RETINAL PATCHING

A NEW APPROACH TO THE MANAGEMENT

OF SELECTED RETINAL BREAKS

By

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ABSTRACT

This thesis examines a new approach to the management of retinal breaks, a retinal patching technique, which could be used to repair retinal breaks by restoring retinal continuity. It is envisaged that this technique could be used as a novel treatment for selected cases where retinal reattachment is currently difficult to achieve, and also to avoid some of the complications of established procedures.

Potential patching materials (17 adhesives and 26 "substrates", both biological and synthetic materials) were evaluated in vitro, in order to select the most suitable for evaluation in an animal model. Bovine eye cup experiments, tissue culture studies and limited organ culture were undertaken to evaluate the physical properties, toxicity and biocompatibility of the materials. Following these experiments two adhesives (octylcyanoacrylate and Tisseel, a fibrin preparation) and two substrates (PVdF and propylene) were evaluated in vivo.

In the animal studies there were three Control Groups (43 rabbits) and two Experimental Groups (37 rabbits). The animal work showed that retinal patching is possible, but difficult. The procedure had to be abandoned in 6 Experimental animals (6/37) compared to 1 Control animal (1/43) because of major operative complications, e.g. dense vitreous haemorrhage (Chi sq test, p=0.028, exact p=0.013). Minor posterior segment operative complications, e.g. inadvertent retinal tears, occurred significantly more often in experimental animals (12/31) compared to controls (4/42)(Chi sq, p=0.003). When octylcyanoacrylate was used to secure the substrate (Experimental Group A) 5/16 (31.3%) eyes developed new tears at the edge of the patch after air/fluid exchange. All developed localised retinal necrosis in the vicinity of the adhesive in the immediate postoperative period. Atrophic retinal holes formed in necrotic retina in 4/10 (40%) animals followed for one week. All eyes with iatrogenic or atrophic retinal breaks subsequently developed retinal detachment. At the end of the period of study (4 weeks) only 2/7 eyes had adherent patches and flat retinas. Generalised epiretinal membranes (ERMs) had developed in 3 of the animals with detached retinas.

When the substrate was applied with Tisseel (Experimental Group B, 15 animals) air/fluid exchange caused fewer problems. However, the fibrin adhesive lysed in all eyes within 14 days, and the patch separated in several animals. Epiretinal membrane formation was a notable feature of patching with Tisseel. At two weeks 3/8 eyes had developed focal ERMs and a further 3 had generalised ERMs. At the end of the study period all eyes had extensive, generalised ERMs (8/8) often
causing marked vitreoretinal traction. Retinal detachment was present in 5/10 eyes examined at 1 week and developed in 3 more eyes during follow up.

Using stratified analysis retinal detachment of any extent and generalised epiretinal membrane formation occurred significantly more often in Experimental Groups animals compared to Controls. Retinal detachment of any extent and generalised epiretinal membrane formation occurred more frequently in Experiment Group B eyes compared to Group A, but this did not reach significance.

Histological examination confirmed the presence of early coagulative necrosis and late retinal atrophy in retina beneath and adjacent to patches made with octyl-cyanoacrylate. The underlying retinal pigment epithelium was also abnormal, with vacuolation and separation of cells from Bruch's membrane. Intense inflammatory infiltration of the choroid and ciliary body characterised eyes where Tisseel was used as the adhesive.

This study has shown that retinal patching is technically possible, but is associated with operative and postoperative complications. Difficulties in delivering, manipulating and applying the patching materials were responsible for some of the complications. Others occurred as a result of toxic and biological effects of the adhesives selected for study. The rabbit was not an ideal model, as the ocular tissues are thin and the eye responds vigorously to insult. Some of the complications may have been less pronounced if another species had been used.

Retinal patching remains a compelling attractive means of managing selected retinal breaks, at least in theory, but involves many practical difficulties which could not be overcome within the constraints of this study.
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CANDIDATE'S OWN RESEARCH AND OBSERVATIONS

The in vitro experiments, surgery, clinical observations, and recording and entering of data were all performed by the candidate. Medical statisticians assisted with analysis of the in vitro and in vivo data after discussion; interpretation of the analysis was done by the candidate. Specimens for electron microscopy were prepared by the candidate and those for light microscopy by laboratory staff. Examination and photography of histological specimens was done by the candidate. All the photographs were taken, developed and printed by the candidate, except colour prints. All figures, tables, illustrations, graphs and text were prepared by the candidate.

ORIGINALITY AND CONTRIBUTION TO MEDICAL SCIENCE

Retinal patching is a technique that has not been explored previously. Adhesives have been evaluated experimentally and used clinically to seal retinal breaks to the underlying retinal pigment epithelium (retinopexy), but the question of restoration of retinal continuity has not been addressed before.

In this thesis adhesives and substrates that could be used to create a retinal patch have been extensively evaluated in vitro by modifying techniques already described, such as cell settlement, but these investigations have not been undertaken before using RPE and retinal glia. The in vivo model was developed and modified using procedures that have already been described for the rabbit, but no other author has attempted to repair retinal defects by patching.

G. (Signature)
CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 THE PROBLEM EXAMINED IN THE THESIS

Retinal detachment (RD) can be divided into rhegmatogenous and non rhegmatogenous RD. In the former retinal breaks or tears (i.e. full thickness defects in the neuroretina) are present, whilst in the latter no breaks are present. Rhegmatogenous RD can be subdivided into simple and complicated RDs, the division being largely determined by practical considerations. Simple RDs respond to conventional techniques, whereas complicated RDs generally require an internal approach using closed microsurgical techniques, with drainage of subretinal fluid (SRF), cryotherapy or laser endophotocoagulation, and internal tamponade with gas or silicone oil. Scleral buckling may also be required.

The retinal reattachment rate for simple RDs is high (84-96%), provided that the right surgical approach is selected and the procedures are correctly applied. The reattachment rate for complicated RDs is not so good (55-80%), however, for a variety of reasons that will be outlined in Section 1.8.1. In addition, some of the techniques used for the management of complicated RDs have potentially sight threatening complications; this is particularly true of internal tamponade with silicone oil.

The concept of retinal patching offers a unique approach to the management of RD. The aim of retinal patching is to restore retinal continuity, an issue which none of the currently used or experimental techniques addresses. With restoration of retinal continuity recruitment of fluid to the subretinal space is prevented, and physiological mechanisms would then be able to promote retinal reattachment, by posterior evacuation of SRF. In the patching technique retinal breaks would be repaired by applying a substrate and adhesive to the internal limiting lamina (ILL) surrounding a break so as to achieve a complete repair (Figure 1.1). The patch would be applied after vitrectomy, with relief of vitreoretinal traction and membrane peeling as required. Conventional methods of "break closure" (i.e. scleral buckling and internal tamponade) and "break sealing" (i.e. cryotherapy or laser photocoagulation) would not be required.

As retinal patching has not been undertaken before it is not possible to review the literature on the subject. What I have attempted to do in the Introduction is to present a rationale for retinal patching by reviewing the current state of knowledge.
concerning factors that maintain attachment of the retina, and the pathogenesis of rhegmatogenous RD. Techniques that are currently used for treating complicated RDs are outlined, including their complications. Where relevant, complications that could, in theory, be avoided if a retinal patching technique were available are highlighted.

Several adhesives have been used experimentally to seal retinal breaks (retinopexy) and some have been used clinically (Figure 1.2). The way in which retinal patching differs from retinopexy, and theoretical reasons why retinal patching may be a superior approach, are put forward.

Consideration is given to the possible indications for retinal patching and finally, the qualities that would be required of ideal patching materials are discussed.

1.2 SCOPE OF INQUIRY

When considering the technique of retinal patching the first problem was to identify materials suitable for repairing retinal defects. A wide range of adhesives and substrates were evaluated using in vitro experiments, to explore their biological and physical characteristics. From this range two adhesives and two substrates were selected for evaluation in an animal model. The animal work was undertaken to assess the technical aspects of applying a patch and to evaluate the complications associated with the technique. The animal work also sought to address whether retinal patching could promote retinal reattachment, whether the patching materials were toxic or stimulated inflammatory or cellular reactions, and to evaluate the outcome of these reactions.

1.3 HISTORICAL PERSPECTIVE

Retinal detachment was first described in a human eye 150 years ago, although it had been observed in animal eyes as early as 1722(1)(from Duke Elder 1962). Detailed fundus examination became possible after the direct ophthalmoscope was introduced in 1851(2), and many cases of RD were reported subsequently. Several theories were put forward to explain the findings which formed the basis for medical therapy and early surgical treatments(1,3)(from Duke Elder and Benson).

Retinal holes were reported by Coccius in 1853(3)(from Benson), although along with many other ophthalmologists at that time he did not appreciate their significance. Miller suggested that retinal breaks were produced by traction of the vitreous on the retina and Leber stressed their frequency in "idiopathic" cases. He
Figure 1.1 Diagramatic representation of retinal patching

A = Adhesive
S = Substrate
R = Retina
RPE = Retinal pigment epithelium

A With suitable materials a patch could be applied to repair a retinal defect in a fluid filled eye without peroperative retinal flattening B Restoration of retinal continuity would promote retinal reattachment
Figure 1.2 Diagramatic representation of retinopexy

A = Adhesive
RPE = Retinal pigment epithelium
R = Retina

A and B Small applications of adhesive applied to seal the edges of a large retinal break
found retinal breaks in 70% of recent RDs, noticed that retinal elevation started in the vicinity of a break and concluded from clinical evaluation and histological study that retinal breaks were a consequence of vitreous degeneration and collapse (from Benson). However, the other theories that held sway then meant that the significance of retinal breaks was not appreciated. Leber changed his original and, as it transpired, accurate view following histo-pathological studies of eyes which were almost certainly complicated by proliferative vitreoretinopathy (PVR). His revised opinion was that vitreous degeneration and traction occurred secondary to retinal break formation.

Leber's original ideas were largely overlooked until they were revived and expanded by Jules Gonin, during the 1920's. Detailed clinical and histological studies led Gonin to conclude that retinal tears were produced by vitreo-retinal (VR) traction. Once retinal continuity was breached, fluid from the vitreous cavity passed through the retinal break into the subretinal space elevating the sensory retina (4). Gonin's theories rapidly gained acceptance, largely because of his surgical success (5,6). He realised that sealing the retinal break to the underlying choroid would promote retinal reattachment. Surgery with this aim in mind was first undertaken in 1919, using scleral cautery (ignipuncture). Gonin's theories were developed by other ophthalmologists who suggested that retinal degeneration could also result in retinal break formation. Poor choroidal and retinal circulation in the periphery were thought to account for these sites being particularly vulnerable (7) (from Tolentino 1976).

Factors that normally sustain retinal attachment, the pathogenesis of retinal break formation and factors that initiate RD and promote retinal reattachment are now more clearly understood. This has influenced the surgical management of RD, with increasingly successful results.

1.4 FACTORS THAT SUSTAIN RETINAL ATTACHMENT

The retinal pigment epithelium (RPE) and neuroretina are derived from different parts of the embryonic optic vesicle. When the retina detaches the neuroretina elevates, leaving the RPE attached to Bruch's membrane. The term RD is therefore not entirely accurate, but has become widely accepted.

Specialised cell junctions do not exist between photoreceptors (PRs) of the neuroretina i.e. rods and cones, and underlying RPE cells. Other anatomical and biochemical mechanisms are responsible for maintaining attachment of the neuroretina to the RPE. The neuroretina can be separated from the RPE much more
readily postmortem than in vivo which suggests that dynamic, physiological mechanisms are also involved(8).

1.4.1 Anatomical Factors:

The RPE is composed of a monolayer of highly specialised, polarised, melanin-containing epithelial cells which underlies the neuroretina in the posterior two thirds of the globe. Scanning (SEM) and transmission electron microscopy (TEM) reveal that the apical surface of RPE cells is composed of a mass of long and short microvilli which interdigitate with cone and rod outer segments (OS). This close association provides some degree of mechanical attachment, with photoreceptor OS and RPE microvilli acting like 'Velcro'(9).

1.4.2 Biochemical Factors:

If the neuroretina is mechanically separated from the RPE of post mortem eyes, SEM shows fractured photoreceptor OSs adherent to RPE microvilli, and fragments of RPE microvilli adherent to photoreceptor OSs. These findings suggest an additional adhesive force between the two cell types. The space between photoreceptor OS and RPE microvilli contains a complex matrix of glycosaminoglycans, glycoproteins and proteoglycans (the interphotoreceptor matrix, IPM). The IPM was initially thought to be amorphous and unstructured, but studies using monoclonal antibodies and specialised staining techniques show that the IPM is in fact a highly structured region (10). Hollyfield has demonstrated that cones are invested by a cylindrical proteoglycan sheath which extends from the outer limiting lamina of the retina beyond the tip of the cone and terminating at the apical membrane of RPE cells (the cone domain) (11). More recently rod domains have also been demonstrated by staining extracted IPM with wheat germ agglutinin conjugated to rhodamine(12). Fibronectin (FN), a well recognised cell attachment factor, has been found in rat RPE apical villi. Hollyfield suggests that FN may act as a biochemical adhesive, binding proteoglycans of PR domains to plasma membranes of both RPE microvilli and cone OS (12). Kain has provided additional experimental evidence for a passive cellular adhesive mechanism from his studies using rabbits (13).

1.4.3 Physiological Mechanisms:

Experimental evidence indicates that there is a net flow of water from the vitreous cavity, through intact retina and RPE, into the choroid(14). If retinal permeability is less than RPE permeability this will tend to create a slight negative pressure in the
space between the retina and the RPE. The retina does have a small, measurable resistance to flow (15), which is greater than that of the RPE (16, 17). Mechanisms that may induce this flux include hydrostatic pressure differences between the vitreous cavity and the orbit, high choroidal osmotic pressure and an active transport pump at the level of the RPE.

A significant pressure difference does exist between the vitreous cavity and orbit (because of intraocular pressure), but only a very small pressure difference has been measured between the vitreous cavity and the subretinal space. Hydrostatic pressure alone would not create much flow of fluid across the RPE, unless it was highly permeable. The fact that the retina does not detach in hypotensive eyes suggests that this mechanism is probably not important in maintaining retinal attachment.

Choroidal capillaries do not have tight junctional complexes. Proteins, ions and water can pass unimpeded from the circulation into the extracellular compartment of the choroid. Conversely, RPE cells and retinal vascular endothelial cells do have tight junctional complexes which prevent the flow of proteins and fluid between cells. The extracellular space of the retina is relatively low in protein therefore, while that of the choroid is high. An osmotic gradient is established between the choroid and the retina and water flows along the gradient, from the vitreous cavity into the choroid. However, the IPM must also exert an osmotic pressure gradient, acting against the osmotic gradient of the choroid, tending to keep water in the subretinal space. One of the roles of the IPM may be to act as a "spacer", to ensure that constant dimensions are kept between photoreceptors OS and RPE microvilli (18).

Experiments have been undertaken which demonstrate the importance of osmotic factors in maintaining retinal attachment. For example, if hyperosmotic solutions are injected into the vitreous cavity RD rapidly ensues (19). If choroidal osmotic pressure is reduced by intravenous infusion of low molecular weight dextran, the rate of clearance of radio-labelled water from the vitreous cavity into the choroid is also reduced (18). Interpretation of these results is difficult because other mechanisms, such as toxic effects on RPE structure and function or damage to retinal vasculature, may also be contributory factors.

The rate of absorption of SRF after successful RD surgery is variable, being quicker in recent RDs and slower in long standing RDs. The protein composition of SRF increases with the duration of the RD and is thought to be one of the major factors determining the rate of absorption of SRF. If physiological saline is injected
into the subretinal space of monkeys it absorbs much more rapidly than if serum is injected, which supports this view(20). However, if osmotic factors were the only mechanisms involved injection of serum into the subretinal space would result in permanent retinal elevation, as the osmotic pressure in the subretinal space and choroid would be the same. Metabolic toxins(21) and hypoxia(22) have also been shown to reduce the rate of absorption of SRF. These findings suggest the presence of an active pump at the level of the RPE.

RPE cells, like all epithelia, have tight junctional complexes. Movement of ions and solutes occurs across cell membranes, rather than between cells. Ions can cross plasma membranes via channels, which are non energy requiring, or via pumps, which require energy provided by ATP. Ions move through channels in a direction determined by chemical or electrochemical gradients, whereas pumps move ions against gradients, which establishes a difference between the intracellular and extracellular environments. RPE tight junctions divide the cell membrane into basal and apical membranes. Each are functionally independent and have different transport mechanisms(23). Current experimental evidence suggests that there is a net flow of potassium and chloride from the subretinal space to the choroid, and a net flow of sodium in the opposite direction(24). Potassium channels exist in apical and basal membranes. Active ATPase pumps in the apical membrane move chloride and potassium into the RPE and sodium from the cell into the subretinal space via bicarbonate and sodium / potassium / chloride co-transporter pumps, respectively (24,25). The site, controlling mechanisms and direction of movement of hydrogen and bicarbonate ions is more contentious, with conflicting evidence being provided by different authors. Some of the discrepancies may be attributed to variations in the species of animals studied and to differing experimental models. Diurnal fluctuations in function and effects of retinal illumination may be other confounding variables(24). What does seem clear is that the transfer of ions across the RPE is a dynamic process which is capable of responding to changes in the metabolic activity of PRs. A constant chemical and electrochemical environment is therefore maintained in the subretinal space. The net movement of ions and solutes across the RPE also determines the movement of water across this cell monolayer. The evidence suggests that the movement of water is from the subretinal space into the choroid(26), although it is not clear at present which pump/s is/are most important. The negative hydrostatic pressure exerted by this mechanism is another factor that contributes to retinal attachment.

In successful RD surgery closure of a retinal break leads to retinal reattachment. It is assumed that the "RPE pump" draws fluid from the subretinal space into the choroid. One rationale for exploring the retinal patching technique is that this
mechanism would promote retinal reattachment after restoration of retinal continuity.

1.5 PATHOGENESIS OF RETINAL BREAK FORMATION:

Retinal breaks form either as a result of vitreous traction exerted on areas of abnormal V-R adhesion, producing retinal tears, or from retinal degeneration, producing atrophic retinal holes.

1.5.1 The Vitreoretinal Juncture:

The vitreous normally completely fills the posterior segment of the globe. It is a gel composed of an ordered meshwork of type II collagen fibres, connected by sulphated glycosaminoglycans, interspersed with hyaluronan and chondroitin sulphate(27). In the body of the gel the collagen fibres form a three dimensional network whereas in cortical gel they are more closely arranged and are oriented parallel to the retinal surface, in close proximity to the inner limiting lamina (ILL). The ILL is a modified basement membrane composed of type IV collagen, proteoglycans, laminin and fibronectin. The ILL differs from other basement membranes as it also contains type I collagen, which increases its tensile strength(28). The ILL separates Muller cell end plates from the vitreous, and TEM reveals a trilaminar structure with a central lamina rara sandwiched between the laminae densa externa and interna(29).

In humans the ILL shows considerable topographical variation, being thickest in the equatorial and posterior zones and thinnest at the macular and vitreous base. In the vitreous base the ILL frequently shows degenerative changes (even in young eyes), with focal defects and loss of underlying Muller cell attachment plaques. More pronounced degenerative changes consist of macrophage infiltration and incarceration of vitreous within degenerative crypts(30).

At the vitreous base cortical vitreous fibres are thick, numerous and are often oriented perpendicular to the ILL. Vitreous fibres appear to insert into the ILL and fibrils can also be observed traversing the ILL. These sites coincide with Muller cell hemidesmosomal junctions(30). The attachment of the vitreous at the vitreous base is therefore structural. The same applies at the optic disc where the thick ILL of the peripapillary retina gives way to a thinned lamina over the optic nerve head. At the transitional zone focal defects in the ILL allow vitreous fibres to become incarcerated between surface glial cells(30). Elsewhere cortical vitreous fibres lie parallel to the retinal surface and routine TEM techniques show no direct
connections between vitreous fibres and the ILL. However, using special fixation
techniques and stains, such as Alcian Blue which stains glycoproteins, biochemical
linkages between vitreous fibres and the ILL become apparent(29). This would
account for the weaker attachment of the vitreous at sites other than at the vitreous
base and disc.

1.5.2 Posterior Vitreous Detachment:

As a result of ageing the vitreous undergoes degenerative changes; the normal
architecture of the gel is lost as collagen fibres coalesce, leaving fluid-filled lacunae
(vitreous syneresis). Should a central lacuna break through the thin premacular
vitreous cortex fluid vitreous can pass into the preretinal space. Ocular movement
and rotational movement of the vitreous results in complete separation of the
vitreous cortex except at the vitreous base (posterior vitreous detachment, PVD).
As long as there are no areas of abnormal V-R adhesion this event is usually
benign, although vitreous opacities can give rise to symptoms of floaters.

Syneresis and PVD occur at an earlier age in myopic and aphakic eyes, and
following intravitreal haemorrhage, posterior segment inflammation, and trauma.
After intra-capsular cataract extraction loss of compartmentilisation of the eye
promotes dispersion of hyaluronic acid from the vitreous, which hastens syneresis.
RD occurs in 0-3.2% of patients following Nd:YAG posterior capsulotomy, with axial
myopia being a risk factor(31). Several mechanisms have been proposed to
account for this, but the most likely explanation is that removal of the barrier
provided by the posterior capsule accelerates vitreous syneresis and PVD(32).
Trauma and posterior segment inflammatory disease are thought to promote
syneresis and PVD as a result of release of proteolytic enzymes by activated
macrophages which invade the vitreous cavity after these insults(33). Ferrous ions,
derived from haemolysed red blood cells, produce free hydroxly radicals which also
degrade hyaluronic acid (34).

1.5.3 Dynamic Vitreoretinal Traction:

If there are focal sites of abnormal adhesion between the vitreous and retina PVD
can result in retinal tears which are said to occur secondary to dynamic vitreous
traction. The rotational energy of the mobile vitreous, induced by ocular movement,
is transferred to the area of focal adhesion. These tears are usually "U" shaped,
sometimes with an avulsed operculum of retina. Their formation is frequently
accompanied by symptoms of flashing lights and floaters.
Lattice degeneration, found in 8% of eyes, is a common cause of retinal tears, and is seen in 30% of eyes with rhegmatogenous RD(35). Histologically lattice degeneration is characterised by circumscribed areas of retinal thinning, disruption of the ILL, loss of retinal capillaries and obliterative fibrosis of larger vessels. Morphological abnormalities are also present in the underlying RPE, and the vitreous appears abnormal. Over affected retina the vitreous shows signs of liquefaction, and is separated from the retina. At the borders of the lattice lesion the vitreous is condensed, and retinal glial outgrowths cause abnormal vitreoretinal attachment(36). PVD may cause retinal tears, which characteristically form at either end of the area of lattice.

Tears secondary to dynamic vitreous traction may also develop in relation to peripheral retinal vessels, causing vitreous haemorrhage. SEM has revealed that the ILL is thin over peripheral retinal vessels, glial cell processes have been observed on the surface of the retina and cortical vitreous is often adherent(37). TEM of posteriorly situated vessels has also shown thinning of the overlying ILL, occasional ILL breaks, degeneration of inner retina and incarceration of vitreous fibrils. Retinal blood vessels thus seem to be sites of V-R adhesion(38) and PVD may cause tractional retinal tears across a blood vessel.

Abnormal V-R adhesion may also occur in areas of chorioretinal scarring secondary to inflammatory disease, trauma or surgical intervention with laser photocoagulation or cryotherapy.

