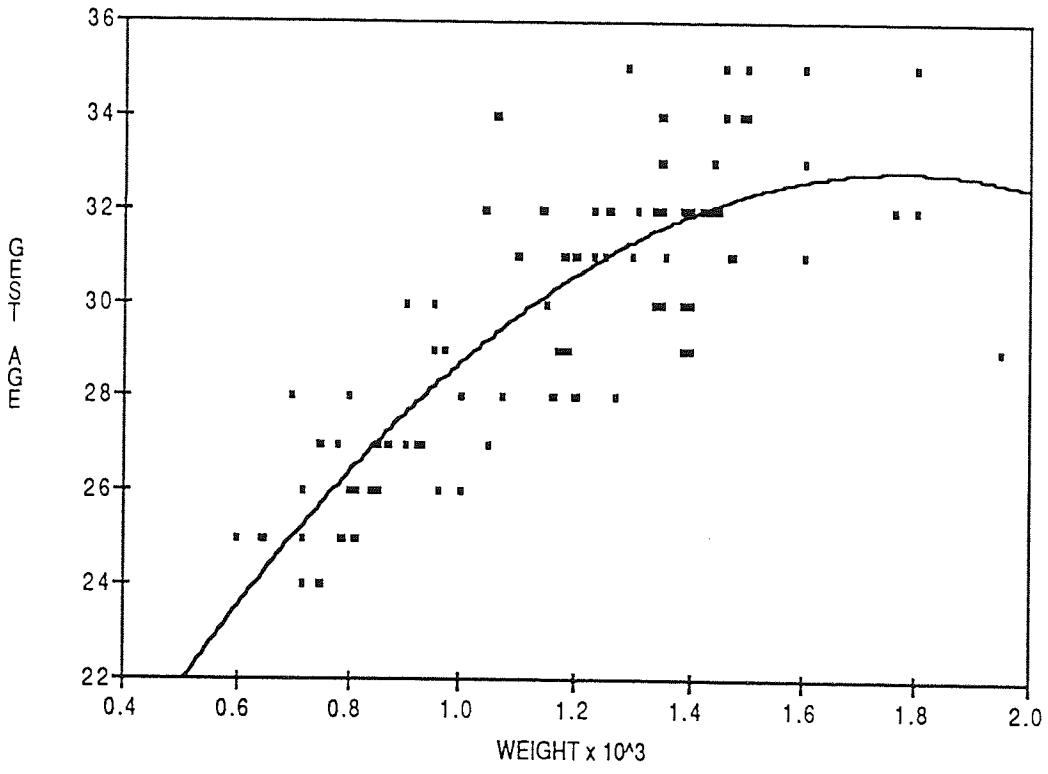


FIG 6.b.4 Second-order polynomial for the dependent variable, PCA ,plotted against the corresponding birth weight. N=96 for the total population of babies refracted. (Gest age = Birth PCA, Weight in grams)

6.2 (c) Babies who weighed less than or equal to 1000 g at birth.

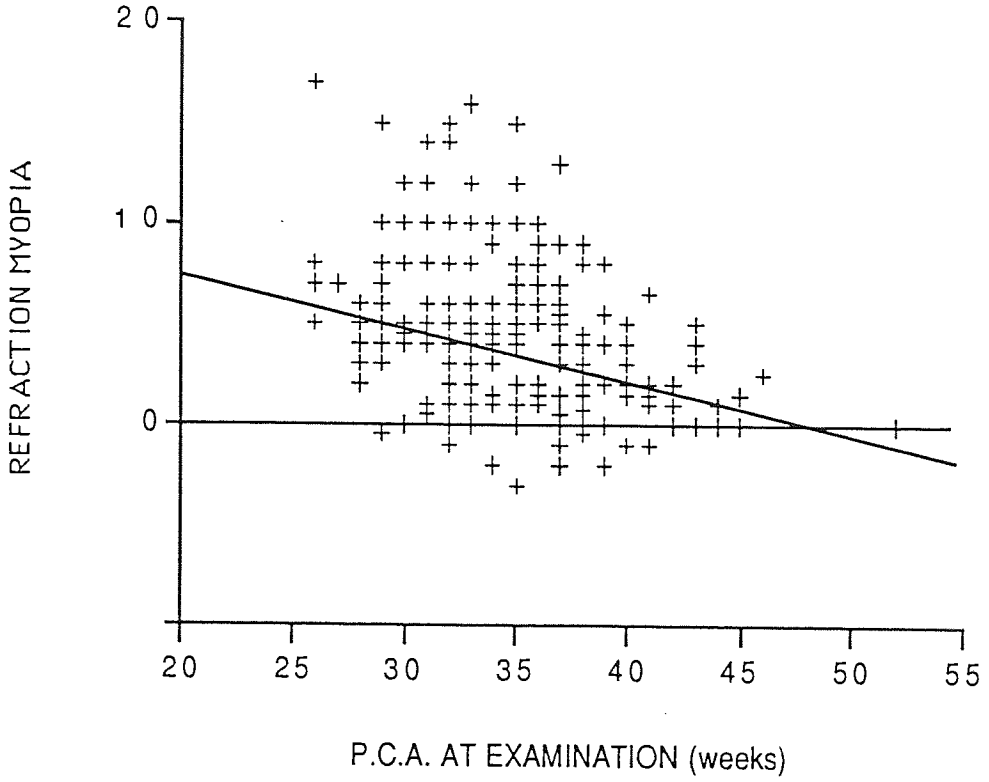


FIG 6.c.1 PCA and refractive error in infants who weighed less than or equal to 1000 g at birth. N=224 observations on a population of 34 babies.

The slope of the linear regression is 17.8 Dioptres of myopia x (-0.38 weeks), indicating emmetropia at the age of 46.8 weeks PCA, the standard error of the estimate being 1.9 dioptres. There is again a poor coefficient of correlation (0.4) but because of the large number of observations the probability is good (0.001) The 'F' ratio is 47.1 and the 't' statistic is -6.9.

When comparing the analysis of this group with either the whole group or those babies that weighed less than or equal to 1250 gms at birth, it can be seen that the confidence limits are somewhat better (Fig 6.c.2).

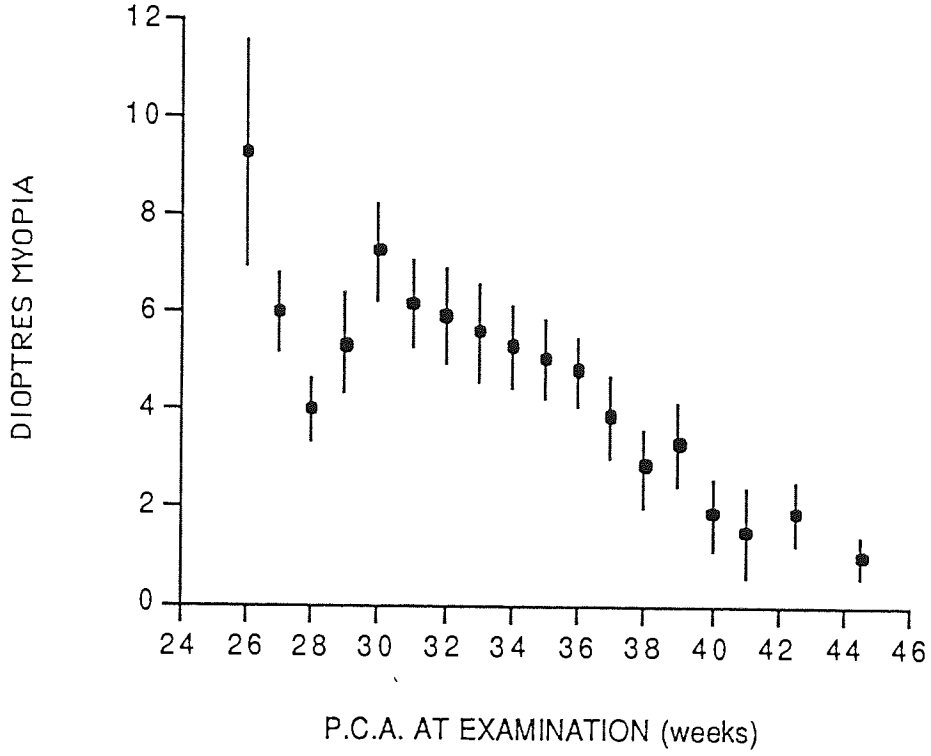


FIG 6.c.2 Refractive errors of all babies who weighed less than or equal to 1000 g at birth plotted against PCA at the time of the examination. Bars = +/- 1SE. N=244 observations on a population of 34 babies.