Many retinal tears caused by dynamic vitreous traction occur in retina that appears normal; these tears and those related to vitreous attachment over retinal vessels usually occur in the equatorial region, or in pre equatorial region of the fundus where there may be irregularities in the posterior border of the vitreous base.

Giant retinal tears, i.e. anterior tears that extend circumferentially for more than 90 degrees of the globe, are caused by dynamic vitreous traction exerted on the posterior border of the vitreous base. They occur in high myopia, aphakia, following trauma and in association with systemic collagen diseases such as Marfan's syndrome. In Wagner- Stickler's syndrome (an autosomally inherited disease with associated systemic abnormalities), the vitreous appears abnormal, with condensations forming strands and veils. Typically the fundus has multiple areas of lattice-like degeneration which are characteristically paravascular and which may extend posterior to the equatorial zone. Multiple areas of abnormal V-R attachment can be detected clinically. In this condition dynamic vitreous traction can give rise
to multiple retinal tears at unusual sites(39) and the risk of bilateral giant retinal tears is high(40).

Dynamic vitreous traction is thought to play a role in the pathogenesis of macular holes, with high myopia and trauma being risk factors (41). Vitreoretinal strands can sometimes be observed preoperatively(42,43), or they may only become apparent during vitrectomy(44). In highly myopic eyes however, V-R strands are often not detectable(45).

1.5.4 Static Vitreoretinal Traction:

Retinal tears can also be caused by static vitreous traction which occurs in the proliferative vasculopathies and following posterior segment trauma. Breaks develop as a result of contraction of abnormal fibrous or fibrovascular tissue growing within the vitreous or on the detached posterior hyaloid.

In the vaso-proliferative retinopathies (e.g. diabetic (PDR) and sickle cell retinopathy, Eales’ disease, ischaemic venous occlusions and retinal vasculitic conditions) fibrovascular proliferations arise from retinal vessels. They lyse and penetrate the ILL and grow on the scaffold provided by the attached posterior vitreous face. This produces areas of abnormal V-R adhesion. As the tissue evolves, cellular mechanisms cause contraction of the membrane which may produce tractional RDs or, if the retina should tear, combined traction/rhegmatogenous RDs. In PDR retinal holes are frequently situated posteriorly, along the vascular arcades, or at the posterior pole(46). The importance of V-R relationships in the natural history of PDR has been shown in a series of 172 patients. Only one eye with complete PVD went on to develop proliferative disease(47) showing that PVD prevents PDR.

1.5.5 Retinal Degeneration:

Retinal holes can also form in areas of retinal degeneration i.e. lattice or snail track degeneration. In these conditions PVD is not implicated in the pathogenesis of the break.

Lattice degeneration is the commonest form of retinal degeneration and small, round, atrophic retinal breaks are characteristically found within the affected area. In a large study by Byer, atrophic retinal holes were found in 35% of eyes with lattice degeneration(48).
Other degenerative conditions that can give rise to retinal holes include retinoschises (outer and inner leaf breaks). Atrophic retinal holes can also develop in the thin, gliotic retina overlying colobomata (49,50).

1.6 PATHOGENESIS OF RETINAL DETACHMENT:

The incidence of RD is approximately 1/10,000/annum, but it has been calculated that only one in 70 retinal breaks gives rise to RD (51). Full thickness retinal breaks have been found in 3% and 2.4% of postmortem eyes (52,53). All eyes with breaks were from elderly patients and PVD was seen in 79%. In one long-term clinical study of 113 patients with asymptomatic retinal breaks none developed RD (54). In another study only 3/108 eyes detached during the period of follow up (51). The conclusions that can be drawn from these studies are that atrophic round holes (where there is no dynamic vitreoretinal traction) and operculated tears (where vitreoretinal traction has been relieved) and asymptomatic "U" tears are common and benign. Symptomatic "U" tears (i.e. those where the formation of a retinal break has not completely relieved vitreoretinal traction) are more likely to lead to RD, with the high risk period being the first three months after the tear occurred.

1.6.1 Initiation of Retinal Detachment:

Movement of posterior segment intraocular fluids is thought to be a mechanism that initiates RD (55) (from Machemer 1984). In eyes with PVD fluid in the retrohyaloid space is less viscous than vitreous gel and flows more rapidly. Rotational eye movements will set up fluid currents and it has been suggested that eddy currents flowing across the edge of a retinal break will tend to elevate it. Once the edge of the tear has elevated retrohyaloid fluid is channelled into the subretinal space. In eyes with atrophic breaks without PVD this mechanism is probably not important, which may explain why these holes rarely lead to RD; if they do, the RD usually progresses slowly, often remains localised and may spontaneously reattach (55).

1.6.2 Derivation of Subretinal Fluid:

Experiments have been undertaken which demonstrate that in rhegmatogenous RD fluid flows from the vitreous cavity, through the retinal defect, into the subretinal space. These findings are relevant to retinal patching; if the retinal break could be repaired further fluid would not enter the subretinal space and physiological mechanisms would evacuate SRF into the choroid, causing the retina to reattach.
Eyes with RDs, particularly chronic RDs, are often hypotonous with mild anterior chamber flare and elevated SRF protein. These findings have several possible explanations. First, the RD could lead to a low grade uveitis with blood retinal barrier (BRB) breakdown and reduced aqueous production. Secondly, eyes with RDs may have altered intraocular fluid dynamics and mild BRB breakdown, but normal aqueous humour production and thirdly, the RPE may have increased permeability.

Several investigators have studied the BRB and intraocular fluid dynamics in patients and in animal models of retinal detachment. Pederson used a monkey model of long standing RD. Fluorescein isothiocyanate/ dextran (molecular weight 70,000) was injected intravenously, to evaluate the BRB. The same tracer was injected into the anterior chamber and later into the vitreous cavity, to assess intraocular fluid dynamics. Results showed the BRB to be intact, as fluorescein did not enter the anterior chamber after intravenous injection and none could be detected in the subretinal space. Fluorescein was cleared more slowly from the anterior chamber of eyes with RDs than from control eyes, and when injected into the vitreous cavity fluorescein accumulated in the subretinal space, where it persisted. These findings support the hypothesis that there are altered fluid dynamics in eyes with RDs, with a slow flow of fluid from the vitreous cavity through the retinal break into the subretinal space. As physiological mechanisms move SRF into the choroid larger protein molecules become sequestered in the subretinal space(56).

Little's clinical studies, using anterior segment fluorophotometry, are at variance with Pederson's. He found increased blood-aqueous barrier breakdown in eyes with retinal detachment compared to fellow eyes, which was not influenced by the duration of the detachment(57).

Tsuboi has demonstrated increased permeability across the RPE in eyes with rhegmatogenous retinal detachment compared to fellow eyes(58).

Subretinal fluid contains a variety of proteins some of which are thought to result from degradation of vitreous and retinal components and others which are serum derived. Rose measured immunoglobulin (IgG, IgA and IgM) levels in serum, SRF and vitreous from patients undergoing vitrectomy for complicated RD to investigate whether mixing of SRF and vitreous fluid took place across retinal breaks. Higher levels of immunoglobulins were found in SRF than in both serum and vitreous, which would suggest either local production or accumulation of these proteins. The varying classes of immunoglobulins were found in different proportions in each of
the fluids, suggesting that mixing of vitreous cavity fluid and SRF did not occur. The investigators support Pederson's conclusions that in eyes with RD there is a net flow of fluid from the vitreous cavity into the subretinal space through the retinal defect, with accumulation of larger molecules as water and ions are evacuated by the RPE into the choroid(59).

Analysis of SRF from eyes with recent and long standing RD shows that in the former only low molecular weight proteins are present, whereas in the latter higher molecular weight proteins, including IgA and IgM are detectable(60). These large proteins cannot usually cross the BRB, suggesting that in long standing RDs the blood-ocular barriers are compromised. In light of the more recent work outlined above, these proteins need not necessarily have been produced locally - they could have accumulated in the subretinal space as a result of generalised breakdown of BRB and abnormal fluid currents within the eye.

1.7 CURRENT SURGICAL PROCEDURES AND ASSOCIATED COMPLICATIONS:

The aims of techniques used in RD surgery are to:

1. relieve vitreo-retinal traction (i.e. to mobilise the retina in the vicinity of a break)
2. close all retinal breaks (to prevent recruitment of further subretinal fluid)
   - internally, using gas tamponade
   - externally, using scleral buckling
3. seal all retinal breaks (to seal the break to the underlying RPE by creating a chorioretinal reaction around the break which heals to form a chorioretinal scar).

In order to plan the surgical approach detailed examination of the posterior and anterior segments of the eye are necessary. This is usually undertaken using the binocular indirect ophthalmoscope(61) with scleral depression, and using a slit lamp with a 3-mirror contact lens and/or condensing lens. The following need to be determined: the number, size, site, shape and configuration of retinal breaks; whether a PVD is present; the degree of V-R traction; the distribution and amount of SRF. An assessment is also made of the mobility of the retina. If PVR has developed the retina loses its mobility and breaks can become distorted, or lost in folds of retina. The retina may also become shortened tangentially and/or antero-posteriorly.

It is usual to describe RDs as either simple or complicated(62) although the distinction between the two is always somewhat arbitrary.
Simple RDs are those which can be expected to respond to conventional surgical methods using a combination of the following: scleral buckling, cryotherapy or post-operative laser photocoagulation, drainage of SRF, and internal tamponade with air or gas. Simple RDs have readily identifiable breaks, which are usually peripheral, small to medium in size, single, or if multiple at the same distance from the equator of the globe. The retina in simple RDs is usually mobile and the media clear. The majority of RDs fall into this category.

Complicated RDs require an internal approach using closed microsurgical techniques (63) and includes those with large retinal breaks, such as giant retinal tears, those with posterior breaks, or multiple breaks at varying distances from the equator, or eyes with marked dynamic or static V-R traction. Eyes with advanced PVR with immobility and shortening of the retina usually require an internal approach(64). Internal tamponade with air, gas or silicone oil, and cryotherapy or laser endophotoocoagulation with or without scleral buckling are used to close and seal retinal breaks after V-R traction has been relieved.

The distinction between simple and complicated RDs is made largely on the grounds of surgical approach, which is influenced to some extent by the expertise, experience and preferences of the surgeon as well as the availability of equipment, drugs, materials and facilities for follow up. There is, therefore, a grey area between the two categories. For example, an internal approach may be required for RDs in which a causative break cannot be found, but where the retina is mobile(65) and for eyes where vitreous opacity or pupillary miosis prevent adequate fundus examination, and some surgeons prefer an internal approach as a first procedure for inferior breaks. Having said this the distinction is, however, a useful one.

Because the anatomical and visual results are good for simple detachments it is only envisaged that retinal patching might have a role in the management of selected, complicated RDs. An outline is given of the techniques currently used, and the complications which can be associated with each technique are described, placing emphasis on those that might be avoided by retinal patching.

1.7.1 Relief of Vitreoretinal Traction:

Vitreo-retinal traction can be relieved indirectly, by scleral buckling(66), or directly, using closed microsurgical techniques.
The aim of scleral buckling is to raise a definitive indent in the sclera in the immediate vicinity of a retinal break so that the whole of the break is supported and associated vitreoretinal traction relieved. This can most effectively be achieved by extending the indent from the posterior limits of the tear up to the ora serrata, producing a "break-ora occlusive buckle"(67). Deeper indents are required for tears with pronounced VR traction or if there is loss of retinal mobility. Indents are usually created by suturing silicone sponges or solid silicone tyres onto full thickness sclera or, less commonly, by inserting these materials into scleral tunnels or pouches.

Scleral buckles, particularly extensive, deep indents, can interfere with uveal, and anterior and posterior segment circulation leading to anterior segment ischaemia, choroidal detachment, exudative RD, uveitis, vitreous haemorrhage and scleral necrosis(68-71). Scleral buckles can cause transient or permanent ocular motility abnormalities, by compromising extraocular muscle action or by stimulating periocular scar tissue(72).

Refractive errors may be induced by scleral buckling. Deep radial indents tend to produce astigmatic errors by altering the curvature of the cornea(73), whereas encircling procedures tend to cause spherical refractive errors by altering the axial length of the eye.

The use of silicone sponges (as opposed to solid silicone tyres) can be complicated by infection, which occurs in 0.1-7.1% of cases. Sponge infection has been shown to result in retinal redetachment and an increased rate of PVR(74).

The correct placing and depth of sutures used to secure an explant is important as inadvertent full thickness needle punctures can cause choroidal haemorrhage, drainage of SRF and iatrogenic retinal holes. If the sutures are not deep enough or too short the explant may extrude or be too shallow, leading to reopening of the break and retinal redetachment. Long circumferential indents produce circumferential shortening of the globe. This may lead to retinal redundancy with "fishmouthing" of breaks, or the development of retinal folds (71).

In complicated RDs closed microsurgical techniques are used to relieve VR traction. Dynamic VR traction can usually be dealt with using vitreous cutters to remove attached gel, whereas fibrovascular proliferation causing static VR traction usually requires the use of picks and scissors to elevate, delaminate and segment epiretinal membranes prior to removal. Methylcellulose or Healonid can be injected under the membranes, which elevates them, making removal easier and less
traumatic. Scleral buckling may also be indicated after vitrectomy to relieve residual VR traction.

Vitrectomy and epiretinal membrane peeling can be complicated by creation of iatrogenic retinal breaks, sclerotomy related retinal tears and fibrovascular ingrowth, vitreous haemorrhage and lens opacities. These complications would not be prevented by retinal patching, as a patch could only be applied after vitrectomy. However, scleral buckling might not be necessary after retinal patching and the complications specifically associated with this technique would therefore be avoided.

1.7.2 Break Closure:

Retinal breaks can be closed by two methods; by scleral buckling, which opposes the break against the RPE, and by internal tamponade, with agents such as air, gases and silicone oil. These techniques are often combined, particularly in complicated RDs. In vitrectomised eyes scleral buckling alone does not usually result in closure of retinal breaks and internal tamponade is also needed. It is thought that in non-vitrectomised eyes the vitreous face may play a part in break closure after scleral buckling. However, this seems an incomplete explanation as, after buckling "U" tears, the flap of the tear, which is the site of vitreous attachment, often remains elevated implying that the vitreous is not opposed against the retina(71).

All the agents that are used routinely for internal tamponade have lower specific gravities than intraocular fluids and are therefore buoyant. With accurate posturing of the patient after surgery the gas or air bubble (or silicone oil) prevents recruitment of SRF and flattens the retinal break against the RPE. Breaks in superior retina are more readily closed than those in inferior retina. Retinal breaks can also be closed by tamponading agents even if they are not flattened against the RPE because of surface tension effects of the tamponading agents, although this is not the predominant mechanism(75).

Temporary tamponade is achieved using air or gases, and prolonged or permanent tamponade with silicone oil. Intravitreal air was advocated by Rosengren in 1938(76) (from Chawla 1973) and popularised in this country by Chawla(76). Air is rapidly absorbed from the posterior segment and if break sealing has not occurred by the time the bubble disappears, the break can reopen and the detachment recur. A longer acting gas, sulphur hexafluoride (SF6) was introduced in 1973 to avoid this problem(77). SF6 absorbs nitrogen and oxygen from the blood, expands
if injected in a pure form and reabsorbs more slowly than air. More recently the perfluorocarbon gases (perfluoroethane, C2F6, and perfluoropropane, C3F8) have been introduced (78,79). These gases persist longer than SF6 gas(80) and tamponade can be pro-longed by repeat injections. The advantage of the longer acting gases is that the extended period of break closure gives time for a chorioretinal scar to form, which seals the break.

Cataract is the most frequent complication of internal tamponade with SF6 and C3F8 gas. Lens opacities are more likely to develop if the gas remains in prolonged contact with the lens, are usually posterior subcapsular and reversible, but can be permanent(79).

Raised intraocular pressure can develop during the post-operative period if too large a volume of undiluted gas is injected or, in aphakic eyes, if peripheral anterior synechiae should form. Air travel is contraindicated as the bubble of gas will expand in the reduced atmospheric pressure of the cabin(81).

Gas may pass through retinal breaks, becoming trapped in the subretinal space. In non-vitrectomised eyes the bubble can pass into the retrohyaloid space, increasing the traction exerted on the retinal break, or producing new tears(82).

The use of silicone oil in RD surgery was advocated by Cibis and popularised by Scott(83,84). Cibis described its use in 33 patients; 5 had giant retinal tears, 13 PVR and the remainder had multiple breaks and retinal atrophy. Partial or complete retinal reattachment was achieved in 23 (70%) of these otherwise inoperable cases(83). Silicone oil is currently used for selected cases, such as those with advanced PVR or static vitreo-retinal traction with posterior breaks, most giant retinal tears, selected inferior breaks, cases with intractable breaks and to prevent severe hypotony. Retinal reattachment takes place because silicone oil closes retinal breaks and not because the retina is forced back against the RPE(85). This clinical impression is supported by experimental studies as silicone oil did not prevent the development of purely tractional RDs in animal models of PVR(86,87). In combined traction/ rhegmatogenous RDs, where it may not possible to relieve V-R traction completely, silicone oil can be used to treat the rhegmatogenous component of the detachment, leaving an area of purely traction RD. This is known as "rhegmatogenous confinement" (Figure 1.3).

Initially silicone oil was injected into the mid-vitreous cavity or retrohyaloid space. The vitreous gel become compressed against the back of the lens. Closed microsurgical techniques were introduced in the 1970s(88), and since then silicone
Figure 1.3 Diagramatic representation of "rhegmatogenous confinement".

A Vitreoretinal traction (indicated by arrows) and a retinal break causing combined traction/rhegmatogenous retinal detachment  

B "Rhegmatogenous confinement" after treating the retinal break with a patch
oil has been used after vitrectomy. This means that the oil is in close contact with
the lens and posterior segment tissues. Silicone oil has a high surface tension and
in the eye tends to form a large, single bubble. In aphakic eyes the bubble has the
potential to pass into the anterior chamber of the eye by occluding aqueous flow
through the pupil (silicone oil pupil block).

Complications were noted soon after silicone oil was introduced. In 1963 Shea
reported glaucoma, keratopathy and lens changes, and cataract and glaucoma
were observed by Dufour in 1968(89)(from Cockerham 1970). In 1967 Watzke
noted similar complications, and cautioned against the use of silicone oil(90). The
pathogenesis of complex RD and some of the reasons why silicone oil induces
complications are now better understood. These factors, combined with advances
in surgical techniques and careful case selection have resulted in a lower incidence
of silicone oil-related complications. However, potentially sight threatening
complications still occur (Table 1.1)(89 -105). This has led to reluctance on the part
of many surgeons to use silicone oil, particularly in the USA(106).

The varying incidence of complications shown in Table 1.1 may, in part, be
explained by case selection. The study by Antoszyk, for instance, which has a high
incidence of keratopathy, was a study of trauma cases, where there may have
been pre-existing corneal damage, or zonular rupture which allowed silicone oil to
pass into the anterior chamber(IOI).

Silicone oil keratopathy, which is more common in aphakic eyes, is characterised
by corneal decompensation and band keratopathy. It develops in eyes where
silicone oil is or has been in contact with the corneal endothelium. In an animal
model endothelial cell loss was observed in eyes where silicone oil was in contact
with the cornea. This was thought to have occurred because the oil provided a
barrier to nutrition(107).

Lens opacities eventually develop in the majority of silicone oil filled phakic eyes.
Histological study has shown silicone oil laden macrophages on the lens capsule,
but no silicone oil in the substance of the lens(92). Lens opacities are also thought
to occur because silicone oil provides a mechanical barrier to nutrition.

Several different forms of glaucoma can occur in silicone oil filled eyes. Pupil block
glaucoma may develop in aphakic eyes if the anterior face of the oil bubble blocks
the peripheral iridectomy and pupil. Chronic angle closure glaucoma can occur
secondary to the formation of peripheral anterior synechiae, and open angle
glaucoma may be aggravated by the presence of silicone oil laden macrophages in the trabecular meshwork (92).

There are other, more contentious issues regarding the use of silicone oil, particularly in relation to whether silicone oil stimulates reproliferation of ERMs and whether it is toxic to the retina. Cockerham and Watzke both noted the presence of episilicone membranes, but because ERMs were not surgically removed at that time little conclusion could be drawn (89, 90). Lewis found reproliferation of ERMs in 19 of 31 eyes (61%), 15 of which subsequently developed recurrent RD (108). Riedel noted reproliferation of epiretinal and subretinal membranes in 40% of his series of 415 patients (103), Federman reported an incidence of 15% (100) and other authors between 3 and 71% (100) (from Federman 1988). The clinical evidence suggests that silicone oil is associated with reproliferation of ERMs, but whether the relationship is causal has yet to be clarified.

This issue has been addressed using animal models of PVR and tissue culture experiments. Cell injection techniques provide a good model for investigating different aspects of PVR. Fastenberg and Lean (109, 110) found no difference in the rate of ERM formation in vitrectomised rabbits’ eyes with and without prior silicone oil injection. These findings are at variance however, with those of Lambrou, but he used a different experimental design (111). Tissue culture experiments have been undertaken to see whether silicone oil has any effect on cell types implicated in the formation of ERMs. Fibroblasts remain viable when plated directly onto silicone oil (112) and human RPE cells grown under silicone oil dedifferentiate and proliferate (113). These studies provide evidence that silicone oil may encourage the development of ERMs by a direct effect on cell behaviour. Lambrou has postulated that silicone oil may stimulate the retina or residual ERM tissue to produce growth factors (111). On the other hand the effect of silicone oil may be purely mechanical. Many factors and processes have been implicated in the pathogenesis of PVR, e.g. inflammation, RPE cell proliferation and effects of growth factors and FN. A bubble of silicone oil will tend to localise these factors in the preretinal space, encouraging ERM formation.

The question of silicone oil retinotoxicity has provoked much debate and stimulated a considerable volume of research, often with conflicting results. Retinae from enucleated silicone oil filled eyes have marked degenerative changes. However, firm conclusions cannot be drawn from this as the eyes were usually surgical failures. Clinical evidence is difficult to interpret as it is hard to distinguish changes due to preexisting retinal pathology from those which could be attributable to toxic effects of silicone oil.
### Table 1.1 Complications of internal tamponade with silicone oil

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<th>Date</th>
<th>Author</th>
<th>Ref</th>
<th>N</th>
<th>FU</th>
<th>K'top</th>
<th>Glauc.</th>
<th>Cat.</th>
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<tr>
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<td>3</td>
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N = number of patients  
FU = minimum follow up  
Glauc = glaucoma  
K'top = keratopathy  
RD = persistent retinal detachment  
BI = basal peripheral iridectomy  
SI = superior iridectomy  
SSG = Silicone Study Group  
a = aphakic  
p = phakic  
Cat = cataract  
hyp = hypotony  
mn = months  
ND = no data  
Yrs = years  
PVR = proliferative vitreoretinopathy
Early studies using electrodiagnostic tests (electro-retinogram, ERG and electro-oculogram, EOG) complicated the issue. Silicone oil filled eyes were found to have almost absent ERGs and EOGs. This was interpreted as evidence of retinal toxicity (114) (from Foerster 1985). However, the findings are probably explained by the fact that silicone oil is an electrical insulator (92). Foerster's clinical studies showed "no consistent changes that could be attributable to a delayed retinotoxic effect" (114) and vision-evoked cortical potentials undertaken on patients before and after removal of silicone oil showed no evidence of optic nerve damage (115).

Experimental studies using animal models have produced conflicting results. In a study by Meredith, ERGs were performed before and after silicone oil or BSS were injected into vitrectomised rabbit eyes. After an initial postoperative decline in B wave amplitude in both groups of animals postoperative values reached preoperative values in both groups by 6 months. Histological study of the retinas from these animals showed little abnormality in either group (116). Similar findings were reported by Ober (117), but are in sharp contrast to the findings of other studies. The rabbit retinae in a study by Gonver showed pronounced degenerative changes, particularly in the outer plexiform layer. The changes were more obvious in the upper retina, where the silicone oil bubble was in direct contact with the retina. Changes were also present when purified silicone oil was used (118). In a similar study on monkeys, consistent degenerative changes were seen in all layers of the retina, particularly in the periphery. These changes were not detected in control eyes which had had injection of BSS rather than silicone oil (119). In another study using primates, light and electron microscopy showed only minimal changes, which were the same in silicone filled eyes and controls and which were attributed to surgical trauma (120). There are several possible explanations for the different findings in these studies. Firstly, different species were used, secondly, surgical techniques were not the same in each study, and finally, silicone oils of varying chemical composition were used.

Although the evidence for retinotoxic effect of silicone oil is controversial there are several theoretical reasons why silicone oil may produce histological changes or functional abnormalities.

Silicone oil is a polydimethylsiloxane, made by polymerisation of dimethylsiloxane-oligomers. The viscosity is determined by the polymer chain length i.e. the molecular weight, but a statistical distribution of molecular weights is an invariable result of the polymerisation process. Viscosity can also be altered by mixing oils of differing molecular weights. The silicone oil that is used clinically is 'medical grade', and is manufactured by several different companies. Physicochemical analysis has
been undertaken of oils from different sources, to determine the molecular weight, viscosity, the percentage of low molecular weight components and the percentage of volatile components (low molecular cyclosiloxanes which vaporise at 37°C).

Silicone oils with viscosities of 1,000 and 5,000 mPas have been analysed (121,122). These studies showed low molecular weight components and volatile cyclosiloxanes in varying amounts. The significance of these findings are that low molecular weight components are thought to lower oil emulsification thresholds. The very low molecular weight, volatile compounds are small enough to diffuse into tissues, and may be responsible for some of the complications observed.

In theory, lipophilic substances will dissolve in silicone oil and this has been investigated by Refojo(123). In an experimental animal model silicone oil was found to extract retinol, with concentrations increasing with time. He also analysed silicone oil removed from patients' eyes and found both cholesterol and retinol to be present. It is not known what effect lipid extraction may have on ocular tissues in terms of structure and function.

Silicone oil has also been analysed for the presence of impurities. Parel analysed 18 different oils and found impurities in amounts varying from 350-4,900 ppm. Arsenic, antimony and cyanide were found amongst others (Parel, unpublished data, presented at ARVO 1989).

Other complications associated with silicone oil include emulsification and refractive errors. Emulsification is seen in most cases, varying from a few tiny droplets in the anterior chamber to an 'inverted hypopyon' or an anterior chamber completely filled with milky, emulsified oil. Emulsification occurs when two immiscible fluids are agitated in the presence of a third substance (an emulsifier, such as proteins). Intraocular proteins may activate emulsification, encouraged by eye movements(75).

Refractive changes occur because silicone has a higher refractive index than intraocular fluids; the + or - change being principally determined by the shape of the anterior surface of the bubble(93,124).
1.7.3 Strategies Adopted to Reduce Complications Associated with Silicone Oil:

The incidence of anterior segment complications is higher in eyes where silicone has entered the anterior chamber. This can be prevented by leaving the lens or anterior gel in situ - should a cataract develop it can be removed later. Leaving anterior gel behind is not advisable as it provides a scaffold for fibrovascular proliferation(125). Ando advocated the use of a basal peripheral iridectomy in aphakic eyes to prevent pupil block glaucoma and to stop silicone oil entering the anterior chamber, as iridectomies at the usual 12 o'clock position often become blocked(126). This measure is effective so long as the iridectomy remains patent(102).

Once retinal reattachment and break sealing have occurred silicone oil could, in theory be removed. Gonvers was the first to advocate using silicone as a temporary measure(127). (Table1.2)(94-5,99,104-5,127-131). In this series of reports patients were only selected for oil removal if they had flat retinas, but retinal redetachment occurred in 12-30% of cases.

Early oil removal reduces the incidence of keratopathy and glaucoma, but cataracts still develop despite very early oil removal(129) although the onset may be delayed (131). In Franks series of patients the visual acuity improved in the majority of patients with flat retinas after silicone oil removal(131). The value of early oil removal needs to be balanced against the risk of retinal redetachment or severe hypotony. Cataracts can be managed by intracapsular extraction, or extracapsular extraction if the oil is not to be removed, and eyes with keratopathy have been successfully grafted(132). A high percentage of eyes with glaucoma can be managed medically(133) although trabeculectomy or Molteno tubes are sometimes required.

The body of evidence seems to suggest that removing silicone oil (in carefully selected patients) is of benefit, not only because the incidence of complications is reduced, but also because the rate of progression of complications seems to be arrested and management more successful(129, 132).

In conclusion, internal tamponade with silicone oil is extremely valuable for the management of complex RDs that until comparatively recently were considered inoperable and the complications need to be seen in that light. It also needs to be born in mind that more conventional procedures are also associated with complications. For example, in the first report of the Silicone Study Group, which
Table 1.2 Temporary use of silicone oil

<table>
<thead>
<tr>
<th>Date</th>
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<th>N</th>
<th>Min FU</th>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>105</td>
<td>36</td>
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SSG = Silicone Study Group
N = number of patients
ReRD = recurrent retinal detachment
Min FU = minimum follow up
Glaucoma op = glaucoma operations
ND = no data

43
randomised patients with PVR to either silicone oil or SF6 gas, keratopathy and glaucoma occurred more frequently in the SF6 treated eyes(104). The reasons why some of the complications seen with silicone oil occur are becoming clearer, and strategies are being adopted to reduce them. The best way to avoid the complications associated with silicone oil would be to introduce techniques that produced equally good results but which make silicone oil unnecessary. If retinal patching were possible then this might be true for some cases.

1.7.4 Break Sealing:

Break sealing is achieved by creating a thermodestructive lesion, using contiguous applications of cryotherapy or laser photocoagulation(134) to surround the break, which heals to form a fibroglial, chorioretinal scar. The healing phase takes 10-14 days so the retinal break must be closed against the RPE while the chorioretinal scar develops.

The importance of break sealing in securing permanent retinal reattachment has been shown by Chignell(135). Forty six cases of simple RD were treated with scleral buckling, and drainage of SRF as indicated, but without cryotherapy. Initial retinal reattachment was achieved in 93%. However, in 9% the retina redetached at a later date due to lessening of the height of the indent and reopening of the original break. However, some RDs caused by small, posteriorly located breaks, such as macular holes, can be successfully treated using vitrectomy and temporary internal tamponade alone.

Cryotherapy is associated with a localised inflammatory response, breakdown of the BRB(136), increased levels of FN in the vitreous(137) and dispersion of viable RPE cells into the vitreous cavity(138). These factors have been implicated in the pathogenesis of PVR(139). Clinically, excessive cryotherapy can cause uveitis, local chorioretinal atrophy and pigment fallout. The latter may compromise postoperative visual recovery if the pigment should settle in the dependent, macular area(140). These complications are more likely in eyes with bullous RDs where it is difficult to monitor cryotherapy applications.

Cryotherapy can also induce localised PVD, producing new retinal breaks(135). These complications can be kept to a minimum by using light, localised applications, or by using laser photocoagulation which is less stimulating (141).

In retinal patching, because the retinal hole is repaired, break sealing would not be necessary.
1.7.5 Drainage of Subretinal Fluid:

Subretinal fluid drainage is not necessary for all simple RDs, but is usually indicated for complicated RDs. In simple RDs SRF is drained externally, whereas in complicated RDs SRF can also be drained internally, via a retinal break or retinotomy. Posteriorly sequestered fluid can also be drained under indirect visualisation by inserting a needle through the posterior coats of the eye (142).

Complications of external drainage include choroidal, subretinal and vitreous haemorrhage (2-9%), vitreous and/or retinal incarceration and retinotomy (0.2-3.3%)(143). Internal drainage is more controlled and less prone to complications. However, subretinal neo-vascularisation and vitreous haemorrhage can occur, due to damage to the RPE, Bruch's membrane and choroid(144).

If patching materials could be delivered in an aqueous environment and which adhered to retina in a fluid-filled eye, peroperative retinal flattening would not be necessary. The complications of SRF drainage would be avoided entirely.

1.8 CAUSES OF FAILURE IN RETINAL DETACHMENT SURGERY:

Retinal reattachment rates are much higher for simple RDs than for complicated RDs, and the visual results better. An outline is given for the reasons why retinal reattachment may not be achieved and why there may be functional (i.e. visual) failure after a technically successful operation.

1.8.1 Anatomical Failure:

A successful outcome will not occur if the wrong surgical approach is selected, if the techniques are incorrectly applied or if unavoidable or insurmountable technical problems arise. The surgical sequence is also important(67). The nature of the RD and attendant pathology has a large part to play in the final outcome and careful preoperative examination is imperative(62,145). Reattachment rates for simple RDs are good, varying from 84% - 96% after one or two operations(67,76,135,146-8). Success is more likely in cases with round holes or dialyses and those with localised RDs, and less likely in aphakic eyes, those with PVR and those in whom a break cannot be identified preoperatively(147).

Retinal reattachment rates for complicated RDs are, however, not so good, varying from 55-80%, multiple operations often being required(104-5,149-153). Clarkson compared reattachment rates in 50 patients who had internal tamponade with
either air or SF6 gas. The gas injected eyes had better reattachment rates which was attributed to longer periods of break closure\cite{154}. The Silicone Oil Study Group (SSG) have undertaken two randomised, multicentre treatment trials of eyes with PVR $\geq$ grade C3\cite{104-5}. Group I eyes had not had previous surgery, while Group II eyes had undergone vitreoretinal surgery. After vitrectomy and peroperative retinal reattachment eyes were randomised to internal tamponade with either SF6 gas or silicone oil in the first study, or C3F8 gas or silicone oil in the second study. The outcomes were macular reattachment rate, visual acuity and complications. In the first study macular reattachment was finally achieved in 80\% of silicone oil filled eyes (Group I) compared to 60\% of those treated with SF6 gas ($p = <0.05$), although repeat surgery was required in 16 eyes in both groups, sometimes using different procedures (data were analysed by the original treatment modality). If the results of the first operation only are compared (Group I eyes only) retinal reattachment was achieved in 13/40 (33\%) eyes treated with SF6 compared to 19/46 (41\%) treated with silicone oil (Chi sq test, $p = 0.4$). The second study showed no significant difference in the final macular reattachment rates of Group I eyes (73\% treated with C3F8 vs 64\% treated with silicone oil) and Group II eyes (73\% vs 61\% respectively). If results of the first operation only are compared for Group I eyes macular reattachment was achieved in 29/67 (43\%) C3F8 treated eyes compared to 27/63 (43\%) eyes treated with silicone (Chi sq test, $p = 0.96$). In Group II eyes the rates were 30/71 (42\%) and 21/63 (33\%) respectively (chi sq test, $p = 0.29$). The authors conclude that results were better using C3F8 compared to SF6 gas, and that C3F8 gas compares favourably with silicone oil.

Retinal reattachment rates for eyes treated with silicone oil show that eyes with advanced PVR have the lowest reattachment rates, varying from 55 - 80\% \cite{104-5,153,155}, with anterior PVR carrying the worst prognosis\cite{125}. Retinal reattachment rates of 85-90\% have been reported for eyes without PVR \cite{97,155-157}.

In these series a significant proportion of eyes treated with silicone oil redetached at a later date, often due to the development or progression of ERMs leading to traction RD, reopening of existing breaks, or the formation of fresh retinal tears. There are several theoretical reasons why retinal patching may prevent the development of ERMs.

ERMs have a cellular component (macrophages, glial cells, RPE cells and fibroblasts) within an extracellular matrix of collagens and FN\cite{158}. Viable RPE cells can be dispersed into the vitreous cavity by cryotherapy and proliferating RPE cells can reach the retinal surface via retinal breaks\cite{88}. Application of a retinal patch would not disturb the RPE and restoration of retinal continuity would prevent
RPE cells reaching the retinal surface. Subretinal and intravitreal fluids have been shown to contain FN and growth factors (possibly derived from RPE cells) which promote proliferation and migration of certain of the cell types implicated in the pathogenesis of ERMs (139) (from Gilbert 1988). Restoration of retinal continuity would confine these substances to the subretinal space. Cryotherapy, which would not be necessary with retinal patching, induces BRB breakdown and has been found to promote ERM formation in experimental models of PVR (136). All these factors offer theoretical reasons why retinal patching may result in a lower incidence of postoperative ERM formation.

1.8.2 Visual Failure:

Visual function may not recover after a technically successful operation due to cystoid macular oedema (CMO), subretinal pigment, retinal folds and failure of macular recovery after macular detachment. Causes of late visual failure include macular pucker, cataract, glaucoma, keratopathy or retinal redetachment. Eyes with complicated RD carry the worse prognosis. Visual results from a series of publications are shown in Table 1.3 (92, 94-6, 99-100, 125). These patients had all undergone vitrectomy with silicone oil tamponade for different types of complicated RD. The findings are not, therefore, directly comparable. 26-63% of patients had early visual acuities of less than counting fingers despite successful anatomical results (defined as complete, or partial retinal reattachment including the posterior pole). The visual outcome of patients in the SSG trials are shown in Table 1.4 (104-5) and early results are similar to the other studies. Early and late visual acuities are reported in the first SSG trial, and late visual acuities are reported in three of the other studies (Tables 1.4, 1.3). In the SSG trial vision deteriorated even in those patients with flat retinas; at 6 months 57% of patients with macular attachment had acuities of ≥5/200, compared to 46% at 24 months. In Leaver’s study 33% of patients had worse vision at follow up, and in the other two studies acuities had dropped below CF in a further 24-40% of patients (Table 1.3). Causes of late visual failure included retinal redetachment and the development of complications. Chan’s data are interesting (96) as the follow up in this group is long, including some of the first patients to have internal tamponade with silicone oil.

Retinal patching would probably not prevent CMO, nor would it influence macular function after macular detachment. Other causes of visual failure, such as retinal folding after scleral buckling, pigment fallout at the macula after cryotherapy, and macular pucker might, however, be prevented, as would some of the other sight-threatening complications of silicone oil.
Table 1.3 Visual acuity after internal tamponade with silicone oil

<table>
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<th>Author</th>
<th>Ref</th>
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<th>Late VA ≥ CF</th>
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<td>Leaver</td>
<td>92</td>
<td>80 ≥ CF</td>
<td>33 deterioration</td>
</tr>
<tr>
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<td>McCuen</td>
<td>94</td>
<td>41 ≥ CF</td>
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<td>Cox</td>
<td>95</td>
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<td>96</td>
<td>90 &quot;functional&quot;</td>
<td>50</td>
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<td>1987</td>
<td>Yeo</td>
<td>99</td>
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VA = visual acuity
ND = no data

Table 1.4 Visual acuity after internal tamponade with silicone oil - results of Silicone Study Group (SSG).

<table>
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<th>At 24 mn ≥ 5/200</th>
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<td>Macular attached 57%</td>
<td>Macular attached 46%</td>
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<tr>
<td></td>
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<td>Overall 46%</td>
</tr>
<tr>
<td>1992</td>
<td>SSG II Group 1</td>
<td>ND</td>
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<tr>
<td></td>
<td>Group 2</td>
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1.9 RETINOPEXY USING ADHESIVES:

The techniques used for break sealing depend on the formation of a chorioretinal scar, which takes up to three weeks to form. During the first week after application retinal adhesion in cryo-treated areas has in fact been shown to be less than in untreated areas (159). Several adhesives have been explored as a novel way of creating a chorioretinal adhesion (retinopexy) (Figure 1.2), the major motivation being that they take effect immediately. The cyanoacrylates adhesives, different preparations of fibrin, mussel adhesive protein and other adhesives have been evaluated.

1.9.1 Cyanoacrylate Retinopexy:

The adhesive properties of alkyl 2-cyanoacrylates, formed by condensation of formaldehyde and cyanoacetates, were discovered in the 1950s. The first commercially available preparation was Eastmann 910, methyl 2-cyanoacrylate. Cyanoacrylate monomers are electronegative and in the presence of anions readily polymerise into a solid. The anions present in ionised atmospheric water molecules or the NH2 group in tissue proteins are sufficient to stimulate polymerisation. During polymerisation heat is generated (160), and water induces hydrolytic degradation with release of formaldehyde and cyanoacetates (161).

Early clinical work demonstrated that Eastmann 901 was toxic to tissues, particularly neural tissue. Higher homologues have been synthesised (i.e. butyl-, isobutyl-, pentyl-, hexyl-, octyl- and decyl-cyanoacrylate) in an attempt to produce less toxic compounds. Both heat generation (160) and release of degradation products (162) are less in the higher homologues, which may explain their better tissue tolerance. Toxicity may also be due to the presence of impurities (163).

Cyanoacrylates were investigated as a potential tool in ophthalmic surgery in 1963. Methylcyanoacrylate was injected into the anterior segment of rabbits' eyes (164-5) and when the higher analogues became available they too were tested in the rabbit (166) (from Refojo 1971). These studies showed the higher analogues to be less toxic than methyl-cyanoacrylate, and that the toxic effects were dose-dependent.

Medical grade n-butyl-cyanoacrylate (Histoacryl) is now used for a variety of anterior segment problems (161, 167). None of the analogues have, however, been found to be suitable for 'sutureless surgery', for example in epikeratophakia, as they are too toxic, do not provide permanent adhesion when applied externally and act as a barrier to normal wound healing (168).
Kurokawa was the first to explore the use of cyanoacrylates for sealing retinal breaks. He used two different preparations and injected them through the sclera into the subretinal space of rabbits' eyes. Both cyanoacrylates produced good adhesion between the retina and the RPE, but caused oedema of the overlying retina (169). In 1973 Spitznas injected minute quantities of Histoacryl into the sclera, choroid, subretinal space and vitreous of rabbits' eyes (170). Minimal anterior segment inflammation was seen, and histological examination showed an early localised inflammatory response with a giant cell reaction occurring after 21 days. When injected into the subretinal space the overlying retina became disorganised and atrophic, with choroidal atrophy and closure of choriocapillaris. Intravitreal injection of Histoacryl caused minimal reaction. Faulborn used N-butyl-cyanoacrylate to repair two giant retinal tears after open sky vitrectomy, applying the adhesive directly onto the edge of the tears whilst holding the retina in place. Retinal reattachment was secured in one eye, and in the other eye the retina was totally detached except in the region of the adhesive (171).

The use of cyanoacrylates in RD surgery is currently being championed by McCuen, North Carolina. He performed preliminary studies in rabbits to compare the strength of adhesion between the retina and RPE surrounding retinotomies treated either with N-butyl-cyanoacrylate or cryotherapy. The adhesive was applied using a specially designed applicator and was delivered directly onto the retinal/RPE surface after vitrectomy, fluid/air exchange and careful drying of the retinal surface. At each time interval (immediately after application and after one week, four weeks and one year) chorioretinal adhesion was greater in cyanoacrylate treated eyes than in those treated with cryotherapy (172). In a second series of experiments the adhesive was delivered in silicone oil filled eyes. Considerable technical problems were encountered, as it was difficult to see the adhesive, and because cyanoacrylates are less dense than silicone the adhesive floated away from the retinal surface. Polymerisation was slower under silicone and the adhesive spread before solidifying. Once again the cyanoacrylate treated retinotomies were more adherent to the underlying RPE than those treated with cryotherapy. Signs of toxicity, in the form of an early halo of retinal oedema and later RPE pigmentary changes, were seen in 84% of eyes. Changes were more pronounced in eyes where larger volumes of adhesive applied. Histological examination of treated retinæ at 24 hours showed inner retinal necrosis, disorganisation of the nuclear layers and loss of photoreceptors. The increased toxicity seen in this model was thought to be due to an insulating effect of the silicone oil with localisation of heat and/or toxic degradation products (173). Further experimental work was undertaken using primates, with similar results (174). In a more detailed study using rabbits, halos of retinal oedema developed around the
drop of adhesive within a few hours of application which gradually faded over the ensuing days. In some cases atrophic retinal holes developed adjacent to the area of cyanoacrylate and there were pigmentary changes in adjacent RPE. Histological examination at 24 hours showed coagulative necrosis of the retina beneath and adjacent to the adhesive, and these areas later became markedly atrophic. Early vacuolation and subsequent RPE atrophy was also seen(175).

Despite the findings of the experimental studies McCuen has used N-butyl-cyanoacrylate in selected patients with complicated RDs in whom previous surgery had failed(176-8). In the first report N-butyl-2-cyanoacrylate mixed 50:50 with lophendylate, an agent that slows polymerisation(179), was used in 25 patients to seal retinal breaks, inadvertent retinal holes or relaxing retinotomies. The majority of the breaks were situated in inferior retina, an area that is difficult to tamponade. Small breaks were treated with a drop of adhesive that covered the entire defect, and larger breaks and retinotomies were treated with confluent applications to the edge of the defect. Retinal reattachment posterior to encircling scleral buckles was achieved in 72% of cases. In only one patient was failure attributed to inadequate sealing of the original break. In one silicone oil filled eye a crescent shaped retinal break developed at the edge of the adhesive, but no other complications that could be attributed to the cyanoacrylate were observed(176).

In the second report nine patients with macular holes were treated with cyanoacrylate retinopexy, with a success rate of 89%. In one patient the adhesive separated from the retinal surface and in another a para-adhesive crescent shaped retinal defect occurred one month after surgery. The authors advocate this technique for RDs arising from macular holes where conventional V-R surgery has failed and who would otherwise be candidates for internal tamponade with silicone oil, or macular buckling(177).

In the third study four patients with retinal detachment secondary to posterior breaks in retina overlying choroidal colobomata were treated with cyanoacrylate retinopexy. All the holes were closed successfully, and the retina reattached in three. In one patient subretinal fluid could not be completely drained, and the adhesive did not adhere properly to the retina(178).

1.9.2 Fibrin Retinopexy:

Prior to the development of good operating microscopes and sutures fibrin was explored as an adjunct to anterior segment surgery(180-2). More recently fibrin has been used to seal leaking glaucoma blebs(183) and in repairing scleral
defects\textsuperscript{(184)}. Fibrin has also been used to secure lamellar corneal grafts and epikeratophakia corneal lenticules, both experimentally and clinically\textsuperscript{(185)}. In the first study fibrin from two sources was used 1) autologous plasma was mixed with bovine thrombin, and 2) a commercial preparation was used, which contains bovine thrombin, human fibrinogen, calcium chloride and aprotonin (a broad-spectrum protease inhibitor that delays fibrinolysis). The major problem encountered was the temporary nature of the fibrin adhesive. This could be partially rectified by using aprotonin, an antifibrinolytic agent.