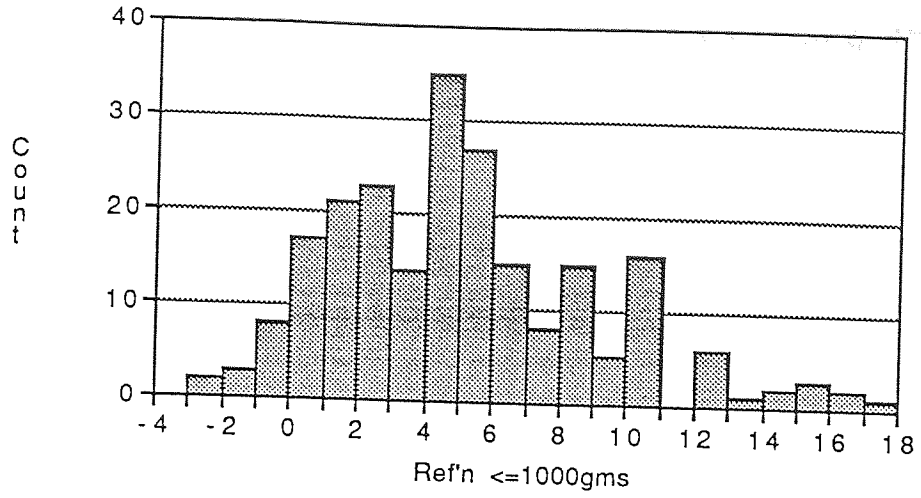


FIG 6.c.3 Distribution of myopic refractive errors in those babies who weighed less than or equal to 1000 g at birth.

MEAN REFRACTIVE ERROR: 4.715 DIOPTRES MYOPIC.

STANDARD DEVIATION: 3.890

SKEW: 0.8 KURTOSIS: 0.6

It can therefore be clearly seen that where there was a significant difference in the population of babies when the dividing line is placed at a birth weight of less than 1251 g This difference is further accentuated by reducing the population to those weighing less than 1001 g and observing the regression towards emmetropia.

When comparing the two groups using analysis of variance there is no statistical difference between them. It has to be clearly understood, however, that the two groups overlap, there being only 106 extra observations in the larger group.

6.2 (d) Analysis of each PCA group by using the number of weeks of age fom birth

If the anaysis is repeated using the weeks of age from birth at the time of the refractive examination as opposed to gestational age at the examination the results shown in Fig 6.d.1 are obtained.

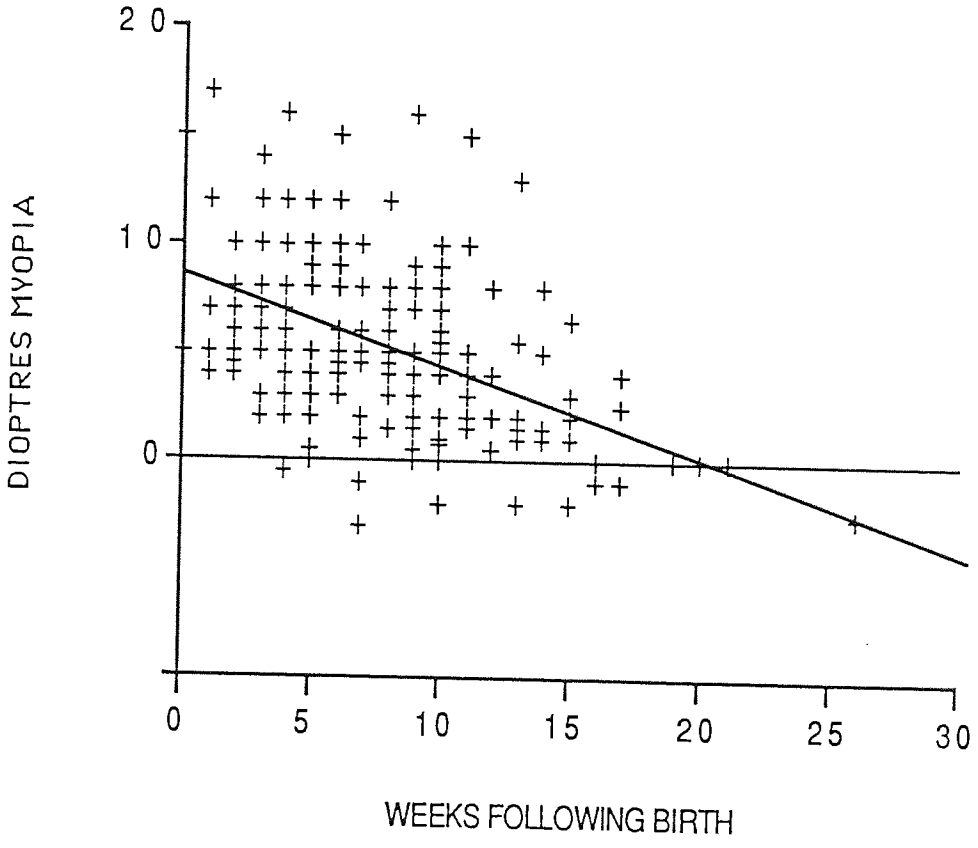


FIG 6.d.1 Weeks since birth and refractive error in babies who weighed less than or equal to 1000 g at birth

Here, with this analysis, the statistical analysis results show a gain in confidence level.

Linear regression 8.0 Dioptres myopia x (-0.4 weeks) predicting

emmetropia at 20 weeks following birth.

Standard error of the estimate 0.5 dioptres

Analysis of variance ('F' ratio) 52.8

't' statistic -7.3 probability 0.001.

Coefficient of correlation 0.5.

This "extra confidence" can be readily be seen by the observation of Fig 6.d.2.

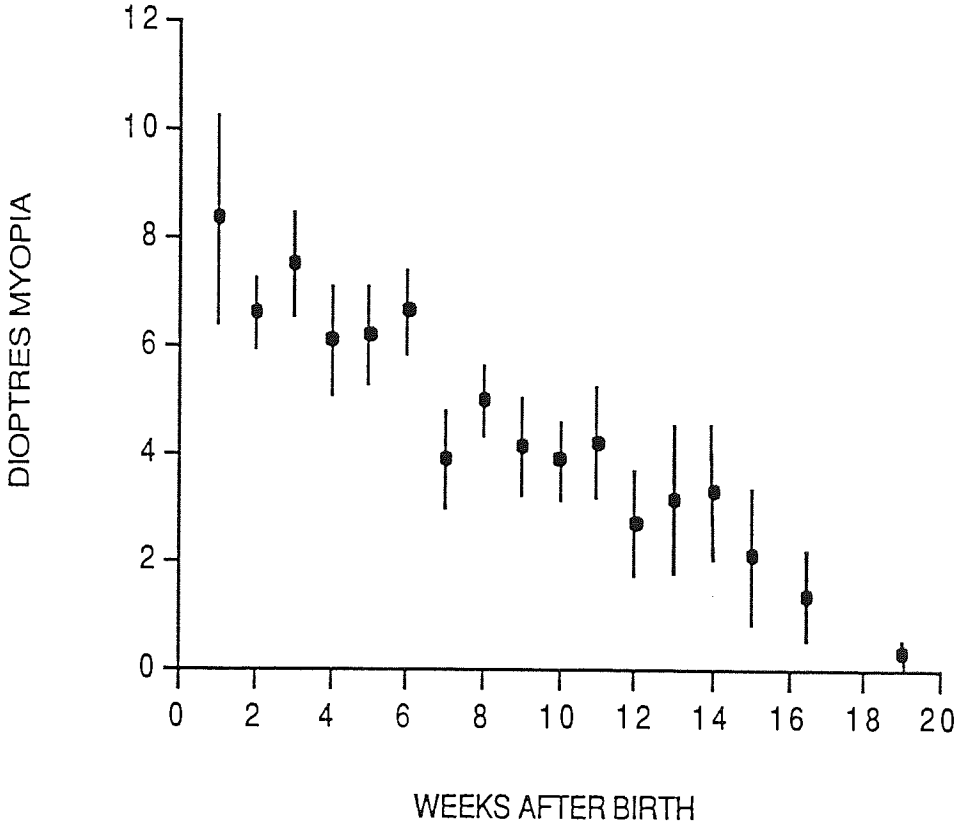


FIG 6.d.2. Refractive state of those babies who weighed less than or equal to 1000 g at birth plotted against the number of weeks since their birth. This represents 224 observations on a population of 34 babies. Bars show +/- one standard error.