Fibrin has also been investigated as a means of sealing retinal breaks. In 1949 Brown treated 30 patients with surface diathermy, drainage of SRF and transcleral injection of thrombin and a small volume of autologous plasma into the subretinal space. Retinal reattachment occurred in 60\% of cases but it was noticed that the subretinal fibrin gradually disappeared\textsuperscript{(180)}. In 1986 Nasaduke undertook experiments using rabbits to investigate possible toxic or inflammatory reactions and to assess fibrin retinopexy. He used either pooled rabbit plasma as a source of fibrinogen or commercially prepared freeze-dried rabbit fibrinogen. The adhesive was delivered using simultaneous injection of thrombin/calcium chloride in one syringe and fibrinogen/aprotonin in a second syringe joined by a Y shaped connector, via a 22g needle. In one group of animals 0.1 cc of fibrin was injected into the mid-vitreous cavity. The fibrin gradually lysed, leaving small vitreous opacities. Histological examination of the retinæ showed no abnormality and ERGs were normal. In a second group of animals a localised RD was created in non-vitrectomised eyes and fibrin injected into the subretinal space, although this could not be achieved in all animals. Clinically and histologically there was no difference between treated eyes and control eyes; all had attached retinas with essentially normal histology. In a third group retinal breaks and RDs were created in vitrectomised eyes, and fibrin injected into the subretinal space. Both treated eyes and controls showed the same rate of retinal reattachment, RD and formation of vitreous membranes. Nasaduke concluded that fibrin was not retinotoxic, but evaluation in the rabbit is unsatisfactory as the retina reattaches spontaneously\textsuperscript{(186)}.

Further experimental and clinical work has been undertaken by Coleman who used either freeze-dried human or rabbit fibrinogen, or autologous plasma, mixed with bovine thrombin, calcium chloride and aprotonin. Experiments were performed on non-vitrectomised rabbit and pig eyes, with injection of fibrin either into the subretinal space after creating a localised RD, or into the mid-vitreous cavity. In the rabbit experiments little inflammatory reaction was seen and retinal reattachment occurred at the same rate in treated and control eyes (who had had a RD created but no subretinal injection of fibrin). No histological evidence of tissue
toxicity was found with any of the fibrin preparations, and clinically none of the eyes developed traction RD secondary to proliferative processes. Two of the four eyes with subretinal injection followed for one year showed histological evidence of chorioretinal adhesion, but this was not found in the other eyes. In the pig model, both treated eyes showed histological evidence of chorioretinal adhesions as the breaks were sealed to the underlying RPE by fibrous scar tissue. Coleman also treated seven patients with fibrin retinopexy, five of whom had macular holes and two who had post traumatic giant retinal tears. In each instance vitrectomy and fluid/air exchange was followed by application of fibrin (made from autologous plasma) to the flattened retinal break. Internal tamponade with gas was used in three eyes. The postoperative inflammatory reaction was mild and in those with retinal reattachment visual recovery was good, suggesting no local toxicity. Four of the eyes with macular holes reattached after this procedure (three had also had intravitreal gas injection) but neither eye with a giant retinal tear reattached. This clinical study shows that fibrin retinopexy has no deleterious effects, but little conclusion can be drawn from such a small study about the effectiveness of fibrin in break sealing, particularly as a high percentage of macular holes respond to vitrectomy and internal tamponade alone. The author concludes that fibrin may have some part to play in peroperative retinal fixation as fibrin does not provide permanent adhesion(187).

Emmerich performed similar experiments to those of Nasaduke, injecting fibrin into the subretinal space of rabbits after vitrectomy. He observed that the degree of postoperative inflammation and ERM formation was dose dependent. ERG changes were not noted. When small volumes of fibrin were injected complete lysis took 7-14 days, but when larger volumes were injected lysis took up to 28 days. From these observations Emmerich concluded that fibrin may have some part to play in temporary break sealing(188).

Further toxicity studies were performed on rabbits by Gerding. Retinal breaks and localised RDs were created in vitrectomised eyes and the breaks plugged with fibrin. Electrodiagnostic testing showed no evidence of toxicity, but ERM formation with or without extensive RD developed (no data is given in the abstract)(189).

1.9.3 Other Adhesives:

Other adhesives that have been evaluated for retinopexy include biological adhesives (mussel glue and transforming growth factor-β (TGF-β)) and synthetic adhesives (acetate polymers and polysiloxanes).
The adhesive that binds mussel byssus threads to rocks is a two component proteinaceous material, each produced by a separate gland which, when the components mix, sets to form a very strong adhesive. The adhesive has properties that might make it desirable for clinical use as it sets in an aqueous environment, is stable in saline and forms strong but flexible bonds. Biochemically mussel adhesive consists of repeated decapeptide units which cross link to form a rubbery solid(190). The adhesive has been extracted from molluscs and has also been produced by genetic engineering by Biopolymers Inc. (mussel adhesive protein, MAP), but the latter has not yet received FDA approval. Mussel protein has been used experimentally for epikeratophakia in rabbits, with promising results(191). In preliminary investigations, pending using the adhesive for retinopexy, Liggett injected large volumes of MAP into the vitreous cavity of rabbits and found no ERG changes nor histological evidence of tissue toxicity(192).

Another biological "adhesive" that has been investigated experimentally is transforming growth factor-β (TGF-β). TGF-β (isoforms 1 and 2) is a growth hormone which stimulates some of the cellular events involved in wound healing, such as macrophage, fibroblast and glial cell migration, and collagen and FN synthesis(193). TGF-β was initially investigated in an animal model to determine whether it stimulated a wound healing response. Retinal breaks were created in rabbit eyes after gas compression vitrectomy and TBF-β delivered to the retina/RPE interface in a hyaluronic acid vehicle. After four days treated eyes had developed areas of localised fibrous tissue between the RPE and choroid which was not seen in control eyes (194). Given these promising results, TGF-β2 has subsequently been used to treat patients with full thickness macular holes(195). One disadvantage of this adhesive however is that as with cryotherapy, a time factor is involved, and internal tamponade would be necessary to close the retinal break whilst the chorioretinal adhesion was forming.

Two further synthetic adhesives have been investigated in experimental retinopexy. Sobol used an acetate polymer to seal retinal breaks. After a very short period of follow up there was good adhesion between the retina and RPE, with no evidence of toxicity(196). Pince has investigated the use of polysiloxanes which can be applied in an aqueous environment but he found evidence of toxicity, with granulomatous inflammation in treated eyes(197).

Despite considerable research the ideal adhesive for retinopexy remains elusive. Those evaluated to date are difficult to deliver, are toxic, provide only transient adhesion, or stimulate ERM formation. The ideal adhesive would have to provide immediate and permanent adhesion or stimulate a purely localised wound healing
response, be non toxic and non inflammatory and be easy to deliver to the retinal surface in an aqueous environment. If a suitable adhesive could be found the management of selected complicated RDs would be greatly simplified and the complications of cryotherapy and internal tamponade would be avoided.

1.10 RETINAL PATCHING:

In previous sections of the Introduction of this thesis the indications and theoretical advantages of retinal patching have been introduced where appropriate. In this final section these are summarised. Possible complications of retinal patching are discussed along with the characteristics that would be required of ideal patching materials.

1.10.1 Indications for Retinal Patching:

Theoretical indications for retinal patching fall into two main categories; first, as a means of treating RDs that are refractory to treatment, and secondly as a means of avoiding the complications of established techniques, particularly those related to internal tamponade with silicone oil.

Retinal breaks can be difficult to close and seal due either to their size, site or configuration, because of associated ocular abnormalities, or the presence of PVR. If there is marked V-R traction or retinal shortening in the area of the break, a retinotomy can be performed to mobilise the retina, treating the edge of the break with cryotherapy, endophotocoagulation (or Histoacryl), followed by internal tamponade (198). Exposing large areas of RPE, however, encourages PVR and may cause hypotony, although the latter may also be due to associated anterior PVR with ciliary body detachment(199). Repairing the immobilised retinal break with a retinal patch would provide an alternative treatment option for these situations, promoting "rhegmatogenous confinement" (Figure 1.3). Peyman attempted to plug retinal defects adjacent to areas of unrelievable VR traction, using autologous tissue (blood clot with fibrous tissue, fibrovascular tissue and lens cortex). He used the term "internal patching", and the technique was successful in two of the three eyes he treated(200). Scott has also used a similar technique using chick allantoic membrane, but without success (personal communication).

Retinal patching may also be useful for selected inferior breaks which are difficult to tamponade. Treatment of RDs arising from macular holes changed since the introduction of closed microsurgical techniques, with improvement in reattachment rates and visual function. Breaks used to be treated with cryotherapy and scleral
buckling, but this technically difficult procedure was associated with a high incidence of complications and poor visual outcome. Gonvers advocated vitrectomy, internal drainage of SRF and internal tamponade without break sealing for eyes with VR macular traction, and in his initial series of six patients retinal reattachment occurred in five eyes (201). Using similar surgical techniques, with or without laser photocoagulation or cryotherapy, retinal reattachment rates for RDs arising from macular holes vary from 59% - 100% (202-6). If initial vitrectomy, internal drainage of SRF and internal tamponade with gas is unsuccessful, further injection of gas and localised macular photocoagulation can promote reattachment. Blankenship has used retrohyaloid gas injection with simultaneous aspiration of retrohyaloid fluid in highly myopic eyes with macular breaks where there is no evidence of vitreo-macular traction. Using this technique as a first procedure in 19 cases his reattachment rate was 47% (45). Koerner reported the results of 23 cases of RD secondary to macular holes. In one group of 8 eyes treatment consisted of vitrectomy, internal tamponade with gas and macular photocoagulation, with a reattachment rate of 75%. In a second group of 15 cases a small drop of autologous blood was applied to the retinal break ("haematotamponade") without photocoagulation, with a 93% reattachment rate. As the blood clot covered the whole of the defect it could be considered to be acting as a biodegradable "patch" (207). Silicone oil is required for refractory cases. Retinal patching would be a useful alternative treatment in these cases to avoid the complications of internal tamponade with silicone oil and the visual sequelae of macular photocoagulation, although the patch would undoubtedly interfere with visual function.

Retinal patching may provide a means of treating retinal breaks in ectactic retina overlying colobomata which cannot be sealed using thermodestructive techniques due to the lack of underlying RPE and choroid. Management of these eyes is particularly problematical as detection of breaks within the ectatic retina is often difficult. The sclera is often ectatic, making scleral buckling hazardous. Internal tamponade with silicone oil currently offers the best hope of success (49).

Retinal patching may also be indicated in the management of RDs that can be reattached successfully using established techniques but where there is a significant risk of sight threatening complications. With the availability of suitable patching materials a retinal patch could be applied to retinal breaks in detached retina, in a fluid filled eye. This would mean that peroperative retinal flattening would not be required and the complications of internal or external drainage of SRF avoided. Thermodestructive techniques would not be required for break sealing and the complications of cryotherapy therefore avoided. Temporary internal tamponade with gas or permanent internal tamponade with silicone oil would not be needed,
making postoperative posturing by the patient unnecessary and avoiding the potentially serious complications associated with the use of silicone oil.

Retinal patching may have some part to play in the management of giant retinal tears and relieving retinotomies although considerable technical difficulties would be encountered in delivering, manipulating and applying a large retinal patch.

Eyes complicated by advanced PVR pose complex management problems and it is difficult to envisage what role retinal patching may have in their treatment, if any. Silicone oil promotes retinal reattachment in these circumstances by virtue of break closure rather than by splinting the retina against the RPE. The advantage of silicone oil, however, is that if retinal shortening is present the buoyant oil bubble forces subretinal fluid inferiorly, and the macular can often be flattened; a situation that would not prevail if a retinal patch were used.

1.10.2 Advantages of Retinal Patching:

As already discussed there are several theoretical reasons why retinal patching may reduce the incidence of postoperative PVR, which occurs in up to 10% of RDs(208). Retinal patching also offers several theoretical advantages over retinopexy using adhesives.

In the patching technique the adhesive would only be applied to the retina, leaving the potentially reactive RPE and choroid undisturbed. By restoring retinal continuity bare RPE would not be exposed, reducing the risk of postoperative ERM formation and hypotony. Peroperative retinal flattening is required in adhesive retinopexy, which is not theoretically required with retinal patching. In addition, after application of a retinal patch the retina would remain mobile and able to flatten naturally against the RPE. Should tangential V-R traction develop postoperatively the retina would maintain its ability to slide over the RPE.

1.10.3 Possible Complications of Retinal Patching and Characteristics of Ideal Patching Materials:

It is possible to postulate that retinal patching may be associated with complications relating both to the technique itself and to the materials used. As a prerequisite, both the adhesive and the substrate would need to be easy to prepare, sterilise and store, so that the materials would be readily to hand when needed.
The main areas of technical difficulty that one could envisage with retinal patching relate to introducing the materials through a small sclerotomy and applying them accurately to the retinal break. Excessive instrumentation is associated with an increased risk of sclerotomy-related peripheral retinal tears, and vitreous base incarceration with the risk of fibrovascular ingrowth. Both these complications might occur, particularly whilst introducing the substrate. A very thin, tough, pliable material would make this a less traumatic procedure. The sclerotomy could be enlarged, but this would mean the loss of an air- or fluid-tight entry site.

Authors who have used adhesives for retinopexy comment on some of the difficulties encountered while delivering adhesives. Problems include polymerisation of one-component adhesives within the delivery system; rapid polymerisation, which can result in instruments becoming adherent to the retina; difficulties in seeing transparent, colourless adhesives and undue spreading of low viscosity, slowly polymerising adhesives. Using two component adhesives is not without problems either; if the components are delivered simultaneously using two syringes with a Y connector the components can mix and set in the needle. If the components are delivered sequentially accurate application is difficult as the first component may spread, and thorough mixing may not take place when the second component is added. The ideal adhesive would be a viscous, one component substance with a polymerisation time that could be manipulated or controlled in some way. The ideal adhesive would also set in an aqueous environment so that the patch could be applied to a retinal break within an area of RD in a fluid filled eye. Good visualisation is more likely under these circumstances, particularly in phakic eyes, as in the air filled phakic eye a biconcave corneal contact lens is required to overcome the refractive change induced by the air bubble. The ideal adhesive would be malleable once set, allowing the area of retina containing the patch to mould itself to the posterior coats of the eye as the retina reattached.

Other possible complications relate to the materials themselves; it would be undesirable if either the adhesive or the substrate were toxic or capable of stimulating an inflammatory reaction. If the materials were toxic they could produce localised granulomatous reactions or tissue necrosis, or more generalised retinal changes. If they stimulated an inflammatory reaction this could initiate mechanisms leading to the development of PVR(139).

The ideal patch would consist of materials that are easy to handle and deliver to the retinal surface and which would form an inert, permanent repair. Should biodegradable materials be used, the patch would only effect a temporary repair. In recent RDs absorption of SRF is rapid and a temporary patch may promote retinal
reattachment, but this may not be true for long standing RDs. A temporary patch may however, produce permanent retinal reattachment under two circumstances; first, if it were to become incorporated into the substance of the retina and secondly if it stimulated a chorioretinal reaction with a wound healing response. The patch might become incorporated into the retina if a glial wound healing response was stimulated. Glial cells are known to migrate through the ILL of the retina in response to a wide number of insults, including inflammation(209), and then proliferate on the inner retinal surface. If the patch stimulated this response glial cells might invade or grow over the surface of the patch, producing a permanent, cellular repair. The second mechanism whereby a temporary patch would produce permanent retinal reattachment would be if a localised chorioretinal reaction was stimulated. This would induce a wound healing response with the formation of a fibrous chorioretinal scar, sealing the retinal break to the underlying RPE. The problem with both of these ideas, however, is that the tissue reactions may not be localised to the area of the retinal patch. If a more generalised wound healing response were stimulated, wide spread ERM formation might well ensue.

A temporary patch could also result in permanent retinal reattachment if it persisted long enough for retinal flattening to take place so that laser photocoagulation could be applied, but this rather defeats the purpose of the technique.

In the first part of this thesis in vitro experiments are described which were undertaken to investigate some of the issues outlined above. A large number of biological and synthetic adhesives and substrates were available for evaluation. The purpose of the in vitro experiments was to select the most suitable materials for testing in an animal model. The in vitro experiments were planned so that after each experiment the number of materials that might be suitable for in vivo investigation could be narrowed down by a process of elimination. Eventually two adhesives and two substrates were selected for evaluation in an animal model.

In the second part of the thesis the animal experiments are described, including the steps taken in developing the animal model. The initial aims of the animal work were to explore the technical and practical questions posed by retinal patching, namely; is retinal patching technically possible? What are the best ways of applying a retinal patch? What are the technical and practical difficulties? and finally what are the complications related to the technique? The further question that needed addressing was whether retinal patching would promote retinal reattachment. Finally, clinical and histological examination was undertaken to look for evidence of toxicity and inflammation and to ascertain the consequences of these reactions.
CHAPTER 2

MATERIALS AND METHODS OF IN VITRO AND IN VIVO INVESTIGATIONS

This chapter is divided into several parts; the materials and methods of the in vitro investigations undertaken to evaluate potential patching materials are described. Then the in vivo experiments are described, including steps taken in developing the animal model.

A) IN VITRO EVALUATION OF ADHESIVES

2.1 AIMS OF IN VITRO INVESTIGATIONS:

At the start of the study 17 biological and synthetic adhesives were available for evaluation in vitro (Table 2.1). The aim of the in vitro experiments was to select the two most suitable adhesives for evaluation in an animal model of retinal patching. The experimental sequence was planned so that selection could be made by a process of elimination.

The initial experiments were undertaken using bovine eye cups to determine which of the available adhesives would adhere to bovine retina. Those adhesives which fulfilled the experimental criteria were then investigated using tissue culture techniques and limited organ culture to evaluate and quantify toxic or cellular reactions.

2.2 MATERIALS:

2.2.1 Biological adhesives:

Glycoproteins:
Fibronectin (FN) and laminin are both naturally occurring basement membrane components involved in cell attachment. Matrogel is a mixture of basement membrane components consisting mainly of laminin and type IV collagen.

Fibrin preparations:
1) bovine thrombin (Sigma) mixed with chick embryo extract reconstituted in phosphate buffered saline (PBS).
2) freeze dried fibrin (Sigma) reconstituted with sodium hydroxide, (25 mg fibrin left for 2 hours in 1 ml 1M sodium hydroxide and then neutralised with 1 ml 1N hydrochloric acid)
Table 2.1 Adhesives available for in vitro investigations.

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<td></td>
<td>Silastic Adhesive A</td>
<td>Dow Corning</td>
</tr>
<tr>
<td></td>
<td>Medical Adhesive 355</td>
<td>Dow Corning</td>
</tr>
<tr>
<td></td>
<td>Polyacrylic Acid</td>
<td>Dow Corning</td>
</tr>
<tr>
<td></td>
<td>Poly-F plus</td>
<td>Dentsply</td>
</tr>
</tbody>
</table>
3) pooled freeze dried rabbit fibrinogen (Sigma) reconstituted in calcium chloride 40 mMol/L mixed with bovine thrombin (4, 20, or 500 IU/ml, Sigma, Immuno Ltd.) reconstituted in aprotonin solution 3 TIU/ml (Sigma)

4) Rabbit plasma (obtained by bleeding a rabbit from an ear vein with 3.8% sodium citrate anticoagulant 1 part citrate to 9 parts blood, and centrifuging at 4c, 3,000 rpm for 7 minutes) mixed with bovine thrombin, as above.

5) Tisseel (Immuno Ltd.), a commercial preparation of fibrin. Pooled freeze dried human fibrinogen 70-110 mg reconstituted in aprotonin after warming to 37°C, and bovine thrombin (4 or 500 IU/ml) reconstituted with calcium chloride 40 mmol/ml after warming to 37°C. The solutions were drawn up in separate syringes, connected to a Y shaped joining piece and attached to the "Duploject" clip for simultaneous delivery via a 20G needle. In addition to fibrinogen the clottable protein content of Tisseel also contains plasma fibronectin 2-9 mg, Factor XIII 10-50 Units and plasminogen 40-129 mg.

As Tisseel contains both human and bovine fibrinogenic components fibrin made from rabbit plasma was assessed as it was hoped to reduce to a minimum immunogenic inflammatory reactions which might be anticipated using Tisseel in the rabbit. Sources of rabbit fibrinogen are commercially available, but rabbit thrombin is not.

Mussel adhesive protein (MAP, Biopolymers Inc.) is a newly developed adhesive which has not yet passed FDA regulations. Small samples were kindly made available by Biopolymers Inc. for in vitro assessment. Both bioengineered MAP and extracted adhesive were investigated. This again is a four component adhesive consisting of lyophilised synthesised or extracted natural mussel protein, sodium chloride buffer, a cross linker and iron peroxide. Two different synthesised mussel proteins and two different cross linkers were assessed (ferrous sulphate and a lysine based compound), using varying concentrations of each component. The protein was mixed with buffer and the cross linker with iron peroxide and small volumes of each drawn up in a micropipette with an air pocket separating the two mixtures, as the volumes of adhesive available were not large enough to use the Duploject system.

2.2.2 Synthetic adhesives:

Cyanoacrylates: Three preparations of N-butylcyanoacrylate were assessed; Histoacryl (B. Braun Melsungen AG), which contains methylene blue and is widely used clinically, avacryl and nexacryl. The latter were made available by CRX Medical as they too
have not yet received FDA approval. The chemical formulations of the avacryl and
nexacryl have been manipulated to provide adhesives of varying viscosities (3 CPS
and 300 CPS respectively) and polymerisation times. Octylcyanoacrylate was also
made available by CRX Medical.

In each in vitro investigation the cyanoacrylates were used pure form or mixed with
equal volumes of lophendylate (Lafayette) which slows polymerisation(179).

Other adhesives:
Medical adhesive 355 and medical adhesive silicone type A have been used in a
clinical setting to bond silicone tubing. Polyacrylic acid and Poly-F Plus (zinc
polycarboxylate) are used in dentistry.

2.3 METHODS:

2.3.1 Bovine Eye Cup Experiments:

The purpose of the experiments undertaken using bovine eye cups was to
determine which of the adhesives adhered to bovine retina.

Freshly enucleated bovine eyes were transported on ice and after cleaning away
excess tissue the anterior segments were removed by making a 360° equatorial
incision. The vitreous humour, which readily separated, was gently removed taking
care not to damage or detach the retina. Small samples of each adhesive were
delivered to the retinal surface of eye cups filled with minimal essential media
(MEM, Gibco) with 10% newborn calf serum (NCS, Gibco) and to the dried retinal
surface of another series of eye cups containing no media. Once it had been
determined which adhesives stuck to the retina four eye cups containing samples
of each adhesive were prepared and filled with MEM + 10% NCS. The eye cups
were then placed on a Luckmann shaking platform set at mark 5 for four hours. At
the end of this period the eye cups were examined to see which of the adhesives
remained adherent to the retina. The cyanoacrylate adhesives were evaluated
alone, or mixed 50:50 by volume with lophendylate.

2.3.2 Tissue Culture Studies:

Those adhesives that remained adherent to bovine retina were assessed in tissue
culture using eye derived cells i.e. bovine retinal glia and bovine RPE, for evidence
of toxicity. The only adhesives that remained adherent to bovine retina using the
methods described above were the cyanoacrylate adhesives, Tisseel and one of
the bioengineered mussel proteins (see Results section 3.1). Of the N-butylcyanoacrylates Histoacryl was selected for study in tissue culture as it is readily available and has been used in ocular surgery. The cyanoacrylates Histoacryl and octylcyanoacrylate were used in a pure form or mixed 50:50 with lophendylate. The fibrin evaluated in tissue culture was that obtained using the 500 U/ml Tisseel preparation. Insufficient volumes of MAP were available for study in tissue culture.