When the group is extended to include those who weighed between 1000 g and 1250 g i.e. those who weighed less than or equal to 1250 g at birth, the results are as shown in Fig 6.d.3.

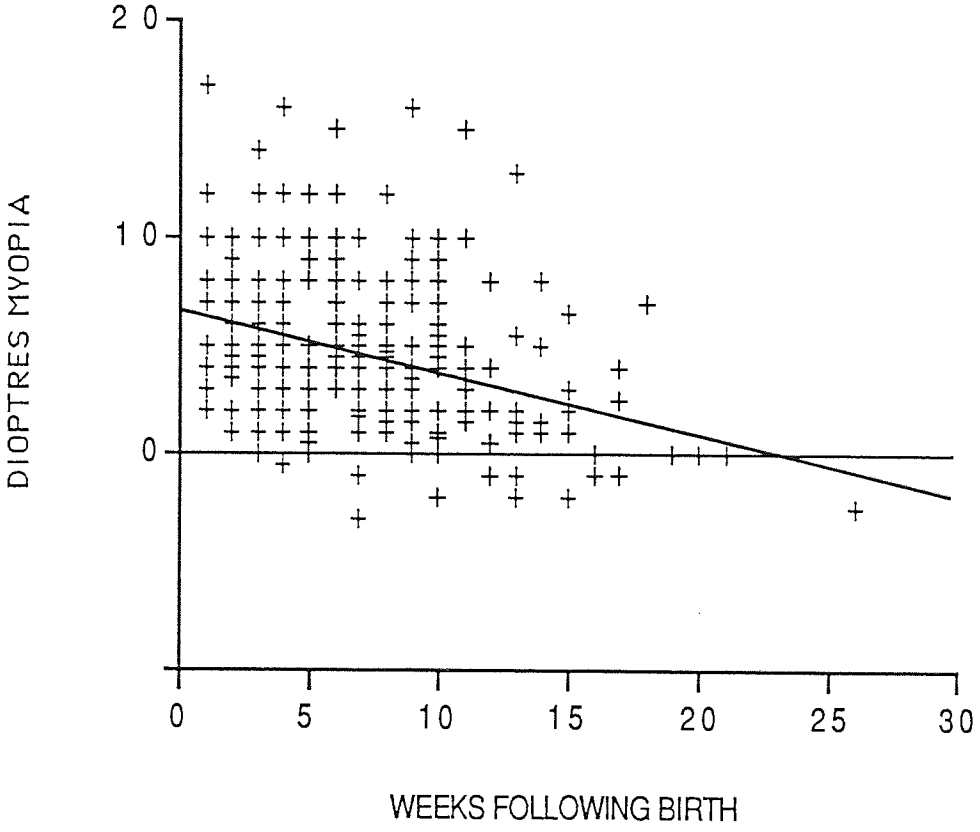


FIG 6.d.3 Scattergram and linear regression of weeks since birth and refractive error in those babies who weighed less than or equal to 1250 g at birth. N=323 observations on 54 babies.

As was found in the analysis of this group previously, the data points are in more of a random order and therefore of less

statistical relevance:

Linear regression, emmetropia at an age of 22 weeks from birth, Regression 6.6 Dioptres myopia x (-0.3weeks).

Coefficient of correlation 0.3

Analysis of variance 'F' ratio 42.5

't' statistic -6.05 p = 0.001.

The coefficient of correlation is reduced by the broadening of the age range in this group.

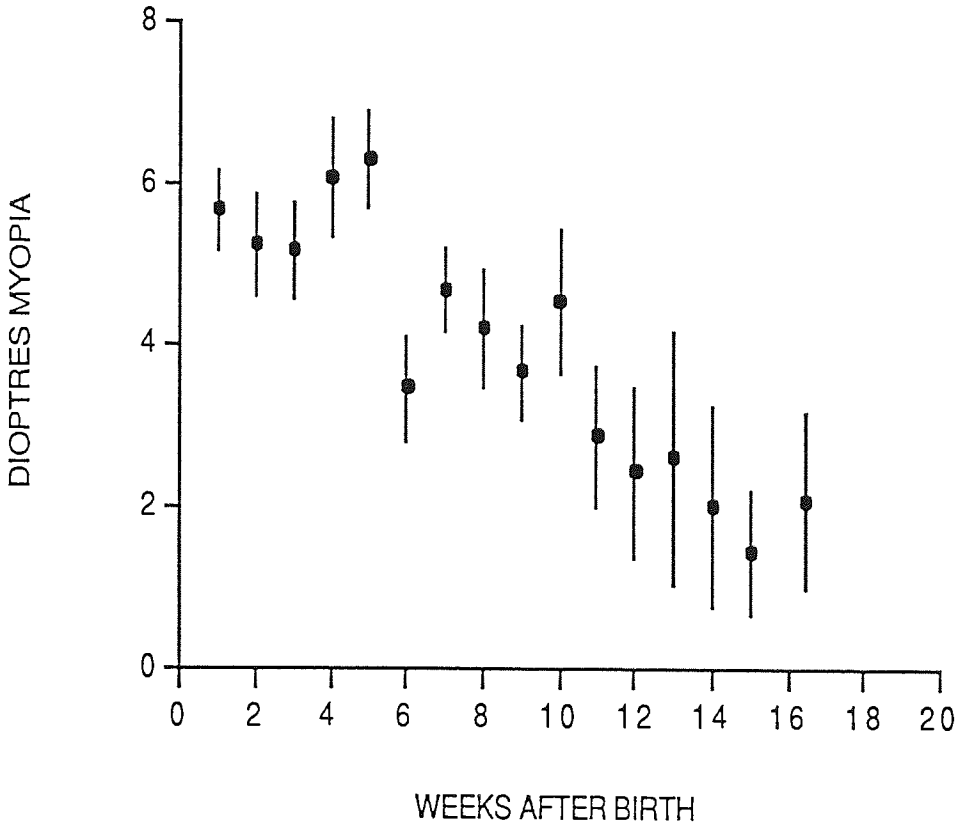


FIG 6.d.4 Means obtained from refractive measurements in those babies weighing less than or equal to 1250 g at birth and plotted against thier age since birth. Bars show +/- 1 SE.

The standard error bars shown in Fig 6.d.4 further show the wider variance of refractive errors when the larger babies are included into the very low birth weight group.

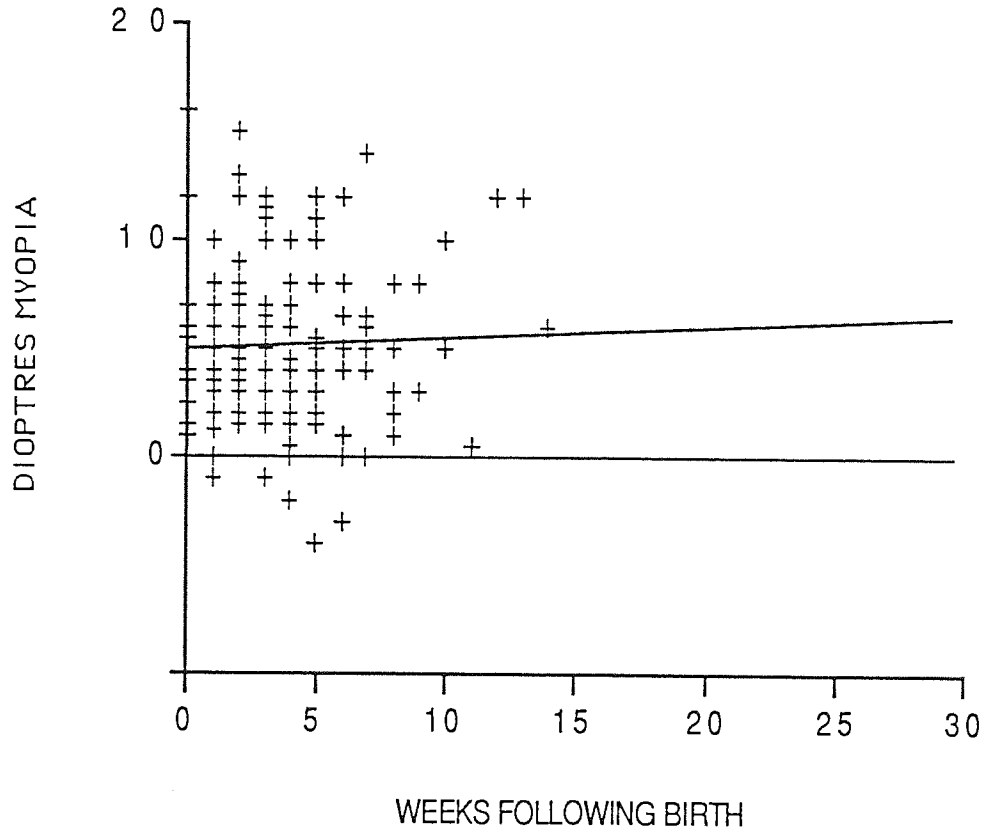


FIG 6.d.5 Weeks since birth and refractive error obtained from those babies who weighed greater than 1250 g at birth. Observations = 323 on 42 babies.

When analysis of this group of low birth weight babies is

conducted it is not surprising that there is no confidence in any regression. Analysis of variance and the coefficient of correlation also indicate no specific trend.

Graphical representation in the form of means and SE bars (Fig 6.d.6) also serves to illustrate the lack of any trends within this group.

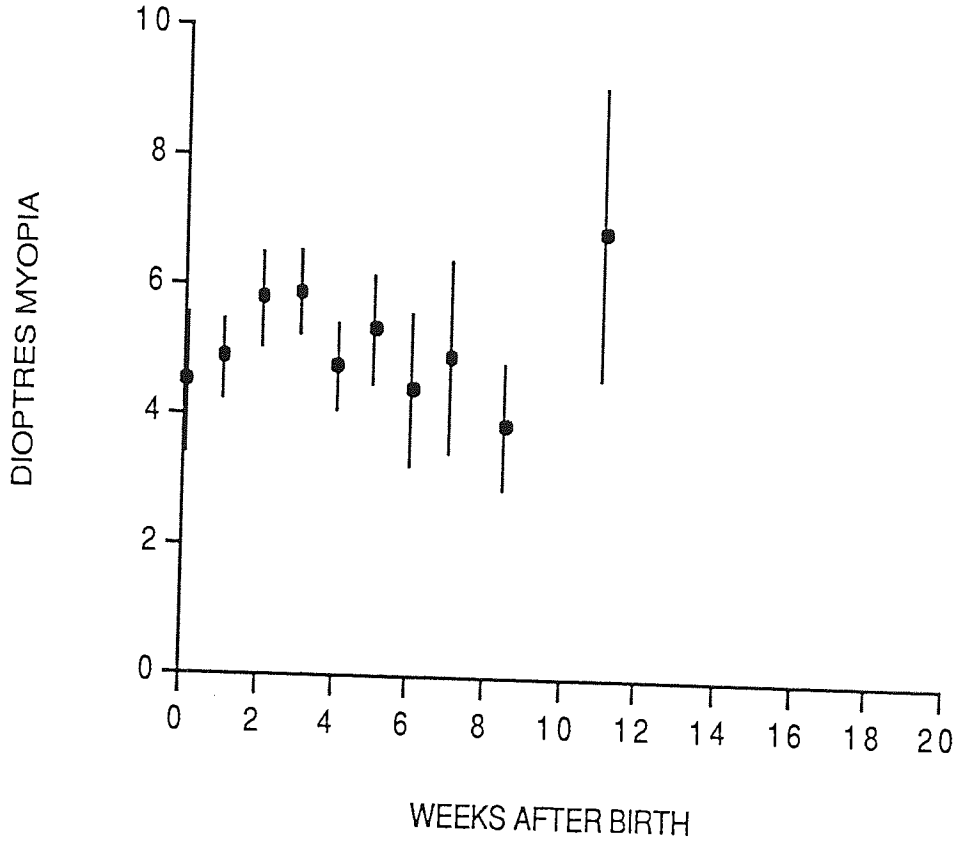


FIG 6.d.6 Weeks since birth and refractive error in those babies who weighed greater than 1250 g at birth. Bars show +/- 1 SE.

This rearrangement of the data using the criteria of the number of weeks since birth, rather than the PCA at the time of the refractive assessment, emphasises the differences previously found when the population has the dividing line drawn at 1250 g birth weight and further accentuates the difference when the dividing line is drawn at a birth weight of equal to or less than 1000 g.

6.2 (e) Babies who had a gestational age of less than or equal to 30 weeks PCA at birth

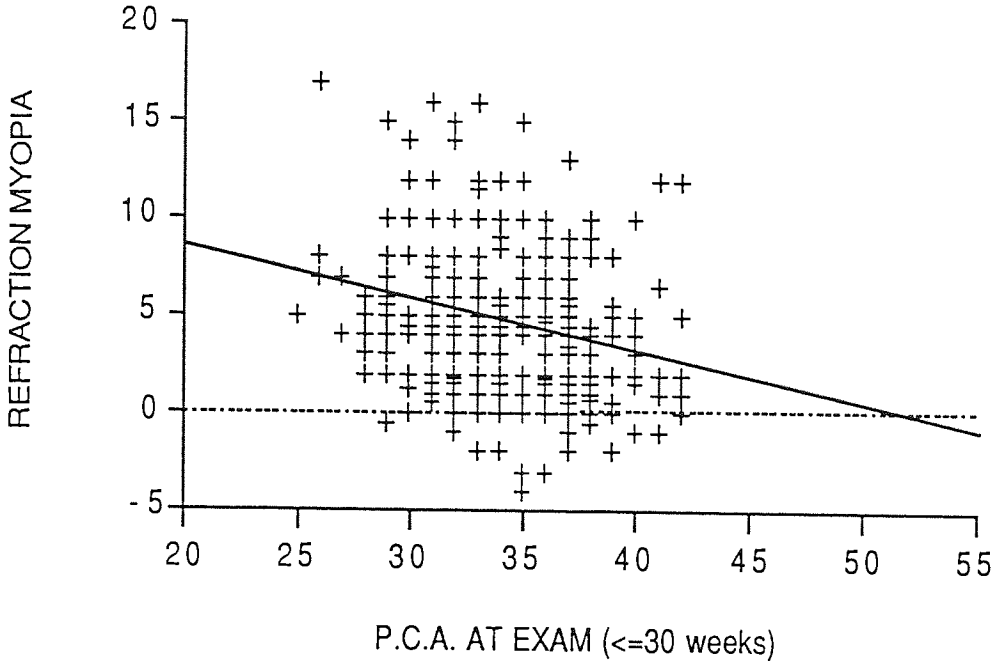


FIG 6.e.1 PCA and refractive error in those babies who had a gestational age of equal to or less than 30 weeks at birth. 329 observations on 53 babies.

From analysis of Fig 6.e.1:

Analysis of variance 'F' ratio 34.8

't' statistic -5.9. p=0.001

Coefficient of correlation 0.3 (reflecting the wide spread of refractive errors at all PCAs).

Regression 14.8 Dioptres myopia x (-0.3 weeks).

Interestingly, the number of babies in this group is one short of the number in the equal to or less than 1250 ggroup. The birth weight analysis of this group is comprised of the following:

33 babies that weighed equal or less than 1000 g at birth.

10 babies that weighed between 1001 and 1250 g at birth.

9 babies that weighed more than 1250 g at birth.

A further understanding of the difference in the birth weight as opposed to estimated gestational age may be gained by further examination of Fig 6.b.4 (page 130), the plot of birth weight versus estimated PCA at birth for the whole group.

Graphical analysis using means and standard errors is shown in Fig 6.e.2.