Bovine retinal glia cell cultures were prepared using the technique described by Savage(210). Freshly enucleated bovine eyes were transported on ice, excess tissue removed and the globes soaked for ten minutes in phosphate buffered saline (PBS) containing penicillin and streptomycin (Gibco). The anterior segments and vitreous were discarded and the retinas removed under sterile conditions, taking care not to disturb the RPE. Several small pieces of retina, approximately 1 cm² were floated in 25 cm² tissue culture flasks containing 10 mls MEM with Earle's salts (Gibco), 15% NCS , 1 ml penicillin and streptomycin and 0.1 ml amphotericin B (0.25 units/ml, Flow). The flasks were incubated at 37°C and 5% CO₂ and the appearance of cells monitored by phase contrast microscopy (Nikon). Once primary cultures had been established the cells were fed twice a week with MEM containing 10% fetal calf serum (FCS, Gibco). Cultures became confluent after three to four weeks at which time the cells were passaged using 2 mls trypsin/EDTA solution (0.25% trypsin, 0.02% EDTA). The glial nature of cells obtained using this technique for primary culture had been determined previously using monoclonal antibodies against glial fibrillary acidic protein (GFAP, an intermediate filament found in normal astrocytes and Muller cells after injury) and vimentin (an intermediate filament found in abundance in Muller cells)(210).

Cultures of bovine RPE cells were established in tissue culture using a modification of the technique described by Edwards(211). Freshly enucleated bovine eyes were transported on ice and soaked for ten minutes in PBS containing penicillin and streptomycin after cleaning off excess tissue. The anterior segments, vitreous humour and retinas were gently removed under sterile conditions taking care not to damage the RPE. The eye cups were filled with EDTA/trypsin solution after placing a plastic tube over the optic nerve head so as not to contaminate the culture with glia and vascular cells from the retinal remnant and optic nerve head. The eye cups were incubated at 37°C for twenty minutes and RPE cells flushed off Bruch's membrane by gentle pipetting. The cell suspension was added to 25 cm² tissue culture flasks containing 2 mls NCS (to neutralise the trypsin), 5 mls of media were added (MEM containing 15% NCS, 5% triptose phosphate broth (TPB), 1% penicillin and streptomycin and 0.2% amphotericin B) and the flasks incubated at 37°C and 5% CO₂. After primary cultures had been established the cells were fed
twice weekly and once confluence reached the cells were passaged using EDTA/trypsin into four daughter cultures. These cultures were fed twice weekly with MEM containing 10% NCS and 5% TPB. The cells remained healthy for eight to ten further passages.

2.3.3 Tissue Culture Toxicity Studies using Cyanoacrylates:

The following tissue culture experiments were performed:

1. 2µL of Histoacryl and 2µL octylcyanoacrylate were added to confluent cultures of glial and RPE tissue culture flasks
2. 2µL of octylcyanoacrylate with and without lophendylate and lophendylate alone were added to confluent cultures of glia and RPE in tissue culture flasks
3. 2µL of Histoacryl was added to confluent cultures of human RPE in tissue culture flasks
4. 2µL of Histoacryl and octylcyanoacrylate with and without lophendylate were added to glial cultures grown on glass (to exclude the possibility that toxic products may be released by the action of the cyanoacrylates on the plastic of the tissue culture flasks)

1) Histoacryl and Octylcyanoacrylate added to glia and RPE cell monolayers:
Six flasks of confluent cultures of each cell type (RPE passage 5 and glia, passage 11) were used for each adhesive. The media was discarded from the flasks, the cell monolayer rinsed with PBS and the excess removed by tilting the flask and aspirating residual fluid with a pipette. If a layer of media was left on the surface of the cells the cyanoacrylate adhesive started to polymerise as soon as it came into contact with the thin film of fluid overlying the cells, spreading widely and not adhering to the cell monolayer. Histoacryl and octylcyanoacrylate were drawn up in fine polythene tubing, as this material does not initiate polymerisation. 2µL of each adhesive were dropped onto the centre of each separate cell monolayer, using a micrometer (Figure 2.1), and allowed to polymerise (Figures 2.2). 2µL were used as this had been found to be sufficient to apply a retinal patch in vitro, and if larger volumes were used the halo of cell death surrounding the adhesive extended to the edge of the tissue culture flask, making measurement impossible. After the adhesive had polymerised (10-20 seconds) fresh media was added and the cells incubated and fed as normal. The flasks were examined daily for a week, using phase contrast microscopy, and every two or three days thereafter for up to 33 days. The area of cell death surrounding the drop of adhesive was plotted on acetate paper at each examination by placing the flasks on a slide reader (Carl Zeiss Jena) and drawing round the area of shadow cast by the drop of adhesive.
Figure 2.1 Micrometer used for delivering small volumes of cyanoacrylate adhesives in the in vitro experiments.
Figure 2.2 2μL drop of Histoacryl added to a confluent culture of bovine retinal pigment epithelial cells growing in a standard tissue culture flask.
Figure 2.3 Cell free zone (arrow) surrounding drop of cyanoacrylate adhesive clearly visible when flask placed on a slide reader.
and by delineating the boundary of dead and healthy cells (Figure 2.3). The area of cell death was determined using an image analyser (MOP Videoplan).

2) Octylcyanoacrylate with and without lophendylate added to glia and RPE cell monolayers:

The experiment was repeated using octylcyanoacrylate alone, mixed 50:50 with lophendylate or lophendylate alone to determine whether lophendylate itself was toxic, or whether the combination of lophendylate and octylcyanoacrylate influenced toxicity. When using the mixture, equal volumes of octylcyanoacrylate and lophendylate were drawn up in a tuberculin syringe and the syringe gently rocked to ensure complete mixing of the components. 2µL of either octylcyanoacrylate, lophendylate, or the mixture were applied to the cells as before, using the micrometer. The cultures were examined and followed as above.

3) Histoacryl added to human RPE cell monolayers:

Human RPE (passage 5) were kindly made available by M. Boulton and similar studies were undertaken using these cells. The media used for human RPE was F10 (Gibco) with 20% FCS, 5% glucose (80 mg/ml), 1% glutamine, 1% penicillin and streptomycin, and 1.5% bicarbonate (7.5% solution).

4) Histoacryl and octylcyanoacrylate with and without lophendylate added to glial cell monolayers grown on glass:

To exclude the possibility that toxicity may have been due to chemical interaction between the cyanoacrylates, or lophendylate, and the plastic of standard tissue culture flasks, similar, short term experiments were undertaken using cells grown on glass. Glia only were used for this experiment as this cell type had been shown in the earlier experiments to be more sensitive to toxic damage and in the retinal patching technique the adhesive would come into direct contact with retinal glial cells rather than RPE cells. 24 dishes were prepared by attaching glass cover slips to the bottom of petri dishes with wax. The petri dishes were sterilised by 24 hours exposure to ultraviolet light. Glia were passaged into the dishes and allowed to grow to confluence. 6 dishes were used for each adhesive. 2µL of Histoacryl or octylcyanoacrylate alone, or mixed 50:50 with lophendylate were allowed to polymerise on the cell monolayer after discarding the media. The area of cell death surrounding each drop of adhesive was measured at 6 hours using the method described above. If cultures were left longer than this the whole cell monolayer separated from the flask.
2.3.4 Preparation of Specimens:

To investigate morphological changes induced by cyanoacrylate adhesives, specimens were prepared for electron microscopy.

Flasks were prepared in a manner similar to experiment 1 and processed for SEM. At different time intervals i.e. days 8, 12, 14, 20 and 33 the media was discarded, the cell monolayer rinsed with warmed PBS with calcium (Dulbecco's, Gibco) and cells fixed with 2% glutaraldehyde in Sorensons buffer for 20 minutes. Cells were postfixixed with 1% osmium tetroxide, dehydrated through alcohol, critical point dried (Polaron), coated with 50nm gold (Polaron) and examined by SEM (Hitachi S-520).

2.3.5 Tissue Culture Toxicity Studies using Tisseel:

Similar experiments were undertaken using Tisseel, the commercial fibrin preparation, to determine whether it was toxic to eye derived cells in tissue culture, and to investigate any morphological changes induced by Tisseel.

1. Tisseel was added to confluent cultures of glia and RPE
2. Tisseel was added to media overlying RPE and glia growing in 24 well plates

1. Tisseel added to glial and RPE cell monolayers:

Six confluent cultures of RPE (passage 7) and glia (passage 10) were used in this experiment. Tisseel (500 IU/ml strength thrombin) was reconstituted, drawn up in the "Duploject" injector (Figure 2.4) and small drops delivered onto the cultures after discarding the media and rinsing the monolayer with PBS. It was not possible to measure the volume of adhesive used, but care was taken to make the applications of equal volume. After refeeding the cells were incubated under standard conditions and examined daily for 15 days by phase contrast microscopy.

2. Tisseel added to the media of cultures of RPE and glia:

To determine whether Tisseel induced morphological changes passage 11 glia and passage 4 RPE were grown to confluence in 24 well plates. The media was changed and 0.05 mls of each of the Tisseel components (4 U/ml thrombin) was added to fresh media so that a fibrin clot formed over the cell monolayer. The cultures were viewed daily for three days using phase contrast microscopy.
Figure 2.4 "Duploject" system used for delivering Tisseel in the in vitro experiments and in the animal model
2.3.6 Preparation of Specimens:

As experiment (1) was non-quantitative flasks were prepared for scanning electron microscopy using the method described above on days 5, 10 and 15.

At the end of experiment (2) (3 days) the media was aspirated from the wells, the fibrin clot was rinsed with PBS containing calcium, fixed with 2% glutaraldehyde, and then processed for scanning electron microscopy.

2.3.7 Organ Culture Studies:

The aims of these experiments were to investigate toxic effects of cyanoacrylates on bovine retina and bovine RPE in limited organ culture.

Retina:
Samples of bovine retina measuring approximately 2 x 2 cm were prepared as described in section 2.3.2. Several different methods of organ culture were assessed using untreated tissue in an attempt to find a method that preserved normal morphology. Methods included placing samples of excised bovine retina between stainless steel rings in petri dishes and incubating under standard tissue culture conditions using a range of different media (i.e. MEM, F10, RPMI with or without glucose and glutamine). After 24, 48 and 60 hours the retina was fixed in 10% formal saline and prepared for light microscopy. Light microscopy showed that even after 24 hours the retina was oedematous, with loss of photoreceptors. By three days the retina had become necrotic. To improve oxygenation samples of retina were placed on nitrocellulose filters which were supported so that the retina was at the air/media interface. However, this method was also unsuccessful in preserving morphology.

Short term "organ culture" was therefore finally undertaken using bovine eye cups. Freshly enucleated bovine eyes were transported on ice, cleaned of excess tissue and the anterior segments and vitreous removed. Ten eye cups were prepared as it was hoped that toxic changes induced by the cyanoacrylate adhesives could be quantified. 2μL drops of Histoacryl and octylcyanoacrylate were delivered to the dried retinal surface of each eye cup using a micrometer (as in Figure 2.5). The drops of Histoacryl, and of octylcyanoacrylate were applied to corresponding quadrants in each eye cup, to avoid the problems of regional retinal morphological variation. A third quadrant acted as control. The fourth quadrant, containing the optic nerve head was not used. The eye cups were filled with MEM containing 10%
Figure 2.5 2μL drops of Histoacryl and octylcyanoacrylate allowed to polymerise on the RPE of a bovine eye cup for limited organ culture studies.
NCS with HEPES as the buffering system and incubated for six hours at 37°C in a warming cabinet.

After incubation the three quadrants of retina were carefully excised and attached to corks with fine stainless steel pins so the retina would stay flat during fixation. Each portion of retina was fixed in 10% formal saline and processed for light microscopy. Sections were cut that passed through the centre of the drop of adhesive as it was hoped that it would be possible to measure the width of any changes.

Retinal pigment epithelium:
Bovine eye cups were prepared as above except that the retina was removed, taking care not to damage the RPE. 2μL drops of Histoacryl and octylcyanoacrylate were applied to corresponding quadrants, as above, and allowed to polymerise. The eye cups were filled with media and incubated as above. After six hours the eye cups were sectioned and the three quadrants from each eye fixed in 2% glutaraldehyde and processed for scanning electron microscopy. Again it was hoped that a quantitative study could be undertaken by measuring the area of change surrounding each drop of adhesive.

B) IN VITRO EVALUATION OF SUBSTRATES

2.4 AIMS OF IN VITRO EXPERIMENTS

At the start of the study 6 biological and 20 thin, synthetic membranes were available for evaluation as potential patching substrates (Table 2.2). The sequence of experiments was designed so that the two most suitable materials could be selected for subsequent animal studies, largely by a process of elimination.

The first experiment was undertaken to determine which of the substrates were tough and malleable enough to be introduced into the posterior segment of bovine eyes through a standard sclerotomy. The second investigation was undertaken on those substrates which could be introduced. The aim of this study was to evaluate and quantify the biocompatibility of these materials, using eye derived cells in tissue culture, to see whether the substrate would support a localised wound healing response.
Table 2.2. Substrates available for in vitro investigations.

| BIOLGICAL SUBSTRATES | | |
|----------------------|-----------------|
| Substrate            | Manufacturer     |
| Bovine anterior lens capsule |                  |
| Bovine Descemets' membrane |              |
| Chick allantoic membrane |                |
| Collagen shield       | Chiron Ophth.    |
| Collagen membrane     | Chiron Ophth.    |
| Fibronectin membrane  | Chiron Ophth.    |

<table>
<thead>
<tr>
<th>SYNTHETIC SUBSTRATES</th>
<th>Thickness (μm)</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose acetate</td>
<td>24</td>
<td>Courthaulds</td>
</tr>
<tr>
<td>Cellulose triacetate</td>
<td>35</td>
<td>AC Converters</td>
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<tr>
<td>Ethylene methacrylic acid copolymer</td>
<td>140</td>
<td>Du Pont</td>
</tr>
<tr>
<td>EVA</td>
<td>45</td>
<td>Dale Products</td>
</tr>
<tr>
<td>Fluorinated ethylene propylene</td>
<td>25</td>
<td>Du Pont</td>
</tr>
<tr>
<td>Hydrogel</td>
<td>50</td>
<td>Fulmer Yarsley</td>
</tr>
<tr>
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<td>100</td>
<td>Fulmer Yarsley</td>
</tr>
<tr>
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</tr>
<tr>
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<tr>
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<tr>
<td>Silicone oil membrane</td>
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<td>Dow Corning</td>
</tr>
<tr>
<td>Steridrape</td>
<td>Unknown</td>
<td>3M</td>
</tr>
<tr>
<td>Vinylidene chloride copolymer</td>
<td>38</td>
<td>Dow Corning</td>
</tr>
</tbody>
</table>
2.5 MATERIALS:

Bovine anterior lens capsules were prepared by removing the corneas from freshly enucleated bovine eyes under aseptic conditions and incising and removing the anterior capsule. Anterior epithelial cells were removed by placing the capsule in trypsin/EDTA solution for 10 minutes and then rinsing vigorously in sterile PBS. Capsules were prepared for scanning electron microscopy to confirm that all anterior epithelial cells had been removed.

Samples of Descemet's membrane were prepared by modifying the technique described by Kenney(212). Bovine corneas were removed and the endothelium and as much stroma as possible was removed by dissection. The corneas were placed in distilled water for four hours, to osmotically shock the tissues. After this further stroma could be removed by dissection. The tissues were then treated with detergents; 3% Triton X-100 for fours hours, followed by distilled water rinse. The tissues were exposed to DNA'se (2,000 Kunitz units, Sigma) in 1M NaCl for 2 hours, and 4% sodium deoxycholate for 2 hours. After rinsing, samples of the tissue were prepared for transmission electron microscopy to confirm that all stromal collagen had been removed.

Chick allantoic membrane was prepared by boiling hens eggs until hard boiled (10 minutes) and when cooled, the membrane lining the shell was removed. Samples were prepared for light and transmission electron microscopy. Special stains were used (Gamori's aldehyde fuschin, with and without oxidation using 10% potassium peroxymonosulphate, Verhoeff iron haematoxylin and Gomori's trichrome) to determine the composition of the membrane.

Hydrogel membranes were reconstituted from their dry form by soaking for 16 hours in 1:7 (by weight) glycerol : distilled water solution, and then rinsing with PBS.

The silicone oil membrane was prepared using a modification of the technique described by Harris(213). 5,000 CS silicone oil (Dow Corning) was spread on a glass slide to form a layer 1-2 mm thick. The slide was placed on a hot plate until the superficial layers of oil had polymerised to form a thin film (5-10 minutes), which could be removed after cooling.
2.6 METHODS:

2.6.1 Bovine Eye Experiments:

The first experiments were undertaken to evaluate the physical properties of potential substrates, with respect to pliability, malleability and toughness, by determining which could be introduced into the posterior segment of bovine eyes through a standard sclerotomy using intraocular forceps. Extraocular tissue was removed from freshly enucleated bovine eyes and a sclerotomy made with a standard MVR blade (20G, Alcon). Samples measuring 2mm2 and 5mm2 of each substrates were prepared and attempts made to introduce each through the sclerotomy using standard intraocular forceps (Bennett Instruments). If the sclerotomy enlarged during manipulation a fresh eye was used. Each substrate that could be introduced into the posterior segment without tearing was assessed to see whether it would flatten onto bovine retina in eye cups.

2.6.2 Tissue Culture Biocompatibility Studies:

This investigation was carried out on those substrates that were tough and plastic enough to be introduced through a standard sclerotomy and which were malleable enough to flatten again onto bovine retina. Quantitative cell settlement studies were undertaken using synthetic membranes as it was possible to prepare samples of each of the same size. This was not possible with the biological membranes, and some of them were not sufficiently transparent for cell counts to be made, and so non quantitative evaluation was undertaken.

Synthetic Materials:

Bovine retinal glia and bovine RPE were grown in tissue culture as previously described; passage 9 RPE and passage 11 glia were used for cell settlement assays. Eight 1cm2 samples of each of the selected synthetic substrates were attached to the bottom of 35mm petri dishes using wax/paraffin to completely seal the edges, and sterilised by 24 hours exposure to ultraviolet light. Eight petri dishes without substrate were used as controls. 1ml of NCS was added to each petri dish and 2 x 105 cells added to each dish. Four petri dishes of each cell type were set up for each substrate, with four controls for each cell type. 3 mls of media were added to each petri dish (MEM with 10% FCS for glia and MEM with 5% TPB and 15% NCS for RPE) and the dishes incubated at 37°C and 5% CO2 for 5 hours. At the end of this period the settlement rate for controls was determined by counting the number of settled and floating cells and calculating the percentage settlement. Media was discarded from experimental petri dishes, the cells fixed with 10%
formal saline and cell settlement determined by counting and totalling the number of attached cells (rounded and spread cells) in five random fields in each dish.

**Biological Materials:**
Samples of each substrate were attached to the bottom of 25 cm² tissue culture flasks under aseptic conditions, using wax/paraffin, and 1 ml of NCS and then 4 x 10⁵ passage 4 RPE cells added. The cells were fed with 5 mls of media (MEM containing 15% NCS and 5% TPB with 1% penicillin and streptomycin) and the flasks incubated under normal conditions. The flasks were examined daily by phase contrast microscopy and once the cells had become confluent the substrates were removed, fixed in 2% glutaraldehyde in Sorensons buffer and processed for scanning or transmission electron microscopy.

**C) IN VITRO EVALUATION OF PATCHING**

**2.7 AIMS OF IN VITRO EXPERIMENTS**

Retinal patches were applied over retinal breaks created in bovine eyes using those adhesives that adhered to bovine retina under the previously described test conditions (see Results 3.1) and those substrates that could be introduced into the posterior segment of bovine eyes through a standard 20G sclerotomy (see Results 3.5). The aims of these investigations were to determine the optimum method of delivering the patching materials and which combinations of adhesive and substrate remained adherent to bovine retina.

**2.8 MATERIALS AND METHODS**

**2.8.1 Bovine Eye Cup Experiments:**

Bovine eye cups were prepared as before. Small retinotomies were created using Vannas scissors and samples of each substrate prepared that were large enough to cover the defect (2-3 mm²). Different methods of application of the patching materials were assessed i.e.

1. applying adhesive to the edge of the break and then placing the substrate on the adhesive
2. coating one surface of the substrate with adhesive and then placing it over the defect
3. putting the substrate in place over the retinal defect and then adding the adhesive to the edges of the substrate.
Figure 2.6 Retinal patch, immediately after application, made from octylcyanoacrylate and PVdF applied to repair a small retinotomy in a bovine eye cup.
Figure 2.7 Same retinal patch as in Figure 2.6, immediately after injecting media under the retina to test the integrity of the patch.
Integrity of the patch was assessed by injecting media through a peripheral retinotomy so that the area of retina containing the patch elevated (Figure 2.6 and 2.7).

Eye cups containing patches (four for each combination) were filled with media and placed on a Luckmann shaking platform set at mark 5 for four hours. At the end of this period the eye cups were examined to determine patches remained adherent.

Retinal patches were created and processed for scanning electron microscopy to examine the retina/adhesive interface and the adhesive/substrate interface. Patches were viewed from the ILL surface, and from the photoreceptor surface to determine, whether adhesive had passed through the retinal break.

D) IN VIVO EVALUATION OF RETINAL PATCHING

2.9 AIMS OF IN VIVO EXPERIMENTS:

Retinal patches were applied to repair retinal defects created in rabbit retinæ after vitreolensectomy using octylcyanoacrylate or Tisseel as the adhesive, and PVdF or propylene as the substrate. These materials were selected on the basis of in vitro evaluation (see Results section 3.1; 3.2; 3.6 and Discussion sections 4.1 - 4.6. The aims of the animal experiments were to address the following questions:

1. Is retinal patching technically possible and what are the best ways of delivering the patching materials?
2. What are the technical problems and complications associated with applying a retinal patch in vivo and what are their consequences?
3. Does retinal patching promote retinal reattachment?
4. What are the toxic, inflammatory and cellular reactions of ocular tissues to the patching materials?
5. What are the consequences of these reactions?

2.10 ANIMALS:

The animals used in the early studies were New Zealand White albino rabbits of either sex, weighing between 2.5 and 3 kg at the time of initial surgery. Later experiments were undertaken using male cross breed Dutch/Lop-eared rabbits weighing between 2.0 and 2.5 kg at the time of initial surgery, as it was found that
visualisation during intraocular manoeuvres was difficult using rabbits with non-pigmented fundi.

2.11 ANAESTHETICS

All operations were performed under general anaesthetic. Premedication with 0.25-0.5 ml of intramuscular Hypnorm (fentanyl citrate 0.315 mg/ml and fluanisone 10 mg/ml, Janssen) was followed half an hour later by 1 ml intravenous Valium (diazepam 5 mg/ml, Roche, diluted 2 in 5 with sterile N saline) via a cannula into an ear vein. Additional intramuscular Hypnorm and intravenous Valium were given peroperatively as required to maintain anaesthesia. Terminal anaesthesia was achieved using 2-4 ml of intravenous Expiral (sodium pentobarbitone 200 mg/ml, CEVA) via an ear vein.

Examination of animals was performed without sedation, or using 0.25-0.5 ml intramuscular Hypnorm half an hour before examination.

All instruments and the substrates were sterilised by soaking in 70% alcohol for 20 minutes, followed by two rinses in sterile distilled water. Surgery was performed on the right eye only of each animal.