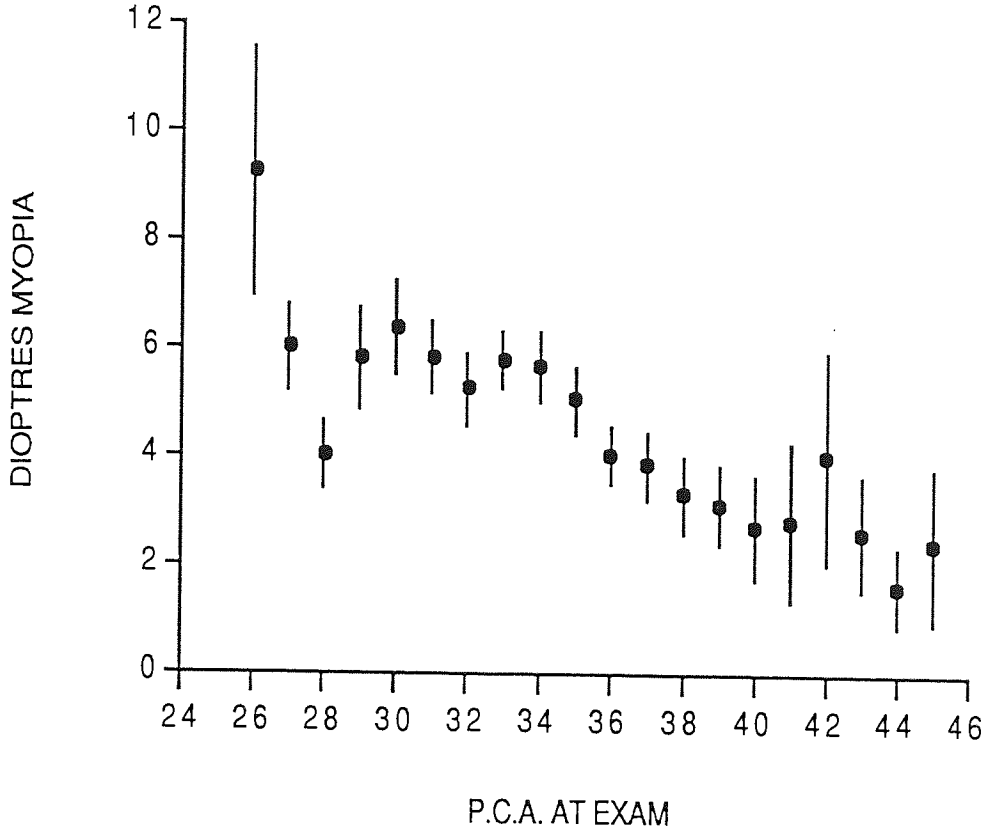


FIG 6.e.2 Refractive error (myopia) plotted as a function of PCA at the time of the examination in those babies who had an estimated gestational age of 30 weeks or less at birth. Means and +/- 1 SE.

6.2 (f) Babies who had a gestational age of more than 30 weeks PCA at birth

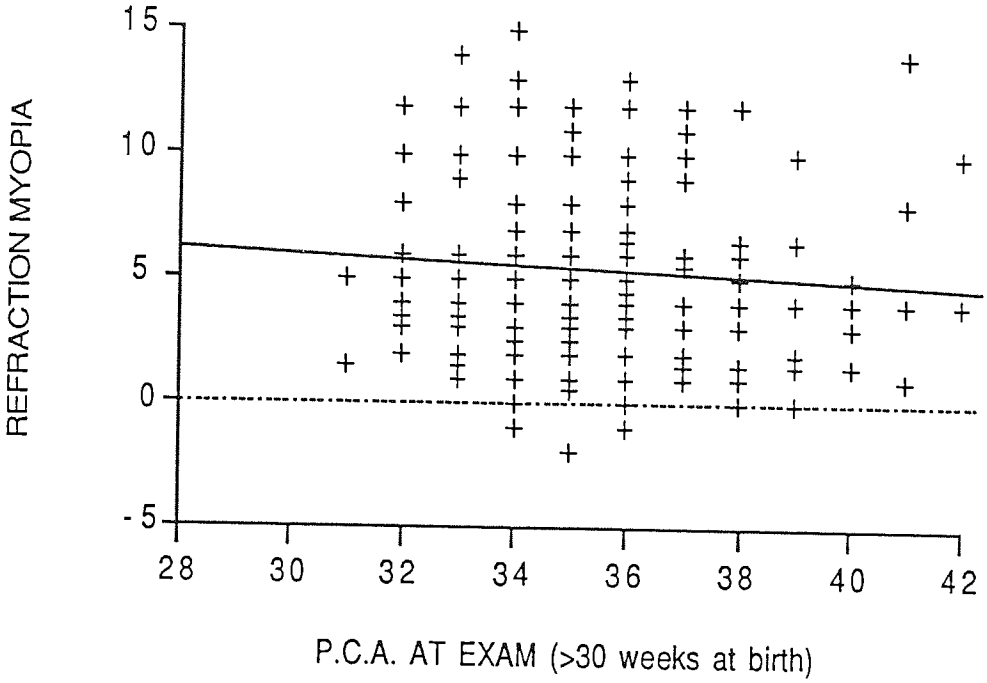


FIG 6.f.1 PCA at the time of the examination and refractive error in those babies who had an estimated gestational age greater than 30 weeks at birth. N=184 observations on a population of 43 babies.

The variations within this group are similar to the larger babies previously analysed in section 6.2 (b), i.e. those babies who had a birth weight of greater than 1250 g There is no statistical evidence to support the regression line included on Fig 6.f.1. The further graphical analysis using the means and standard error bars shown below in Fig 6.f.2. further illustrates this point.

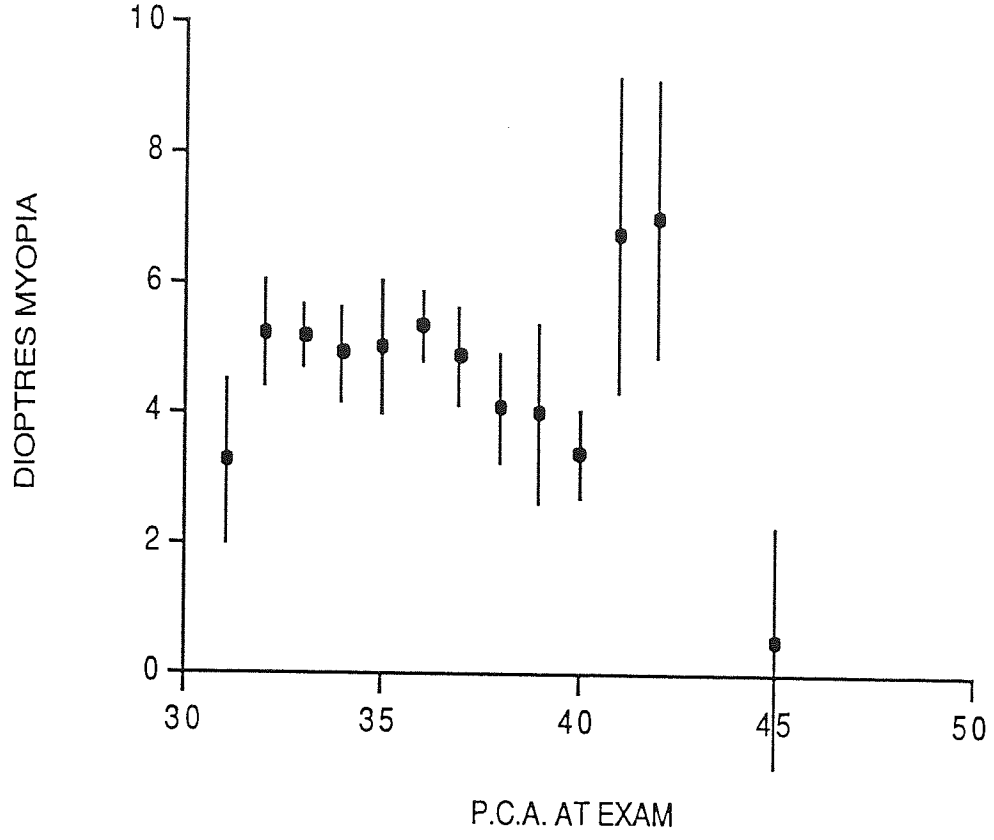


FIG 6.f.2 Refractive error (myopia) in babies with an estimated PCA of greater than 30 weeks at birth plotted against PCA at the time of examination. Means and +/- 1 SE.

The standard error bars of the analysis of those babies that were refracted at a PCA of greater than 40 weeks are extended owing to the small numbers seen. Most of the healthier babies have been discharged by this time.

(g) Analysis of the estimated PCA groups by using the weeks of age from birth that they were examined.

If the previous exercise is repeated and the alternative plots are produced using the division criteria unchanged at 30 weeks PCA but plotting the number of weeks since birth the results shown in Fig 6.g.1 are obtained.

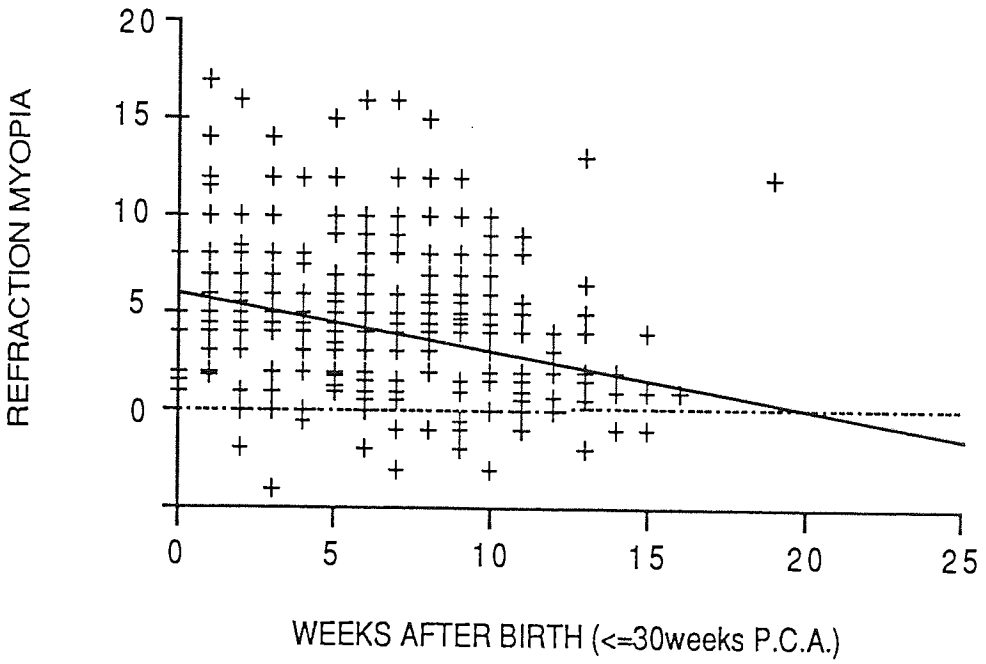


FIG 6.g.1. Age in weeks versus refractive error in those babies who had a gestational age of less than or equal to 30 weeks at birth.

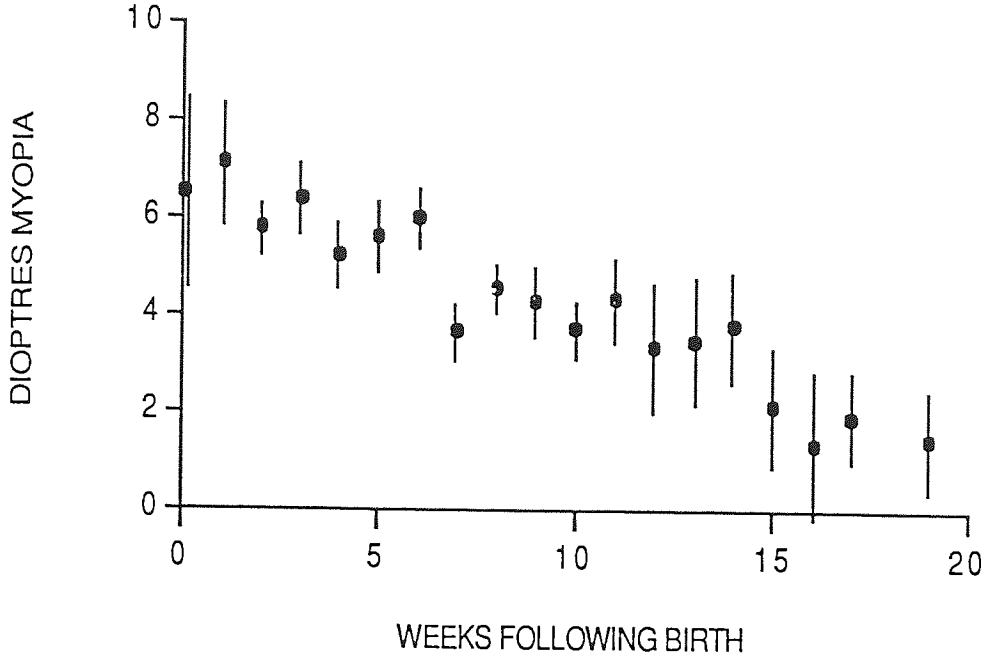


FIG 6.g.2 Babies who had an estimated PCA of less than or equal to 30 weeks at birth. Means and +/- 1 SE.

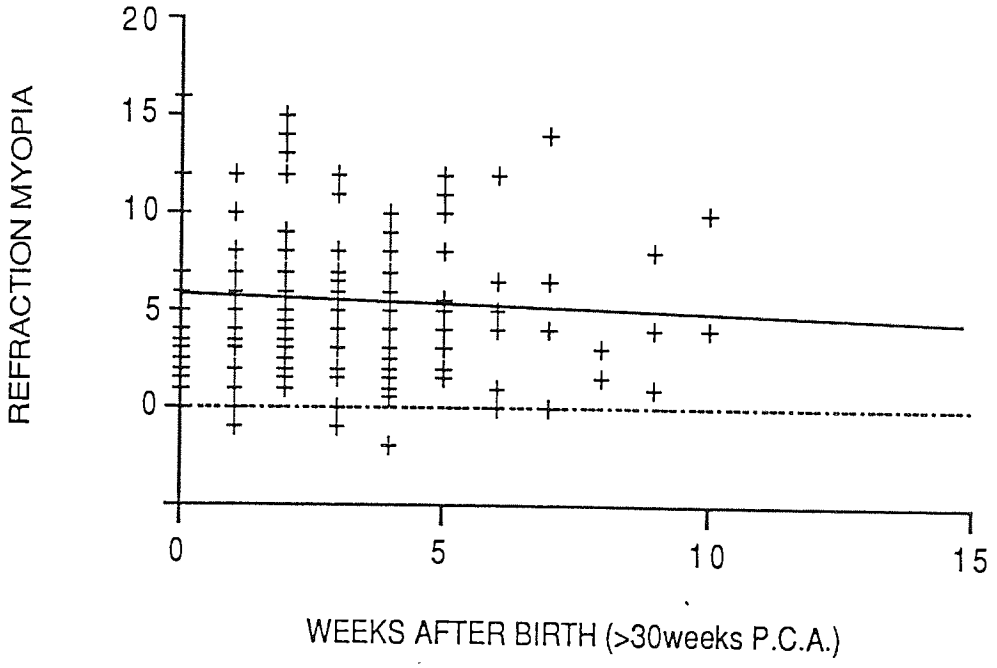


Fig 6.g.3 Weeks since birth and refractive error in those babies who had a gestational age of greater than 30 weeks at birth.

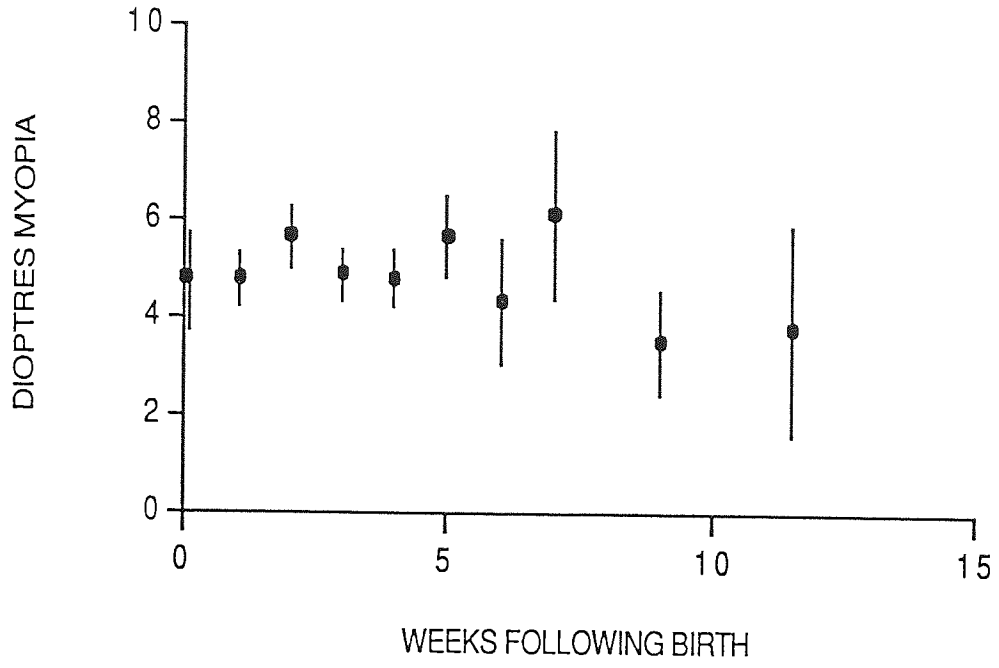


Fig 6.g.4 . Babies who had an estimated PCA of greater than 30 weeks at birth. Means and +/- 1 SE.