2.12 SURGICAL PROCEDURES:

Retinal patching is a technique proposed for the management of selected retinal breaks requiring an internal approach after vitrectomy. In order to reproduce as closely as possible the conditions in which a retinal patch would be applied in the human situation retinal patches were applied to rabbit retinae after vitrectomy. For anatomical reasons vitrectomy in the rabbit requires a sequence of surgical procedures and modification of the techniques used in humans. The rabbit eye has a very narrow pars plana, long, delicate, highly vascular ciliary processes, a large crystalline lens and adherent, fluid vitreous. Instruments cannot be passed through the pars plana without risk of damaging the ciliary processes and lens and so sclerotomies have to be made more posteriorly, passing through peripheral retina. Pretreatment with peripheral retinal cryotherapy reduces the risk of retinal detachment from this approach. Although more fluid than human vitreous, rabbit vitreous humour is adherent to the retina both peripherally and over the medullary rays. Mechanical vitrectomy has been shown to induce traumatic, morphological changes in the retina(214). The vitreous can be induced to detach peripherally using a gas compression technique ("gas compression vitrectomy")(215), although the vitreous remains attached over the medullary rays. Even after gas compression
vitrectomy adequate mechanical vitrectomy cannot be accomplished without
damage to the lens, and in any case, gas injection causes lens opacities which
would prevent good visualisation of the fundus.

In order to prepare the rabbit eye for retinal patching a sequence of operations was
therefore required:

1. Monitored peripheral retinal cryotherapy to subsequent sclerotomy sites
   Animals left for a minimum of 2 weeks
2. Intravitreal injection of 0.4 mls of 100% C3F8 gas, to induce PVD
   Animals left for a minimum of 2 weeks
3. Vitreolensectomy

2.12.1 Peripheral Retinal Cryotherapy:

Pupillary dilation was with 10% phenylephrine (Richard Daniel) and 1% tropicamide
(Alcon), and topical anaesthesia using 0.3% Benoxinate. After induction of general
anaesthesia the right globe was gently prolapsed and maintained in this position by
placing polythene tubing around the globe, posterior to the equator and secured
with artery forceps. The conjunctiva was opened via fornix based flaps at three
sites (6, 2 and 10 o’clock) and a partial thickness 8/0 nylon scleral marking suture
(Ethicon) placed 3 mm from the limbus. Monitored transcleral cryotherapy was
placed contiguously in two circumferential rows to treat peripheral retina at these
sites (usually 4 applications, 2-8 mm from the limbus), using a Keeler indirect
ophthalmoscope and KR Med MC 1,000 cryotherapy unit. The conjunctiva was
closed with 6/0 Vicryl (Ethicon) and the eye treated with one drop of Gentamicin
(Nicholas).

The rabbits were undisturbed for two weeks, to allow chorioretinal adhesions to
develop.

2.12.2 Gas Compression Vitrectomy:

Pupillary dilatation, topical and general anaesthesia and globe prolapse as above.
The conjunctiva over the inferior sclerotomy site was opened, the presence of a
chorioretinal adhesion confirmed by indirect ophthalmoscopy and a preplaced 8/0
nylon mattress suture inserted into the sclera 4 mm from the limbus. Pure C3F8
gas was drawn up in a tuberculin syringe through a 22µm millipore filter and
attached to a canula made from a 30G needle and fine polythene tubing. The
tubing was flushed with gas and the point of the needle inserted through treated
choroid 4 mm from the limbus, directing the needle posteriorly so as to avoid the lens. The rabbit was then placed in a head up position (so that the gas floated away from the tip of the needle into the upper part of the vitreous cavity) and 0.2 ml of gas injected under indirect ophthalmoscopic visualisation. Intraocular pressure was monitored by checking the patency of the central retinal artery and once the intraocular pressure had fallen sufficiently for retinal circulation to be re-established the remaining 0.2 ml of gas was injected. The needle was then slowly withdrawn and pressure immediately applied over the injection site with a sterile cotton wool tip for 5 minutes, to prevent egress of gas or fluid vitreous. The preplaced mattress suture was then tied, the conjunctiva closed and one drop of Gentamicin added. The intraocular pressure was checked half an hour later by observing central retinal artery perfusion.

In a group of ten rabbits intraocular pressures measurements were taken during this procedure, using a Perkins hand held applanator (Clement Clarke) and fluorescein mixed with Benoxinate. Measurements were made immediately prior to and after gas injections, every five minutes for half an hour, after one hour and daily for a week thereafter. The volume of the intraocular gas bubble was also estimated daily for one week; if the lower meniscus of the bubble was level with the centre of the pupil the bubble was said to occupy 50% of the posterior segment, if the lower meniscus could easily be seen by indirect ophthalmoscopy the fill was estimated as 80% and if it was not visible, 100% fill. Percentage fills between 50% and 80% and less than 50% were judged by eye.

These animals were also examined daily on the slit lamp and the degree of cataract formation and anterior segment inflammation noted, using the grading system outlined below.

Every animal undergoing gas compression vitrectomy was examined macroscopic and by indirect ophthalmoscopy on the day of maximal gas expansion (day 4) to check that the gas bubble had not over expanded, producing shallowing of the anterior chamber and/or raised intraocular pressure.

The animals were left undisturbed for a minimum of two weeks to allow posterior vitreous detachment to take place.

2.12.3 Vitreolensectomy:

Topical and general anaesthesia and globe prolapse as above. To ensure maximal and prolonged pupillary dilation the pupils were dilated using 10% phenylephrine,
1% tropicamide and subconjunctival injection of 0.1-0.2 mls of Mydricaine No 1 (McCarthy Medical) after topical anaesthesia. Surgery was performed under aseptic conditions using an operating microscope (Zeiss Op Mi 6).

After prolapsing the globe the conjunctiva was opened at the three pretreated sites and a preplaced 6/0 Dexon (Davis and Geck) mattress suture placed 5mm from the limbus at the six o'clock site after confirming the presence of a chorioretinal scar. A sclerotomy was created at this site using a 20G MVR blade (Alcon) and a 2.5 mm infusion canula attached to 500 mls Hartmanns solution (Baxter) secured. Sclerotomies were created at the other two sites; the 2 o'clock site was plugged and the 10 o'clock site used for lensectomy and vitrectomy. Lensectomy was performed using a fragmatome (Berkeley Engineering) and vitrectomy using a Peyman Vitrophage and Visc vitreous cutters. A core vitrectomy was performed and if peripheral vitreous had not spontaneously detached it could be induced to detach with relative ease by gentle aspiration. Adherent vitreous over the medullary rays was removed as extensively as possible. As the rabbit eye is prone to haemorrhage, the intraocular pressure was kept as high as possible during surgery, by raising the bag of infusion fluid.

2.13 DEVELOPMENT OF THE ANIMAL MODEL

As various problems were encountered using the rabbit as an experimental model, the technique evolved with experience. Initially the lens was completely removed, to allow better visualisation of the posterior segment during subsequent retinal patching surgery after fluid/air exchange, but this induced a marked inflammatory reaction. The animals were therefore left for a further two weeks to allow intraocular inflammatory reactions to quieten before operating a fourth time to patch the retina. It was found however, that fluid/air exchange in the aphakic rabbit eye caused intense pupillary miosis and a marked inflammatory response with peroperative fibrin formation. The miosis could not be reversed despite intensive treatment with mydriatics nor by adding 1 ppm adrenaline to the infusion fluid. Sphincterotomy resulted in haemorrhage and a marked inflammatory response. Pupillary miosis and postoperative inflammation were greatly reduced if the anterior capsule was left in situ, after removing anterior epithelial cells. Per- and postoperative fibrin formation was also greatly reduced if low molecular weight heparin was added to the infusion fluid (Sigma, Mol. Wt. 4-6,00, 20 mg/500 mls Hartmanns solution)(216). However, leaving the eyes two weeks to quieten following vitreolensectomy prior to operating a fourth time to apply a retinal patch resulted in poor posterior segment visualisation due to wrinkling and opacification of the anterior capsule (the posterior capsule could not be preserved as it is extremely thin).
The surgical sequence that was finally adopted for retinal patching consisted of monitored peripheral retinal cryotherapy followed two weeks later by injection of 0.4 mls of 100% C3F8 gas. A minimum of two weeks later vitreolensectomy was performed, preserving the anterior capsule and adding low molecular weight heparin to the infusion fluid. Creation of a retinal break with or without patching of the retinal defect was undertaken after vitreolensectomy, during the same operation.

2.14 EXPERIMENTAL GROUPS:

In this study there were six different groups of animals: 3 Control Groups and 3 Experimental Groups.

Control Group 1.
These animals underwent vitreolensectomy followed by creation of a small retinal break and localised retinal detachment.

Control Group 2.
This group of animals had vitreolensectomy followed by fluid/air exchange and then air/fluid exchange alone.

Control Group 3.
This group of animals underwent vitreolensectomy combined with creation of a small retinal break followed by fluid/air and then air/fluid exchange.

Experimental Group A.
In this group of animals a retinal break was created and repaired with a patch using octylcyanoacrylate as the adhesive and propylene or PVdF as the substrate, after fluid/air exchange. Air/fluid exchange was performed at the end of the procedure.

Experimental Group B.
These animals had vitreolensectomy with creation of a retinal break that was patched using Tisseel as the adhesive and PVdF as the substrate after fluid/air exchange. Air/fluid exchange was performed at the end of the procedure.

Experimental Group C.
As above, but the adhesive used was fibrin made from autologous plasma and bovine thrombin 4 IU/ml reconstituted in calcium chloride.
The purpose of having Control Group 1 animals was to observe the natural history of rhegmatogenous RD in the vitrectomised rabbit eye in order to address the question "does retinal patching promote retinal reattachment". The other two Control Groups were included so that the clinical and histological consequences of vitreolensectomy alone or in combination with an unpatched retinal break could be studied. Fluid/air exchange was performed in these eyes as this was a necessary prerequisite to patching using the selected adhesives.

In Control Groups 2 and 3 eyes intraocular forceps and a blunt needle of the same calibre as the adhesive delivery systems were introduced and removed. The only way in which Control group 3 differed from the Experimental Groups A, B and C therefore, was that no retinal patching materials were introduced or applied.

There were three Experimental Groups, where retinal defects were repaired with different adhesives, in order to compare the technical problems, clinical findings and complications of patching using biological and synthetic adhesives.

2.14.1 Surgical Procedures for Control Groups:

Vitreolensectomy was performed in all groups, as described above, preserving the anterior capsule and using low molecular weight heparin in the infusion fluid. In Control Group 1 eyes a small retinal break was created below the medullary rays using a blunt 20G needle, taking care not to damage the RPE, and Hartmanns fluid was slowly injected through the break to create a localised RD. It was not possible to inject equal and measured volumes of fluid in each eye as some fluid tended to escape round the edge of the break, but care was taken that the area and height of retinal elevation was as equal as possible (5x5 disc diameters). Fluid/air exchange was not performed in these eyes as this would have resulted in retinal flattening.

In Control Group 2 eyes vitreolensectomy alone was performed followed by fluid/air exchange using an aquarium air pump (Atlantis 9,000S) (connected to the infusion canula via plastic tubing and a 22μm millipore filter) and a modified Charles flute needle. Air/fluid exchange was then carried out. In Control Group 3 eyes the procedure was as for Control Group 2 eyes, except that a small retinal break was created in inferior retina.

2.14.2 Surgical Procedures for Experimental Groups:

In the experimental groups vitreolensectomy was performed, a small retinal break made in inferior retina and fluid/air exchange undertaken. The preretinal space was
dried as extensively as possible, using a modified Charles flute needle, and the retinal defect was then repaired using a retinal patch made of either PVdF or propylene, using either octylcyanoacrylate (Group A), Tisseel (Group B), or autologous fibrin (Group C) as the adhesive. The substrates were trimmed to a size large enough to cover the retinal break (approximately 2 mm²), introduced into the posterior segment using intraocular forceps (Beckett) and placed over the retinal break. Both the substrates evaluated were transparent and very difficult to see when placed on the retinal surface in an air filled eye. It was found that colouring the substrate with an indelible felt pen prior to sterilisation made visualisation easier. After the substrate was in place the preretinal space was dried again and the adhesive delivered so that the substrate was completely sealed to the retina.

When patching using octylcyanoacrylate a modified version of the delivery system designed by McCuen was used (Figure 2.8)(171). This consisted of a polythene reservoir attached to teflon tubing (Du Pont) which is fine enough to pass through the bore of a blunt 20G needle. The delivery system was designed so that the tip of the teflon tubing could be retracted into the needle during entry into the eye to prevent intraocular fluids from entering the tip of the tubing and initiating polymerisation. A minute amount of 1,000 cs silicone oil was also drawn up into the tip of the tubing, after the cyanoacrylate and separated from it by a small air pocket, to prevent intraocular fluids from coming into contact with the adhesive. Once within the eye the tubing could be advanced and minute volumes of adhesive dropped onto the substrate by applying gentle pressure to the reservoir.

The Duploject system was employed when applying autologous fibrin and the Tisseel preparation (4 IU/ml thrombin). When the 500 IU/ml thrombin strength was used the needle blocked and so the two components were delivered separately and sequentially. In each instance a blunt 20G needle was used.

When the adhesives had set air/fluid exchange was performed, the sclerotomies closed using the preplaced 6/0 Dexon suture and the 8/0 nylon sutures, the conjunctiva closed with 6/0 Vicryl and Gentamicin drops applied. The fundus was then examined by indirect ophthalmoscopy and the findings recorded.

2.15 CLINICAL EXAMINATION OF ANIMALS

2.15.1 Anterior Segment Signs:

Animals in each group were examined daily for the first week following surgery and weekly thereafter until sacrifice. The maximum period of follow up was four weeks.
Figure 2.8 Delivering system used for applying octylcyanoacrylate in the animal model. Fine teflon tubing (T) is attached to a reservoir (R). Pressure on the depressor (D) expresses a small volume of adhesive.
Figure 2.9. Slit lamp photograph of anterior segment of an albino rabbit showing method of assessing anterior segment inflammation.
Anterior segments were examined by slit lamp bimicroscopy (Pocklington slit lamp) before pupillary dilatation (Figure 2.9). Inflammatory signs were graded using a modification of the system used by Hiscott(209); ocular injection 1-5; corneal opacification 1-5; anterior chamber flare 1-5; iris reaction 1-5 (albino rabbits only); and opacification of the anterior lens capsule 1-5. In each instance grade 1= none, grade 2 = mild, Grade 3 = moderate, grade 4 = pronounced and grade 5 = severe. The scores were totalled to give an Anterior Segment Index of inflammation (ASI).

2.15.2 Posterior Segment Signs:

The posterior segment was examined by slit lamp bimicroscopy and by indirect ophthalmoscopy after dilating the pupil with 10% phenylephrine and 1% tropicamide. Vitreous inflammatory signs were graded 1-5 as above. Obscuration of fundus details by haemorrhage or inflammatory reaction was graded 1-5, with grade 1 indicating a clear view, grade 5 total obscuration with intermediary grades 2, 3 and 4. Scores were totalled to give a Posterior Segment Index of inflammation (PSI). Fundi were examined by indirect ophthalmoscopy. The following grading system was used to record the degree of retinal detachment and ERM formation:

<table>
<thead>
<tr>
<th>Grade</th>
<th>Retinal detachment</th>
<th>ERM formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flat retina</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>≤ 1 quadrant</td>
<td>Mild focal ERM</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 1 - 2 quadrants</td>
<td>Marked focal ERM</td>
</tr>
<tr>
<td>4</td>
<td>&gt; 2 - 3 quadrants</td>
<td>Mild generalised ERM</td>
</tr>
<tr>
<td>5</td>
<td>&gt; 3 quadrants - total RD</td>
<td>Marked generalised ERM</td>
</tr>
</tbody>
</table>

The presence of retinal breaks was denoted + or -, and their site recorded.

2.16 PHOTOGRAPHY:

Fundus photographs were taken using a hand held fundus camera (Keeler KOWA RC) and Ektachrome 100 HC film. Anterior segment photographs were taken using a Zeiss camera attached to the Pocklington slit lamp and flash unit.

2.17 ENUCLEATION AND FIXATION OF EYES:

Animals were sacrificed at varying time intervals and the globes prolapsed and enucleated. A postequatorial stab incision was made and the globes fixed in 10% formal saline for light microscopy, or in 2% glutaraldehyde in Sorensons buffer for electron microscopy.
2.18 PREPARATION OF SPECIMENS FOR MICROSCOPY:

2.18.1 Light Microscopy:

Eyes were fixed in 10% formal saline and the anterior segments of selected globes removed after processing to 70% alcohol (particularly those where fundus examination was not possible prior to sacrifice) and macrophotographs taken of the posterior segment. The remaining globes were processed whole and embedded in wax. Calottes were made such that subsequent sectioning included the region of the retinotomy, or of the retinal detachment, or the area of retina where the patch had been applied, and the optic nerve. Sections were stained with haematoxylin and eosin, and examined and photographed (Reichert microscope, Panatomic X film). Selected sections were also stained with Masson Trichrome, Alcian Blue, MSB (Martins Scarlet Blue) and MGP (Methyl Green Pyronin Y).

2.18.2 Electron Microscopy:

Globes were fixed in 2% glutaraldehyde in Sorenson’s buffer and the anterior segments removed after a minimum of 6 hours fixation. Macrophotographs were taken of the posterior segment, the globes sectioned and selected areas processed for SEM or TEM. These included the area of retina where the retinal break had been created with or without a patch, sections of peripheral retina and anterior segment structures.

2.19 SELECTION OF SPECIMENS FOR HISTOLOGY

Animals were sacrificed after cryotherapy and gas compression vitrectomy to confirm histologically that cryotherapy produced a chorioretinal scar, and to determine whether gas compression vitrectomy caused morphological change in the retina. Animals in each of the experimental groups were sacrificed at the following time intervals; after two days, one week, two weeks and one month. If more than one animal in the same Experimental or Control group was sacrificed at the same time interval, one eye was processed for light microscopy and the other for electron microscopy. If only one eye was available at a given time point a decision was made regarding which type of processing would give the most useful histological information. A few animals had to be sacrificed at other time intervals because they developed severe diarrhoea.
CHAPTER 3

RESULTS OF IN VITRO AND IN VIVO INVESTIGATIONS

In the first part of this chapter the findings of the in vitro experiments undertaken to evaluate potential patching materials are presented. In the second part of the chapter the clinical and histological findings of retinal patching in the animal model are reported.

A) IN VITRO EVALUATION OF ADHESIVES

3.1 BOVINE EYE CUP EXPERIMENTS:

The following adhesives remained adherent to the retinal surface of bovine eye cups under the test conditions and when applied to the dried retinal surface of bovine eye cups containing no media:

1. all the cyanoacrylates with or without lophendylate
2. the Tisseel fibrin preparation (both 4 and 500 IU/ml strength thrombin)
3. one of the mussel adhesive proteins (synthetic protein 2, using iron sulphate as the cross linker)

Fibrin preparations 1,2 and 3 formed clots which did not adhere to bovine retina and fibrin made from rabbit plasma gave inconsistent results; 0% adherence using 4 IU/ml thrombin, 25% adherence using 20 IU/ml thrombin and 50% adherence using 500 IU/ml thrombin.

None of the adhesives investigated adhered to the retina of bovine eyes filled with media. This occurred for several reasons:

1. some of the adhesives had a lower density than the media and floated away from the retinal surface (all the cyanoacrylate preparations with or without lophendylate)
2. they could be delivered to the retinal surface but would not adhere (medical adhesive silastic type A, medical adhesive 355)
3. aqueous preparations dispersed (fibronectin, laminin, Matrogel)
4. the multicomponent adhesives with an aqueous component also dispersed (fibrin preparations, mussel adhesive protein, polyacrylic acid and poly-F plus)
3.2 TISSUE CULTURE TOXICITY STUDIES:

These investigations were undertaken using Histoacryl, octylcyanoacrylate and Tisseel, as these adhesives adhered to the dried retinal surface of bovine retina.

3.2.1. Cyanoacrylates:

1) Histoacryl and octylcyanoacrylate added to RPE and glia:

Morphological changes were observed in RPE and glial cells adjacent to the adhesive as early as one hour after application - cells had retracted and rounded up. At three hours these changes were more pronounced (Figure 3.1). At 24 hours cultures containing Histoacryl demonstrated three concentric zones around the adhesive (Figure 3.2, 3.3). Immediately adjacent to the adhesive cells appeared to have been "fixed". Surrounding this zone was a cell free zone, where cells had died and separated from the flask, leaving cytoskeletal remnants (Figure 3.3). The third zone consisted of rounded, damaged cells while cells at a further distance from the adhesive appeared normal. At 24 hours flasks containing octylcyanoacrylate did not show a zone of fixed cells and had a much narrower cell free area (Figure 3.4).

Over the next few days the area of cell death increased in flasks to which Histoacryl had been added, RPE cultures reaching a maximum by day 7 and glia by day 2. The area of cell death did not increase in RPE cultures to which octylcyanoacrylate had been added, but did increase slightly by the second day in glial cultures (Table 3.1). The maximum area of cell death surrounding Histoacryl (glia, 390 +/- 136 mm² and RPE 339 +/- 53 mm²) was significantly different from that surrounding octylcyanoacrylate (glia 20 +/- 14 mm² and RPE 11 +/- 4 mm²) for both cell types (Anova at p = 0.05)(Figure 3.5). Although the maximum area of cell death surrounding glia with each adhesive was greater than that for RPE, this did not reach significance (Anova at p = 0.05).

Over the next few days the "fixed" cells adjacent to the drops of Histoacryl separated from the flask. Healthy cells from the edge of the cell free zone began to migrate into and repopulate the cell free area (Figure 3.6, 3.7). After 5-7 days cells were also seen to be dividing in this area. This occurred with both cell types and in flasks containing both types of cyanoacrylate. Eventually cells reached the edge of the adhesive. SEM of glial cultures to which octylcyanoacrylate had been added showed cells migrating onto the adhesive (Figure 3.8). A plot of the rate of repopulation of the cell free area for both types of cells and adhesives was undertaken by calculating at each time interval the area of cell death as a
percentage of the area of maximum cell death (Figure 3.9). There was no significant difference between the rate of repopulation by cell type or adhesive type.

2) Octylcyanoacrylate with and without lophendylate and lophendylate alone added to RPE and glial cultures:

When lophendylate alone was added to glial and RPE cultures no morphological changes were observed in either cell type, with no evidence of cell toxicity or death. Small droplets of lophendylate were visible on the surface of some cells.

The maximum area of cell death was recorded at 24 hours in all cultures except those where octylcyanoacrylate with lophendylate had been added to RPE cells; here the maximum area was recorded on day 4 (Table 3.2 and Figure 3.10). The maximum area of cell death was significantly greater in flasks containing lophendylate mixed with octylcyanoacrylate, and this was true for both cell types (glia, 227 +/- 38 mm² compared to 125 +/- 60 mm²; RPE 99 +/- 37 mm² compared to 54 +/- 14 mm²) (Anova at p = 0.05). This was particularly true for glia (p less than 0.05, Students t test) compared to RPE (p = 0.05). This experiment confirmed the findings of the previous experiment that glia are more sensitive to the toxic effects of cyanoacrylates than RPE and this time the findings were significant whether lophendylate was added or not (p = 0.001, t test).

3) Histoacryl added to human RPE:

This was a non-quantitative experiment in which the same morphological changes and evidence of cell death were observed as for bovine cells.