Figs6.g.1-6.g.4 readily show that a significant confidence level is obtained only when comparing age since birth and the regression towards emmetropia in the more premature age group.

In this group of 53 babies, where the total number of refractive examinations was 329, the predicted age of attaining emmetropia is 22.6 weeks of age with a standard error of the estimate of 0.4 dioptres. The 'F' ratio is 40.8 on the analysis of variance. Large numbers and wide variance again gives a poor coefficient of correlation of 0.3 but the 't' statistic of -6.4 shows $p = 0.001$.

6.3 Summary and analysis of refractive findings

In at attempt to rationalise the significant findings, Table 6.1 has been prepared which ranks the results of analysis of varience of the various sub categories that have been studied. Also included is the predicted age of attaining emmetropia. Categories not included are those of babies who weighed more than 1250 g at birth or those who had an estimated gestational age of greater than 30 weeks PCA at delivery. These latter groups have no statistical significance and no confidence levels regarding a regression towards emmetropia.

CONDITION ANALYSED	PREDICTION OF EMMETROPIA	F-RATIO
Weeks / <=1000 g	20 weeks old	52.8
PCA at Exam. / <=30 weeks at birth	20.1 weeks PCA	52.1
PCA at Exam. / <=1250 g	49.3 weeks PCA	47.4
PCA at Exam. / <=1000 g	46.8 weeks PCA	47.1
Weeks / <=1250 g	22 weeks old	42.5
Weeks / <=30 weeks at birth	22.6 weeks old	40.8

Table 6.1 Ranking of results of analysis of variance of sub-categories studied

It is interesting to speculate that the previously reported incidence of myopia associated with prematurity came primarily from reports having populations of babies that were similar in composition to the older and heavier babies seen in

this study. This would not appear to be unreasonable, as it is only recently that paediatricians have been able to expect the survival of premature babies weighing less than 1000 g or those that have a gestational age of less than 30 weeks.

Recent studies like those of Sharr *at al.* (1984) found little or no association with prematurity and myopia. Her study was conducted on populations that had modern technology available at birth allowing, it is assumed, not only those of very low birth weight to survive but to attain as near as possible stable metabolic and environmental conditions for their survival.

Although this study would perhaps imply that low birth weight babies, as opposed to very low birth weight babies, retain their myopia significantly beyond the expected time of delivery; there is no reported long term evidence of any significant level of sustained refractive problems observed at the Child Assessment Centre in Coventry.

As observed there is within all the groupings a wide distribution of refractive errors at all post-delivery and post-conceptual ages. This must clinically exacerbate the problem of differentiating the normal from those who may subsequently be discovered to have a high significant refractive error.

One aspect of this study that is intriguing is the apparent fall and rise in myopia between the ages of 26 and 32 weeks PCA as seen in Fig 6.c.2, (page 132) which shows the means and S.E.

bars for those babies weighing less than or equal to 1000 g. These trends are also evident in Fig 6.a.3, (page 125) the less than 1251 g birth weight group. This phenomenon also shows as irregular refractive errors in Figs 6.d.2 (page 135) and 6.d.4, (page 137) where the number of weeks since birth is analysed for the very low birth weight groups.

This variation is seen within the first few weeks of life and coincides with a time when the babies have survived long enough to be subjected to an increase in routine feed volumes. The unit policy was to increase the routine feed volumes to 200 ml per kilogram as soon as possible.

CHAPTER 7

REVIEW OF RESULTS

7.1 Retinopathy and screening criteria

This study reports on the state of retinal development and refractive state on the eyes of 138 babies between September 1982 and December 1984. During this period a total of 186 babies were observed, the criteria for inclusion in this study was survival to discharge and a minimum of three observations. Clinical oservations were made on a weekly basis.

Flynn *et al.* 1987) started a study in 1981 where they constantly monitored oxygen therapy in 214 infants with birth weights of 1300 g or less (1987). 119 infants (55.5%) were diagnosed as having ROP, most of whom were diagnosed 35-45 weeks PCA. Of the remainder, 83 infants (39%) were classified as negative and 12 (5.5%) were of indefinite diagnosis.

Of those diagnosed as having ROP, 65% showed signs of regression while under observation and 53% of these regressed completely whereas the remainder were still regressing at the last examination.

The infants were first examined at 32 weeks PCA and every 2 weeks thereafter. Almost 70% of the first examinations were during the first 6 weeks of life, but most initial positive

examinations were not until weeks 7-9. When taking PCA into account most first examinations were made between weeks 32 and 37. Most of first diagnoses were made during weeks 35-40 although a significant proportion of positive diagnoses were made earlier than 35 weeks (10%) and some later, after 40 weeks (18%).

If the results of Flynn's study are broken down into two birth weight groupings with the dividing line drawn at 900 g, the following may be extrapolated:

Birth weight	500-899 g
46 first examined at	8.9+/- 3.6 weeks of life
Diagnosed as ROP	41 (89%)
Age at diagnosis	9.4+/- 3.5 weeks of life
Negative at first examination	5 (11%)

Birth weight	900-1300 g
168 first examined at	4.7+/- 3.3 weeks of life
Diagnosed as ROP	78 (46.4%)
Age at diagnosis	5.7+/- 3.4 weeks of life
Negative at first examination	90 (53.6%)

A paper published by Archambault and Gomolin (1987) reported a study of premature infants in Montreal. They examined 157 newborn infants that had a birth weight of 2000 g or less seen between January 1983 and December 1985.

All their babies were given oxygen therapy to maintain a partial

pressure O_2 above 55 mm Hg requiring a FiO_2 of between 85% and 100%. Vitamin E was given by mouth once oral feeds were tolerated.

Birth weights were broken down into the following groupings:

Measure	1000 g	1001-1500 g	1501-2000 g	Total
Number	42	56	56	157
Avg. Wt.	844 gms	1267 gms	1745 gms	1324
Avg.PCA	27.3 weeks	30 weeks	32.4 weeks	30.1
Avg.PCA (on exam)	36 weeks	35 weeks	35 weeks	35.3

Out of this population of infants 15% (N=24) were diagnosed as having ROP. It was found to occur in 43% of the lowest birth weight group, 8% in the middle group and 2% in the highest birth weight group.

The grades or retinopathy were; one grade 4 from the middle birth weight group, seven Grade 2 from the lower two groups and sixteen Grade 1. All but one of these grade 1 retinopathies were found in the lower birth weight groups (13 in >1001 g 2 in 1001-1500 g). Unfortunately the study did not report how many regressed, or indeed progressed.

Brown , Biglan and Stretavsky (1987) examined the eyes of 2986 neonates from January 1977 through to December 1985. They again used birth weight as one of the criteria for screening, the second criteria being exposure to excessive oxygen. They were classified

under the Kingham system which integrates well with the international classification of ROP.

From this group of infants they found the incidence of ROP to be 56 (1.9%) ROP grades 3 or 4, the mean birth weight of these being1042 g for grade 3 and 971 g for grade 4. The conclusions that were drawn from this study were that screening should cover all babies that weighed less than 1600 g at birth and that had been exposed to added oxygen for more than 50 days. This is a decrease on the limits recommended by the American Academy of Pediatrics and the American College of Obstetrics and Gynaecology (Braun and Cafalo 1983) who suggest the upper limit to be 2000 g or an exposure to added oxygen.

This thesis attempts to limit the differentiation between birth weight and PCA at birth (see Fig 5.9 page 95). A greater emphasis on estimated PCA at birth is marginally supported from statistical analysis of my results (see Table 5.4, page 101). Fielder *et al.* (1986) also reported that the use of PCA is more reliable in the determination of the time to examine pre-term babies for ROP.