4) Histoacryl and octylcyanoacrylate with and without lophendylate added to glia grown on glass:

In this experiment only one measurement was made at six hours and the findings of this study were at variance with the previous experiment. The effect of mixing lophendylate with Histoacryl significantly reduced its toxic effect as the area of cell death surrounding Histoacryl was much greater than that surrounding the mixture (346 +/- 51 mm² compared to 79 +/- 3 mm²) (t test, p = 0.014) (Table 3.3 and Figure 3.11). Reduced toxicity was not seen however, when lophendylate was mixed with octylcyanoacrylate; there was a slight but insignificant increase in toxicity (84 +/- 15 mm² compared to 115 +/- 30 mm²) (Anova at p = 0.05). Again, Histoacryl alone was more toxic than octylcyanoacrylate alone (t test, p = 0.018).
Figure 3.1 Light micrograph of bovine retinal glial cells in tissue culture

A Confluent culture of bovine retinal glia, passage 7, before adding 2μL of Histoacyrl adhesive (Magnification x 250). B Same culture as above 3 hours after adding Histoacyrl. Area immediately adjacent to adhesive. Cells are rounding up and separating from the flask (Magnification x 250).
Figure 3.2 Light micrograph of bovine retinal glial cells

A 24 hours after adding 2μL of Histoacryl. A zone of cells immediately adjacent to the Histoacryl (H) have become "fixed" (FC). B At 24 hours. More peripherally cells have died and separated from the flask (DC). At a distance from the Histoacryl cells appear healthy (HC)(Magnification x 100).
Figure 3.3 Scanning electron micrographs 8 days after adding 2µL of Histoacryl to confluent cultures of bovine retinal glial cells

A "Fixed" cells (FC) adjacent to drop of adhesive (H), with more peripheral cell free zone (CFZ)(Mag x 50).  B Cytoskeletal remnants in cell free zone (Mag x 400).  C Healthy peripheral retinal glial cells (Mag x 1.6K).
Figure 3.4 Light micrograph 3 days after adding 2µL of octylcyanoacrylate (O) to confluent cultures of bovine retinal glial cells (Magnification x 100)

The area of cell death is narrower than with Histoacryl. There is no zone of "fixed" cells.
Table 3.1 Area of RPE and glial cell death surrounding 2μL drops of Histoacryl and octylcyanoacrylate (mm²).

<table>
<thead>
<tr>
<th></th>
<th>Histoacryl</th>
<th>Octylcyanoacrylate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glia</td>
<td>RPE</td>
</tr>
<tr>
<td>Day 1 (SD)</td>
<td>387 (± 131)</td>
<td>268 (± 30)</td>
</tr>
<tr>
<td>Maximum area</td>
<td>390 (± 136)</td>
<td>339 (± 53)</td>
</tr>
<tr>
<td>Day</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>
Figure 3.5 Graph of area of maximum area of cell death surrounding 2μL drops of Histoacryl and octylcyanoacrylate added to confluent cultures of bovine RPE cells and retinal glial cells

The area of maximum cell death surrounding Histoacryl was significantly different from that surrounding octylcyanoacrylate for both cells types (Anova at p = 0.05)
Figure 3.6 Light micrographs 15 and 23 days after adding 2µL of Histoacryl to confluent cultures of bovine retinal glial cells (Magnification x 100)

A 15 days. Some of the fixed cells have separated from the flask. Peripheral, healthy cells are migrating towards the drop of Histoacryl (H) (Magnification x 100).

B 23 days. Cells have migrated up the edge of the drop of Histoacryl
Figure 3.7 Scanning electron micrograph 12 days after adding 2 μL of Histoacryl to a confluent culture of bovine retinal glial cells

A Healthy peripheral cell migrating towards the area of adhesive, under a dead cell (Mag x 1.2K) B Healthy peripheral cell migrating towards the area of adhesive, over a dead cell (Mag x 2.2)
Figure 3.8 Scanning electron micrographs of bovine retinal glial cells migrating onto a drop of octylcyanoacrylate A Mag x 1.1K B Mag x 1.6K
Figure 3.9 Graph showing the rate of repopulation of the cell free area after adding 2μL of Histoacryl and octylcyanoacrylate to cultures of bovine RPE and glia

The rate of repopulation is not different for either cell or adhesive type
Table 3.2 Area of RPE and glial cell death surrounding 2μL drops of octylcyanoacrylate with and without lophendylate (mm²).

<table>
<thead>
<tr>
<th></th>
<th>Octylcyanoacrylate alone</th>
<th>Octylcyanoacrylate + lophendylate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glia</td>
<td>RPE</td>
</tr>
<tr>
<td>Day 1 (SD)</td>
<td>125 (± 60)</td>
<td>54 (± 14)</td>
</tr>
<tr>
<td>Maximum area</td>
<td>As above</td>
<td>As above</td>
</tr>
<tr>
<td>Day</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>


Figure 3.10 Graph of maximum area of cell death surrounding 2μL applications of octylcyanoacrylate with and without lophendylate added to cultures of bovine retinal glia and RPE.

The area of cell death surrounding octylcyanoacrylate with lophendylate was greater than octylcyanoacrylate alone for both cell types (Anova at p = 0.05). The area of glial cell death was significantly greater than the area of RPE cell death (p < 0.05, Students t test)
Table 3.3 Area of cell death surrounding 2μL drops of Histoacryl or octylcyanoacrylate alone or mixed with lophendylate added to glial cultures grown on glass (mm²).

<table>
<thead>
<tr>
<th></th>
<th>Histoacryl alone</th>
<th>Histoacryl + lophendylate</th>
<th>Octylcyanoacrylate alone</th>
<th>Octylcyanoacrylate + lophendylate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area (SD)</td>
<td>346 (±51)</td>
<td>79 (± 3)</td>
<td>84 (± 15)</td>
<td>115 (±30)</td>
</tr>
</tbody>
</table>
Figure 3.11 Graph of area of cell death after application of 2μL of Histoacryl and octylcyanoacrylate to cultures of bovine retinal glia grown on glass.

Histoacryl alone is more toxic than octylcyanoacrylate (p = 0.018, Students t test). Histoacryl is less toxic when mixed with equal volumes of lophendylate (p = 0.014, Students t test).
3.2.2 Tisseel:

1) Tisseel added to confluent cultures of glia and RPE:

When Tisseel was added to confluent cultures of bovine RPE, no toxicity was observed at any stage. The cells in the immediate vicinity of the drop of adhesive appeared normal. The fibrin clot started to lyse and separate from the cell monolayer by day 4 in all flasks, and had completely lysed by day 6.

There was no evidence of toxicity when Tisseel was added to glial cultures, indeed the cells were seen to be migrating onto the surface of the clot as early as 24 hours after application (Figure 3.12). This was confirmed by scanning electron microscopy of a culture fixed at 5 days (Figure 3.13). The migration of cells onto the surface of the clot progressed over the period of observation (15 days), and at no time was there evidence of clot lysis.

2) Tisseel added to cultures of RPE and glia grown in 24 well plates:

After adding Tisseel to the media overlying the confluent cultures a loose fibrin clot formed throughout the well. On day 1 RPE and glial cells were observed migrating into the clot, attached to the fine fibrin strands. Both cell types had taken on a spindloid morphology. On day 3 more cells were observed within the fibrin clot and it appeared as if the fibrin strands were being drawn in towards the cells. (Figure 3.14) The clot overlying RPE cells began to retract and separate from the monolayer on day 4, and had completely lysed in all wells by day 6. The clot overlying glial cells did not lyse.
Figure 3.12 Light micrographs of confluent cultures of bovine retinal glial cells before and after adding Tisseel (T)(Magnification x 100)

A Confluent culture before adding Tisseel. B Same culture 24 hours later. There is no evidence of toxicity C Same culture, 10 days after adding Tisseel. Cells are migrating onto the fibrin clot
Figure 3.13 Scanning electron micrographs 5 days after adding Tisseel to confluent cultures of bovine retinal glial cells

A (Mag x 370) B (Mag x 2.1K) Glial cells have migrated onto the surface of the Tisseel fibrin
Figure 3.14 Light micrograph of bovine RPE cells growing in a loose fibrin clot formed by adding Tisseel to the culture media (Photograph taken at 3 days, Magnification X 100).

Cells have grown onto the scaffold provided by fibrils of fibrin
3.3 ORGAN CULTURE STUDIES:

3.3.1 Retina:

The quadrants of retina to which the cyanoacrylates had been added showed several changes compared to controls. The retina underlying the drop of adhesive was thrown up into folds and showed evidence of necrosis i.e. oedema, loss of organisation, loss of photoreceptor OS and pyknotic nuclei. These changes extended beyond the edge of the adhesive to varying degrees. It was not possible to quantify these changes to compare Histoacryl with octylcyanoacrylate as

1. the control retinae sometimes showed similar but less pronounced changes
2. the degree of morphological abnormality varied widely within each adhesive type
3. it was difficult to determine with any degree of certainty the extent to which the changes extended beyond the edge of the adhesive
4. the adhesive had separated from several of the sections
5. it was difficult to determine whether the sections had passed through the centre of the drop of adhesive.

However, the results show that Histoacryl and octylcyanoacrylate both induce morphological changes in bovine retina which are suggestive of tissue toxicity.

3.3.2 Bovine Retinal Pigment Epithelium:

The quadrants of retina to which the cyanoacrylates had been added showed some morphological changes. In many instances there was an area of cell loss surrounding the drop of polymerised adhesive. Cells near the adhesive were oedematous, vacuolated, with loss of microvilli (Figure 3.15) Again, it was not possible to quantify changes in RPE morphology in this study as

1. there was considerable morphological variation in control quadrants, with some quadrants showing healthy RPE cells whereas others contained many cells with loss of microvilli
2. in some specimens the adhesive separated during processing
3. there was a wide range in the degree of change within each adhesive type.

However, as for the retina, this experiment suggests that cyanoacrylate adhesives are toxic to bovine RPE in limited organ culture.
Figure 3.15 Scanning electron micrographs of bovine RPE following limited organ culture after application of 2μL drops of Histoacryl

A Low power showing bare Bruch's membrane where Histoacryl applied (Mag x 40)
B Low power of RPE adjacent to drop of adhesive (Mag x 800)
Figure 3.15 continued. Scanning electron micrographs of bovine RPE following limited organ culture after application of 2μL drops of Histoacryl

C High power of RPE cells adjacent to Histoacryl. The cells are vacuolated with loss of microvilli. Some photoreceptor debris can be seen (Mag x 3K)
D Healthy RPE at a distance from the drop of adhesive (Mag X 2.1K)
B) IN VITRO EVALUATION OF SUBSTRATES

3.4 PREPARATION OF SUBSTRATES:

Scanning electron microscopy demonstrated that treating bovine anterior lens capsule with trypsin/EDTA solution for ten minutes removed anterior epithelial cells, but TEM of samples of Descemet's membrane showed that stromal collagen fibres were still present after treatment.

The staining techniques used for the chick allantoic membrane showed it to be mainly eluin, a form of elastin.

3.5 BOVINE EYE EXPERIMENTS:

Of the 26 materials available for investigation as patching substrates 7 of the 20 synthetic membranes (PVdF, EVA, Hydrogel 50 µm, nylon 6.6, polycarbonate, propylene and Steridrape) and 3 of the 6 biological membranes (bovine anterior capsule and Descemets membrane and chick allantoic membrane) could be introduced through a standard 20G sclerotomy using intraocular forceps. In some instances, however, only the smaller size (2 mm²) could be introduced. The materials that could not be introduced were either too bulky, would not deform sufficiently or were too fragile and tore. Most of the substrate samples that could be introduced were malleable enough to flatten onto the surface of bovine retina.

3.6 TISSUE CULTURE BIOCOMPATIBILITY STUDIES:

3.6.1 Synthetic Materials:

Quantitative cell settlement studies were undertaken using the following synthetic substrates: polycarbonate, hydrogel (50 µm thickness), PVdF, EVA, Steridrape, nylon 6,6 and propylene. It was not possible to visualised cells growing on the EVA material and on the Steridrape as they were not transparent and so cell settlement was not studied using these substrates.

The cell settlement rate for control dishes was 73% for glia and 63% for RPE after five hours. Figures for the total number of cells settled for controls and for each of the substrates are given in Table 3.4 and Figure 3.16. Glial cell settlement was significantly higher than RPE settlement in control dishes (825 +/- 153 cells compared to 425 +/- 41, p = 0.0009, t test) and on the PVdF membrane (847 +/- 316 compared 235 +/-154 cells, p = 0.0001, t test) and insignificantly higher on all
other substrates where cell counts could be made except for propylene, where no
glia settled at all. The PVdF membrane had the highest cell settlement for both glia
and RPE, which did not differ significantly from controls (glia: PVdF 847 +/- 316
compared to control 825 +/-153 cells; RPE: PVdF 235 +/- 154 compared to control
425 +/- 41 cells) (Anova, at p = 0.05). The other membranes had significantly less

cell settlement with the propylene membrane having the lowest cell settlement.

Considerable morphological variation was observed in both RPE and glial cells
depending on the substrate on which they had settled (Figures 3.17 and 3.18). Glial
cells that had settled on the PVdF membrane were well attached and spread and
appeared similar to control cells. However, those that had settled on the hydrogel,
nylon and polycarbonate membranes were still rounded with very small cell

processes.

RPE cells that had settled in the control dishes and on the nylon membrane had a
rounded morphology and many had spread, with a few active cell processes. This
was in sharp contrast to the cells that had settled on the other membranes,
particularly PVdF, where the cells had taken on a spindle shape. Cells on the
hydrgel and polycarbonate membranes had settled but had not spread and
remained rounded.

3.6.2 Biological Membranes:

Non quantitative evaluation of biocompatibility was undertaken using bovine
anterior lens capsule, bovine Descemet's membrane and chick allantoic
membrane, using RPE cells. Phase contrast light microscopy showed that the cells
grew readily on anterior capsule and Descemet's membrane but they were only
revealed on the chick membrane by electron microscopy as this material is opaque
(Figure 3.19). RPE cells growing on anterior lens capsule had a similar morphology
to controls; in both cases a monolayer of hexagonal cell was demonstrated, with
cells showing contact inhibition. This was in contrast to those growing on
Descemet's membrane where they had taken up an elongated shape. Scanning
electron microscopy showed RPE cells growing on the fibrillar surface of the chick
allantoic membrane and transmission electron microscopy also showed cells
invading the membrane (Figure 3.20).
Table 3.4 Settlement of glia and RPE on synthetic substrates.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Glia</th>
<th>RPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>825 (± 153)</td>
<td>425 (± 41)</td>
</tr>
<tr>
<td>PVdF</td>
<td>847 (± 316)</td>
<td>235 (± 154)</td>
</tr>
<tr>
<td>Nylon</td>
<td>317 (± 204)</td>
<td>90 (± 83)</td>
</tr>
<tr>
<td>Polycarbonate</td>
<td>108 (± 63)</td>
<td>70 (± 52)</td>
</tr>
<tr>
<td>Hydrogel</td>
<td>63 (± 29)</td>
<td>22 (± 10)</td>
</tr>
<tr>
<td>Propylene</td>
<td>0</td>
<td>12 (± 10)</td>
</tr>
</tbody>
</table>
Glia cell settlement was higher than RPE settlement in control flasks ($p = 0.0009$) and on the PVdF substrate ($p=0.0001$). The PVdF membrane had the highest cell settlement which did not differ significantly from control flasks (ANOVA at $p = 0.05$).
Figure 3.17 Light micrographs of bovine RPE cells settled onto synthetic substrates. Photographs taken after 5 hours showing settled (spread) and unsettled (rounded) cells (Magnification x 100).

A Control Substrate - tissue culture flask. B Substrate - PVdF.
Figure 3.17 Continued. Light micrographs of bovine RPE cells settled onto synthetic substrates. Photographs taken after 5 hours showing settled (spread) and unsettled (rounded) cells (Magnification x 100).

C Substrate - Hydrogel D Substrate - Polypropylene.
Figure 3.17 Continued. Light micrographs of bovine RPE cells settled onto synthetic substrates. Photographs taken after 5 hours showing settled (spread) and unsettled (rounded) cells (Magnification x 100).

E Substrate - Nylon  F Substrate - Polycarbonate.
Figure 3.18 Light micrographs of bovine retinal glial cells settled onto synthetic substrates. Photographs taken after 5 hours showing settled (spread) and unsettled (rounded) cells (Magnification x100)

A Control Substrate - tissue culture flask. B Substrate - PVDf
Figure 3.18 Continued. Light micrographs of bovine retinal glial cells settled onto synthetic substrates. Photographs taken after 5 hours showing settled (spread) and unsettled (rounded) cells (Magnification x 100)

C Substrate - Hydrogel  D Substrate - Polypropylene
Figure 3.18 Continued. Light micrographs of bovine retinal glial cells settled onto synthetic substrates. Photographs taken after 5 hours showing settled (spread) and unsettled (rounded) cells (Magnification x 100)

E Substrate - Nylon  F Substrate - Polycarbonate
Figure 3.19 Light micrographs of bovine RPE cells growing on biological membranes. Photographs taken after 4 days.

A Control. B Bovine anterior capsule (Magnification x 100).
Figure 3.19 continued. Light and scanning electron micrographs of bovine RPE cells growing on biological membranes. Photographs taken after 4 days.

C Bovine Descemet's membrane (Magnification x 100). D Chick allantoic membrane (SEM). A sheet of cells is growing on the fibrillar surface (Mag x 600)
Figure 3.20 Light and transmission electron micrographs of bovine retinal glial cells growing on biological membranes

A Control (48 hours, Mag x 100). B Bovine anterior capsule (48 hours, Mag x 100). C Chick allantoic membrane (TEM) (Day 5, Mag x 5.5K).
C) IN VITRO EVALUATION OF RETINAL PATCHING

3.7 BOVINE EYE CUP EXPERIMENTS:

Retinal breaks in bovine eyes were repaired with PVdF and propylene (the most and least biocompatible synthetic materials), bovine anterior capsule, Descemets membrane and chick allantoic membrane using Histoacryl, octylcyanoacrylate, Tisseel (4 IU/ml thrombin) and fibrin made from rabbit plasma and 4, 20, 500 IU/ml thrombin. It was found that the easiest way of applying a retinal patch was to place the substrate over the retinal break and then apply the adhesive to the edges of the material. Using the other methods described it was found that the adhesive had often set on the substrate or on the retina before the two were apposed and there was an increased likelihood of instruments becoming adherent. If the cyanoacrylates were applied around a retinal break before placing the substrate on the adhesive SEM showed that the adhesive tended to spread through the retinal defect.

After placing the eye cups containing retinal patches filled with media on a Luckmann shaking platform for four hours all the retinal patches using Histoacryl, octylcyanoacrylate and Tisseel remained adherent. Those made from fibrin formed from rabbit plasma gave varying results with 25% adherence when the 4 and 20 IU/ml strength thrombin was used and 50% adherence with the 500 IU/ml strength thrombin.

D) IN VIVO EVALUATION OF RETINAL PATCHING

In this section the results of the animal experiments are presented. After outlining details of the number of animals used the clinical findings for each procedure are described, including operative complications. Postoperative clinical data and analyses are then presented, followed by the findings of histological examination of tissues.

3.8 TOTAL NUMBER OF RABBITS AND DATA AVAILABLE

A total of 132 rabbits were used (Table 3.5). Descriptive clinical details and grading system data are available from 76 animals from six surgical groups. Grading system data from 53 animals has been analysed statistically.
3.8.1 Data excluded or not available:

Twenty eight animals were used in developing the animal model. Thirteen animals died under anaesthesia, and on two occasions equipment failure meant the procedure had to be abandoned and the animals sacrificed. Two animals developed RD after gas compression vitrectomy due to vitreous loss at the time of gas injection, and 1 animal was sacrificed as complications developed during vitreolensectomy. Six animals were sacrificed because of complications during retinal patching. Six animals who developed postoperative endophthalmitis were also sacrificed. Data is therefore available from 76 rabbits (Table 3.5).

3.8.2 Time Points of Available Data:

Clinical examination was undertaken on days 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28. Follow up was for one month as it became apparent during the study that this was long enough to observe and document the consequences of retinal patching. Animals were sacrificed at 2, 7, 14 and 28 days after surgery. Selection for sacrifice was not random and was biased in favour of those with greater postoperative complications, such as vitreous haemorrhage, so that full clinical details could be obtained on those followed up for longer. In some animals data is incomplete; for example animals operated on early in the study vitreous cells and flare were not graded, only the degree of vitreous opacity. In other animals posterior segment data is not available due to the presence of dense vitreous haemorrhage.

3.9 NUMBERS OF RABBITS IN EACH GROUP

<table>
<thead>
<tr>
<th>Group</th>
<th>Surgical procedure</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group 1</td>
<td>CG1 Vitreolensectomy, retinotomy, RD</td>
<td>13</td>
</tr>
<tr>
<td>Control Group 2</td>
<td>CG2 Vitreolensectomy only</td>
<td>16</td>
</tr>
<tr>
<td>Control Group 3</td>
<td>CG3 Vitreolensectomy, retinotomy</td>
<td>13</td>
</tr>
<tr>
<td>Experimental Group A</td>
<td>EXPA Retinal patching using octylcyanoacrylate</td>
<td>16</td>
</tr>
<tr>
<td>Experimental Group B</td>
<td>EXPB Retinal patching using Tisseel</td>
<td>15</td>
</tr>
<tr>
<td>Experimental Group C</td>
<td>EXPC Retinal patching using autologous fibrin</td>
<td>3</td>
</tr>
</tbody>
</table>

The number of animals in which complete or incomplete clinical data were available in each group is given in Table 3.6. Data from EXPC has not been included in the statistical analysis as the group is so small, and results will be presented and discussed separately. Clinical data from 73 rabbits has been analysed statistically.
Table 3.5 Number of animals used in the thesis.

<table>
<thead>
<tr>
<th>Total number of rabbits</th>
<th>132</th>
</tr>
</thead>
<tbody>
<tr>
<td>Used in developing animal model</td>
<td>28</td>
</tr>
<tr>
<td>Animals excluded:</td>
<td></td>
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<tr>
<td>Anaesthetic deaths</td>
<td>13</td>
</tr>
<tr>
<td>Equipment failure</td>
<td>2</td>
</tr>
<tr>
<td>Preexisting detachment</td>
<td>2</td>
</tr>
<tr>
<td>Surgical complications</td>
<td>7</td>
</tr>
<tr>
<td>Endophthalmitis</td>
<td>4</td>
</tr>
<tr>
<td>Animals included in results</td>
<td>76</td>
</tr>
</tbody>
</table>

Table 3.6 Number of rabbits with complete or incomplete data available at each time point for each group.

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>CG 1</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
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<tr>
<td>4</td>
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<tr>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td>28</td>
<td>6</td>
</tr>
</tbody>
</table>
3.10 OBSERVATIONS FOLLOWING CRYOTHERAPY AND C3F8 GAS INJECTION

3.10.1 Following Cryotherapy:

Due to the lack of intraocular pigmentation chorioretinal reactions were not visible following monitored cryotherapy in albino rabbits, but in Dutch rabbits cryotherapy induced an area of chorioretinal oedema which developed into a visible chorioretinal reaction by 10 days. No eyes developed any per- or postoperative complications at this stage.

3.10.2 Following Gas Injection:

The mean intraocular pressure measurement in ten rabbits prior to injection of the first 0.2 mls of C3F8 gas was 3 +/- 1 mmHg which rose to 48 +/- 10 mmHg immediately after injection (Table 3.7). After 15 minutes this had fallen to 23 +/- 9 mmHg and rose to 58 +/- 4 mmHg after injecting the remaining 0.2 mls of gas. In no animal was the central retinal artery closed for longer than 15 minutes. At one hour the mean intraocular pressure was 14 +/- 5 mmHg which fell to 11 +/- 3 mmHg on the first postoperative day. On day 4, the day of maximum gas expansion, the mean intraocular pressure was 6 +/- 3 mmHg, which fell to the preoperative value at one week.