Of the 87 babies that were left in this study, following the pruning with regard to weight, time and number of serial examinations (see page 94), the incidence of maximum observed ROP is as follows:

No retinopathy	55.2%
Grade 1	26.4%
Grade 2	9.2%
Grade 3	6.9%
Grade 4	2.3%

These figures complement other contemporary studies mentioned before in this chapter. No absolute comparisons can be made between studies for the following reasons:

1. The differences in screening strategy.
2. The recognition of grade 1 ROP and in particular the clinicians' criteria for abnormal early changes.
3. The low numbers of grade 4 ROPs.
4. Different treatment regimens and available technology at different hospitals.

This study was conducted with stricter criteria than most other studies in so far that the number of observations on each individual and the time interval between observations was more stringent. Also the criteria for babies to be included in the final analysis enhanced these criteria, enabling a clinical recommendation.

When recommending from my results that all infants of equal to or less than 30 weeks PCA or less than 1251 g at birth should have routine ophthalmoscopic examinations from 33 to 37 weeks PCA, there is a proviso. The proviso is that others outside this category

may develop RLF but that my results show there is only a slight risk of this. Only the results in the upper right hand quadrant of Fig 5.9, (page 95) are being dismissed.

Between 1949 and 1984 there have been 14 reports of full-term babies that have developed ROP. The reports cover 64 infants, all of whom received little or no oxygen. Dixon and Paul (1951) suggested that some full-term infants are possibly born with retinal development that lags significantly behind that of other organ systems, leading to a process comparable to that in premature infants.

There have been also 12 reports on 95 infants born between 1950 and 1983 who had low birth weight (<2500 g) who have developed ROP but never received oxygen or received little oxygen. Brockhurst (1975) suggested that these infants were excessively responsive to the normal increase in arterial oxygen saturation that occurs at birth.

This research confirms that the ocular morbidity in the Special Care Baby Unit at Coventry Maternity Hospital from ROP is satisfactory and that the use of a Monocular Indirect Ophthalmoscope for screening is satisfactory. This ophthalmoscope also provides the neonatologist with an instrument that is much more acceptable for their use, especially as the level of skill required is less than with conventional indirect ophthalmoscope systems.

7.2 Refraction

Keith and Kitchen (1983) studied 111 infants who weighed less than 1500 g at birth. 33% showed some ocular defect:

Strabismus	19%
ROP	10%
Optic atrophy	2.7%
Refractive error	17%

Nissenkorn *et al.* (1983) examined 155 premature infants for the presence of ROP and myopia. Of these, 42 were found to have ROP, and of these 42, 21 (50%) had myopia between -0.25D and -15.00D (average -4.00D). Of the 113 infants that had no sign of ROP, 18 (15.9%) had myopia between -0.25D and -4.00D (average -1.50D).

This indicates a significant relationship between ROP and myopia. Whether the myopia is axial, corneal or lenticular has been questioned but 10 measurements made by Nissenkorn *et al.* showed that a variety of factors could cause or effect the myopia.

Anterior segment abnormalities have been known to occur in association with ROP, and recent attention has been directed mainly to anomalies in the corneal curvature. Hittner *et al.* (1979) reported abnormally steep keratometry readings associated with shallowing of the anterior chamber. Microphthalmia was reported by Terry (1945), King (1950), Reese *et al.* (1954) and Cohen *et al.* (1964), and this was confirmed by ultrasonography by Gitter *et al.* (1968) and Steindler (1973).

Schaffer Quinn and Johnson (1984) compared 26 infants that had totally resolved ROP with a group of 38 infants that had no history of ROP. They found that 7.9% of the normal group developed mild myopia but in the resolved ROP group 17% were myopic. In the resolved ROP group the myopia was more severe in two-thirds of the cases.

Kelly and Fielder (1987) reported eight infants with ROP whose corneal diameters were abnormally small, ranging from 9.5 mm to 10.5 mm. In six the condition was unilateral and in two bilateral.

Kelly and Fielder did not consider a retrolental membrane to be the sole cause of delayed growth as this membrane only developed in four eyes in three infants. They concluded that growth may be adversely affected during the severe acute stage of ROP and is not necessarily a sign of advanced cicatricial disease. They also found that, with the exception of one case, the microcornea was present in the eye with the most advanced disease. Kelly and Fielder found myopia in four of the eyes and no refractive error in two eyes. The hypothesis that the myopia of prematurity was directly associated with microcornea could not be proven.

It has been clinically observed by neonatologists that babies appear to be a little exophthalmic during this period of high volume/high solute feeding. It is tempting to speculate that these changes in observed refractive error reflect the peterm baby's limited ability to control its plasma osmolality precisely. It has been clinically observed that the preterm infant has a limited ability to handle water and solute when compared with a full term

infant (Al-Danham *et al.* 1984). The physiological corridor down which the preterm infant must proceed for fluid and electrolyte balance is therefore narrow. These infants can neither conserve water or sodium in the presence of a relative deficit nor excrete a relative excess.

The question of effects of electrolyte balance, plasma osmolality, aqueous outflow and intraocular pressure pose a question with these sub acute buphalmus looking babies.

Tonometry by any means proved unsuccessful because the eyes were small, palpebral apertures small and the eyelids tight. Also, what information was obtained with a Digilab pneumatic tonometer, intraocular pressures were apparently normally in the region of less than 10 mm Hg., the question of increased pressure and axial myopia due to very elastic eyes is therefore only assumed.

As previously mentioned (page 68) most studies into the pathenogenesis of the myopia associated with prematurity were carried out on infants that were of post term age. This study demonstrates the high and variable levels of myopia that exist in the preterm period. It also hypothesises that the state of the general physiology of the baby, and in particular the plasma osmolality, may have a bearing on the eventual outcome. The apparent differences in the trend towards emmetropia in the different birth weight groups could therefore be as a result of the inability of the older premature baby's eyes to adapt to changes in their aqueous dynamics. However the work by Schaffer *et al.*

(1984) would not appear to support this. It is therefore comforting to read the report by Scharr *et al.* (1984) who found no association between prematurity and myopia.

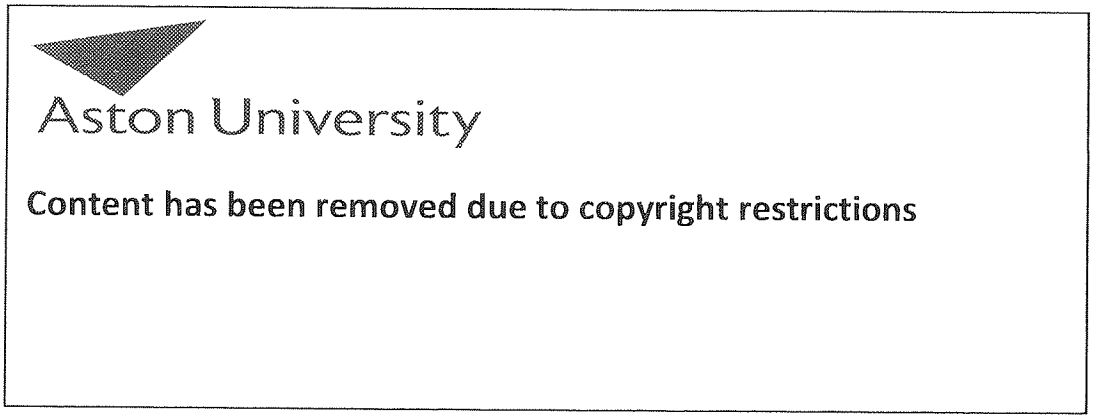
From all the literature on ocular morbidity, in the form of refractive disorders, especially myopia associated with premature birth, the extent, severity and the type of baby at risk remains obscure. This study shows what is happening refractively in the pre-term period to babies with different birth weights and PCAs.

Further research is required to understand the reasons for the different rates of change in refractive status during the pre-term period as well as the ocular characteristics of myopic eyes of babies of premature birth.

This study did not support an association between grades of ROP and refractive status.

APPENDIX I

Submission to the Clinical Research and Ethical Committee
Warwickshire Postgraduate Medical Centre



APPENDIX 2 Example of record sheet used and classification criteria: Kieth and International.

APPENDIX 5 Blood oxygen analysis during stay in the SCBU

APPENDIX 6

Supporting Publications

1. Linfield, P. B. and Davies, J. G. (1984)

   A study of the change in mean sphere refractive errors obtained from very low birth weight infants.

   Trans. First International Congress British College of Ophthalmic Opticians (Optometrists) London UK

2. Linfield, P. B. and Davies, J. G. (1987)

   Screening for the Retinopathy of Prematurity

   Ophthal. Physiol. Opt. **7** 401-402

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