On the first postoperative day the mean estimated volume size of the gas bubble was 68% of the posterior segment volume, which rose to 89% by day four, the day of maximum expansion (Table 3.8). At one week the estimated volume had fallen to 75% and by two weeks only a small bubble of gas usually remained.

Lens opacities, starting as superior posterior subcapsular opacities developed to varying degrees in all eyes, but in none did this progress to dense cataract formation. By the time vitreolensectomy was performed the opacities had usually regressed, leaving localised, central posterior subcapsular opacities.

On the first day after gas injection the eyes were slightly inflamed over the injection site, but this rapidly cleared. Anterior segment inflammatory reactions were minimal and these again regressed so that by the time the eyes were operated on again the anterior segments were quiet. In two eyes vitreous prolapsed through the sclerotomy at the time of gas injection. The retinae in both rabbits detached over the next two weeks.
Table 3.7 Mean intraocular pressure measurements in ten rabbits after intravitreal injection of C3F8 gas (mm Hg).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0B</th>
<th>0A</th>
<th>5</th>
<th>10</th>
<th>15B</th>
<th>15A</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>IOP</td>
<td>3</td>
<td>48</td>
<td>40</td>
<td>36</td>
<td>23</td>
<td>51</td>
<td>45</td>
<td>36</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>SD ±</td>
<td>1</td>
<td>10</td>
<td>13</td>
<td>13</td>
<td>9</td>
<td>4</td>
<td>12</td>
<td>9</td>
<td>12</td>
<td>5</td>
</tr>
</tbody>
</table>

B = before gas injection  
A = after gas injection

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
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<tr>
<td>IOP</td>
<td>11</td>
<td>9</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>SD ±</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3.8 Volume of C3F8 gas in posterior segment during first week after intravitreal injection of 0.2 mls or pure gas (as estimated % fill)(N=10).

<table>
<thead>
<tr>
<th>Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
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<tbody>
<tr>
<td>% fill (SD ±)</td>
<td>68</td>
<td>78</td>
<td>86</td>
<td>89</td>
<td>84</td>
<td>78</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>9</td>
<td>8</td>
<td>5</td>
<td>8</td>
<td>9</td>
<td>8</td>
</tr>
</tbody>
</table>
3.11 TECHNICAL ASPECTS IN CONTROL AND EXPERIMENTAL GROUPS

3.11.1 Control Groups:

Once the technique of vitreolensectomy in the rabbit had been mastered, there were no particular technical difficulties thereafter up to this experimental stage. The rabbit eye tends to bleed readily, and this was kept to a minimum by raising the intraocular pressure, i.e. by raising the infusion bag. Peroperative fibrin formation was not a problem once low molecular weight heparin was used (all Control and Experimental Group animals).

3.11.2 Experimental Group A:

Several technical difficulties were encountered whilst repairing retinal defects with octylcyanoacrylate and PVdF in an air filled eye. These relate both to introduction and application of the substrate, and to delivery of the adhesive.

Introducing the substrate through the sclerotomy invariably resulted in enlargement of the sclerotomy, with loss of an air-tight seal. This led to hypotony, loss of good visualisation of the posterior segment and an increased risk of vitreous haemorrhage. A preplaced 6/Dexon purse string suture sited round the sclerotomy helped to maintain an air-tight seal, but this was not always successful. Introducing the propylene substrate was much more problematic than the PVdF membrane, and so PVdF was used for all eyes.

Considerable difficulty was encountered in accurately placing the substrate over the retinal break, as it was hard to free the material from the jaws of the forceps, even using a blunt needle introduced through the third sclerotomy. Once on the retinal surface it was difficult to see the transparent material, but this problem was solved by colouring the substrate with ink from an indelible felt pen. Manoeuvring the substrate until it was accurately placed over the retinal defect was difficult, mainly because of surface tension effects. Additional retinal holes were sometimes created during this procedure.

The cyanoacrylate delivery system proved effective as the adhesive rarely polymerised within the tubing. Applying minute quantities was a problem as the drop of adhesive tended to flow back up the outside of the teflon tubing rather than remaining as a discrete drop at the end of the tube. If a thin film of preretinal fluid was present the octylcyanoacrylate tended to spread widely before polymerising;
great care was therefore taken in drying the preretinal space with the modified flute needle prior to delivering the adhesive.

The octylcyanoacrylate took 1-2 seconds to polymerise after application which was long enough, in most instances, to allow the tubing of the delivery system to be removed from the patch to prevent the patch and tubing from sticking together. Although evaluated in the in vitro experiments lophendylate was not used in any animals as this would have made the groups too small.

Air/fluid exchange was performed at the end of the operation. Care was taken to do this in as controlled a way as possible, but this was not always possible. While changing the air pump to the infusion line the eyes tended to collapse as soon as the intraocular pressure dropped because the rabbit eye has little scleral rigidity. When this happened the infusion fluid was run in quickly to restore intraocular pressure, to reduce the considerable risk of vitreous haemorrhage. The posterior segment was examined at the end of the procedure, and the findings noted. Air/fluid exchange was not performed in two animals. The reasons for this, and the consequences are reported in the next section.

3.11.3 Experimental Group B:

The same technical difficulties were encountered in introducing and applying the substrate as described above.

In this group of animals Tisseel was used as the adhesive and PVdF as the substrate. In only one animal was the 500 IU/ml strength thrombin used; the remainder were treated with 4 IU/ml. When using the stronger concentration of thrombin the needle of the Duploject blocked, as the fibrin clot formed very rapidly as the components mixed. Sequential delivery was tried, but the first component spread before the second could be delivered. Small, localised applications could not be made. All subsequent procedures were carried out using the Duploject system and 4 IU/ml strength thrombin. Even using the Duploject system it was difficult to apply minute amounts of Tisseel as the adhesive tended to spread widely before setting to form a fibrin clot. It was necessary to wait at least five minutes after application for the fibrin clot to form and adhere to the retina before undertaking air/fluid exchange. Meticulous drying of the preretinal space with the flute needle helped to make the clot stick to the retina. If the patch separated from the retinal surface during air/fluid exchange the fibrin was removed as extensively as possible with the vitrophage and the patch reapplied.
PVdF is hydrophobic, and it tended to float to the top of the drop of unclotted adhesive, floating away from the retinal surface and break.

3.11.4 Experimental Group C:

Patching using fibrin prepared from autologous plasma and bovine thrombin 4 IU/ml was only attempted in three eyes as many problems were encountered. Delivery was undertaken using the Duploject system and to aid visualisation of the adhesive fluorescein was added to the plasma component. In 1 eye the substrate would not adhere to the retina despite repeated attempts and waiting ten minutes for the clot to form. In two eyes the patch did remain adherent, but again this was after repeated attempts. In one of these eyes examination showed that the adhesive had spread very widely, forming a thin film covering virtually the whole of the inferior retina.

3.12 PEROPERATIVE COMPLICATIONS

Complications were divided into major and minor complications; severe complications necessitated abandoning the procedure.

3.12.1 Major Peroperative Complications:

Seven animals had to be sacrificed during surgery because of insurmountable intraoperative complications encountered during vitreolensectomy and/or application of a retinal patch (Table 3.9). 3 eyes developed uncontrollable vitreous haemorrhage after introduction of the substrate. One eye developed extensive retinal holes whilst manipulating the substrate over the retinotomy. In one eye a hole in the anterior lens capsule led to intense pupillary miosis and haemorrhage into the anterior chamber and in 2 further eyes where octylcyanoacrylate was being used instruments became adherent to the retina, resulting in huge tears as they were withdrawn. In 6 of these 7 eyes therefore, the procedure had to be abandoned because of severe complications related to retinal patching while in only one control eye was the procedure abandoned. Retinal patching was therefore attempted in 37 eyes and had to be abandoned in 6 (16%), compared to 1 out of 43 (2%) control animals (Chi square test, p = 0.028). As the numbers in each group are small Fisher's exact test was used, which also showed a significant difference between Control and Experimental Groups (Exact p = 0.013).
3.12.2 Minor Peroperative Complications:

Intraoperative complications which occurred that were not serious enough to necessitate abandoning the procedure included controllable vitreous haemorrhage, inadvertent small retinal holes, anterior capsular holes, zonular rupture and haemorrhage into the anterior chamber (Tables 3.10 and 3.11). There were significantly more posterior segment complications in the Experimental Groups (total 12/31) compared to Controls (total 4/42) (Chi square test, M-H p = 0.003), but significantly more anterior segment complications in Control Group eyes (total 10/42) compared to Experimental Group eyes (total 1/31)(Chi sq test M-H, p = 0.003). The reason for the higher incidence of anterior segment complications in Control animals will be discussed later.

3.13 POSTOPERATIVE VITREOUS HAEMORRHAGE

Postoperative vitreous haemorrhage (i.e. occurring during or immediately after air/fluid exchange, present on day one, or developing later) that was dense enough to prevent fundal examination occurred in a total of 10 rabbits (13.7%) (Table 3.12). Vitreous haemorrhage occurred more frequently in Experimental Group rabbits 7/31 (22.6%) compared to Control Group animals 3/42 (7.2%), but this did not reach significance (Chi square test, M-H, p = 0.06; EXACT p = 0.09). Two eyes that were patched using fibrin developed late vitreous haemorrhage, occurring on days 3 and 5 (6.5%); this was not seen in the other Control or Experimental Groups, but was not a statistically significant finding.

3.14 POSTOPERATIVE ENDOPHTHALMITIS:

Four eyes developed endophthalmitis during the postoperative period. Three were in Control Groups and one was in Experimental Group B. These animals were sacrificed as soon as the diagnosis was made and have not been included in the descriptions (below) or in the statistical analysis, nor was tissue prepared for histology. Endophthalmitis occurred in 3/45 Control animals compared to 1/32 Experimental animals (Chi sq. test, Fisher Exact p = 0.64).
Table 3.9 Major peroperative complications

<table>
<thead>
<tr>
<th>Event</th>
<th>Control Groups (n=43)</th>
<th>Experimental Groups (n=37)</th>
<th>Chi Sq (M-H) (Exact p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitreous haemorrhage</td>
<td>0</td>
<td>3 (8.1%)</td>
<td>p = 0.058 (p = 0.095)</td>
</tr>
<tr>
<td>Anterior chamber haemorrhage</td>
<td>1 (2.3%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Retinal holes</td>
<td>0</td>
<td>3 (8.1%)</td>
<td>p = 0.058 (p = 0.095)</td>
</tr>
<tr>
<td>Total:</td>
<td>1 (2.3%)</td>
<td>6 (16.2%)</td>
<td>p = 0.029 (p = 0.045)</td>
</tr>
</tbody>
</table>

Table 3.10 Minor posterior segment peroperative complications.

<table>
<thead>
<tr>
<th>Event</th>
<th>Control Groups (n=42)</th>
<th>Experimental Groups (n=31)</th>
<th>Chi Sq (M-H) (Exact p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitreous haemorrhage</td>
<td>2 (4.8%)</td>
<td>3 (9.7%)</td>
<td>p = 0.41 (p = 0.64)</td>
</tr>
<tr>
<td>Small retinal holes</td>
<td>2 (4.8%)</td>
<td>8 (25.6%)</td>
<td>p = 0.01 (p = 0.01)</td>
</tr>
<tr>
<td>Large retinal holes</td>
<td>0</td>
<td>1 (3.2%)</td>
<td>p = 0.24 (p = 0.43)</td>
</tr>
<tr>
<td>Total</td>
<td>4 (9.5%)</td>
<td>12 (38.7%)</td>
<td>p = 0.003</td>
</tr>
</tbody>
</table>
Table 3.11 Minor anterior segment peroperative complications

<table>
<thead>
<tr>
<th>Event</th>
<th>Control Groups (n=42)</th>
<th>Experimental Groups (n=31)</th>
<th>Chi Sq (M-H) (Exact p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holes in anterior lens capsule</td>
<td>5 (11.9%)</td>
<td>1 (3.2%)</td>
<td>p = 0.19 (p = 0.23)</td>
</tr>
<tr>
<td>Anterior chamber haemorrhage</td>
<td>2 (4.8%)</td>
<td>0</td>
<td>p = 0.22 (p = 0.50)</td>
</tr>
<tr>
<td>Zonular rupture</td>
<td>2 (4.8%)</td>
<td>0</td>
<td>p = 0.22 (p = 0.50)</td>
</tr>
<tr>
<td>Total</td>
<td>9 (21.4%)</td>
<td>1 (3.2%)</td>
<td>p = 0.003</td>
</tr>
</tbody>
</table>

Table 3.12 Postoperative vitreous haemorrhage

<table>
<thead>
<tr>
<th>Time</th>
<th>Control Groups (n=42)</th>
<th>Experimental Groups (n=31)</th>
<th>Chi Sq (M-H) (Exact p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>2 (4.8%)</td>
<td>5 (16.1%)</td>
<td>p = 0.11 (p = 0.13)</td>
</tr>
<tr>
<td>Day 2</td>
<td>1 (2.4%)</td>
<td>0</td>
<td>p = 0.39 (p = 1.00)</td>
</tr>
<tr>
<td>Late</td>
<td>0</td>
<td>2 (6.5%)</td>
<td>p = 0.10 (p = 0.18)</td>
</tr>
<tr>
<td>Total</td>
<td>3 (7.2%)</td>
<td>7 (22.6%)</td>
<td>p = 0.06 (p = 0.09)</td>
</tr>
</tbody>
</table>
3.15 POSTOPERATIVE CLINICAL OBSERVATIONS:

All eyes developed some degree of opacification of the anterior capsule. This did not interfere with fundal examination or grading of posterior segment findings.

3.15.1 Control Group 1 (N = 13):

In this group a localised retinal detachment was created below the medullary rays after vitreolensectomy. All the animals in this group were Dutch rabbits. Clinical findings are summarised in Table 3.13.

Peroperative findings:
In one animal an second, inadvertent retinal break was created. In 3 eyes holes were made in the anterior capsule.

Findings during the first postoperative week:
Vitreous haemorrhage occurred in two animals (day 1 and 2), preventing fundus examination. The area of retinal detachment spontaneously reattached in all 11 animals where the retina was visible, between day 1 and 5, mean 3.3 days (SD 1.2 days)(Figure 3.21 and 3.22). One animal developed subtotal RD, which was not related to the retinotomy, and which presumably arose from an undetected sclerotomy related break. Five animals were sacrificed during or at the end of the first week.

Findings on day 14:
In all 8 rabbits the view of the fundus was good. Another animal developed a sclerotomy related RD, and the RD which had developed during the first postoperative week progressed to total RD with development of generalised ERMs. The retinae in the other 6 rabbits remained flat. Both animals with RD were sacrificed.

Findings on day 21:
In all 6 rabbits the view of the fundus was good and no eye showed any abnormality apart from mild RPE changes in the vicinity of the flat retinal break. No animals were sacrificed.

Findings on day 28:
In all 6 rabbits the view of the fundus was good and no eye showed any abnormality apart from under the original area of induced retinal detachment where there was patchy RPE hypertrophy (Figure 3.23). All 6 animals were sacrificed.
Table 3.13 Clinical observations in Control Group 1 animals (N=13).

<table>
<thead>
<tr>
<th>Animal code</th>
<th>Strain</th>
<th>Operative complications</th>
<th>Postop. VH</th>
<th>Day re-attached</th>
<th>Other problems</th>
<th>Day sacrificed</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG1A</td>
<td>Dutch</td>
<td>Hole in ant cap</td>
<td>No</td>
<td>2</td>
<td>None</td>
<td>2</td>
</tr>
<tr>
<td>CG1B</td>
<td>Dutch</td>
<td>Hole in ant cap</td>
<td>Yes, day 2</td>
<td>No view</td>
<td>No view</td>
<td>5</td>
</tr>
<tr>
<td>CG1C</td>
<td>Dutch</td>
<td>Retinal hole</td>
<td>No</td>
<td>1</td>
<td>None</td>
<td>7</td>
</tr>
<tr>
<td>CG1D</td>
<td>Dutch</td>
<td>None</td>
<td>Yes, day 1</td>
<td>No view</td>
<td>No view</td>
<td>7</td>
</tr>
<tr>
<td>CG1E</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>4</td>
<td>None</td>
<td>7</td>
</tr>
<tr>
<td>CG1F</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>3</td>
<td>ReRD, day 14</td>
<td>14</td>
</tr>
<tr>
<td>CG1G</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>5</td>
<td>Re RD, day 7</td>
<td>14</td>
</tr>
<tr>
<td>CG1H</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>3</td>
<td>None</td>
<td>28</td>
</tr>
<tr>
<td>CG1I</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>3</td>
<td>None</td>
<td>28</td>
</tr>
<tr>
<td>CG1J</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>4</td>
<td>None</td>
<td>28</td>
</tr>
<tr>
<td>CG1K</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>5</td>
<td>None</td>
<td>28</td>
</tr>
<tr>
<td>CG1L</td>
<td>Dutch</td>
<td>Hole in ant cap</td>
<td>No</td>
<td>3</td>
<td>None</td>
<td>28</td>
</tr>
<tr>
<td>CG1M</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>3</td>
<td>None</td>
<td>28</td>
</tr>
</tbody>
</table>

ReRD = retinal redetachment  
Postop VH = postoperative vitreous haemorrhage  
ant cap = anterior lens capsule
Figure 3.21 Fundus photograph of Control Group 1 eye

Dutch rabbit 2 days after creating a retinal break and localised retinal detachment.
Figure 3.22 Fundus photograph of Control Group 1 eye

Same eye as Figure 3.21, 5 days after surgery. The retina is reattaching spontaneously
Figure 3.23 Macrophotograph of Control Group 1 eye sacrificed 28 days after surgery

The retina has reattached leaving an area of RPE hypertrophy
3.15.2 Control Group 2 (N = 16):

This group underwent vitreolensectomy only. Four animals were Dutch rabbits and 12 Albino. Clinical findings are summarised in Table 3.14.

**Peroperative findings:**
Anterior segment complications occurred in 3 animals (zonular rupture in 2 and anterior capsule holes 1). In 1 eye an inadvertent retinal break was created while inducing posterior vitreous detachment.

**Findings during the first postoperative week:**
Slight vitreous haemorrhage occurred in 2 animals immediately after surgery, but this was not dense enough to obscure the view of the retina. Three animals developed focal ERMs on the edge of the medullary rays. The other 14 animals showed no abnormalities (Figure 3.24). Two of the animals without abnormality were sacrificed.

**Findings on day 14:**
In all 14 rabbits the view of the fundus was good. Three more rabbits developed focal ERMs, again at the edge of, or on the medullary rays. No eyes developed retinal detachment or generalised ERMs. No animals were sacrificed.

**Findings on day 21:**
One of animals with focal ERMs had developed posterior synaechiae, and fundus examination was not possible. The view of the fundus in the remaining 13 animals was good. One more animal had developed focal ERMs, and one of the animals that had previously developed ERMs had developed a subtotal retinal detachment. In 8 eyes the retina appeared normal. Six animals were sacrificed, including 3 with focal ERMs, 1 with retinal detachment and 2 with normal retinae.

**Findings on day 28:**
The fundus was visible in 7 of the 8 animals examined at this time period. One more rabbit had developed focal ERMs. All 8 animals were sacrificed. The retinae of all animals followed for four weeks were flat.
Table 3.14 Clinical observations in Control Group 2 animals (N=16).

<table>
<thead>
<tr>
<th>Animal code</th>
<th>Strain</th>
<th>Operative complications</th>
<th>Postop. VH</th>
<th>Other postoperative complications</th>
<th>Day sacrificed</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG2A</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>None</td>
<td>2</td>
</tr>
<tr>
<td>CG2B</td>
<td>Albino</td>
<td>None</td>
<td>No</td>
<td>None</td>
<td>5</td>
</tr>
<tr>
<td>CG2C</td>
<td>Albino</td>
<td>None</td>
<td>Slight</td>
<td>Focal ERM, day 7</td>
<td>21</td>
</tr>
<tr>
<td>CG2D</td>
<td>Albino</td>
<td>None</td>
<td>No</td>
<td>None</td>
<td>21</td>
</tr>
<tr>
<td>CG2E</td>
<td>Albino</td>
<td>None</td>
<td>No</td>
<td>Focal ERM, day 14</td>
<td>21</td>
</tr>
<tr>
<td>CG2F</td>
<td>Albino</td>
<td>None</td>
<td>No</td>
<td>None</td>
<td>21</td>
</tr>
<tr>
<td>CG2G</td>
<td>Albino</td>
<td>None</td>
<td>Slight</td>
<td>RD day 2, Focal ERM, day 14</td>
<td>21</td>
</tr>
<tr>
<td>CG2H</td>
<td>Albino</td>
<td>None</td>
<td>No</td>
<td>None</td>
<td>21</td>
</tr>
<tr>
<td>CG2I</td>
<td>Albino</td>
<td>None</td>
<td>No</td>
<td>Focal ERM, day 7, no view from day 14</td>
<td>28</td>
</tr>
<tr>
<td>CG2J</td>
<td>Albino</td>
<td>Hole in ant cap</td>
<td>No</td>
<td>Focal ERM, day 21</td>
<td>28</td>
</tr>
<tr>
<td>CG2K</td>
<td>Albino</td>
<td>Zonular rupture</td>
<td>No</td>
<td>Focal ERM, day 5</td>
<td>28</td>
</tr>
<tr>
<td>CG2L</td>
<td>Albino</td>
<td>Zonular rupture</td>
<td>No</td>
<td>Focal ERM, day 28</td>
<td>28</td>
</tr>
<tr>
<td>CG2M</td>
<td>Albino</td>
<td>Retinal hole</td>
<td>No</td>
<td>Focal ERM, day 14</td>
<td>28</td>
</tr>
<tr>
<td>CG2N</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>None</td>
<td>28</td>
</tr>
<tr>
<td>CG2O</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>None</td>
<td>28</td>
</tr>
<tr>
<td>CG2P</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>None</td>
<td>28</td>
</tr>
</tbody>
</table>

Postop VH = postoperative vitreous haemorrhage  
ant cap = anterior lens capsule
Figure 3.24 Fundus photograph of Control Group 2 eye immediately after vitreolensectomy (Albino rabbit)

Small tags of vitreous (V) remain adherent to the disc (D) and edge of the medullary rays (MR)
3.15.3 Control Group 3 (N = 13):

This group of animals underwent vitreolensectomy with creation of a retinotomy. All animals in this group were Dutch rabbits. Clinical findings are summarised in Table 3.15. Data from a further 3 animals is not included as they developed endophthalmitis.

Peroperative findings:
In one eye a hole was made in the anterior capsule, and in 2 eyes there was haemorrhage into the anterior chamber at the end of the procedure.

Findings during the first postoperative week:
One animal developed a vitreous haemorrhage on the first postoperative day which precluded fundus examination. One animal developed focal ERMs on the edge of the medullary rays; the fundi of the remaining 12 animals was normal apart from the retinotomy, which remained flat in all animals. Three animals were sacrificed; the rabbit with the vitreous haemorrhage and 2 with normal findings.

Findings on day 14:
The view of the fundus was good in all 10 animals. One animal developed focal ERMs and subtotal retinal detachment, which did not involve the posterior pole. Two animals were sacrificed; one was normal, but the other had focal ERMs.

Findings on day 21:
The fundus was visible in all 8 animals. Another rabbit had developed focal ERMs, and the subtotal RD had progressed to a total RD. No animals were sacrificed at this time point.

Findings on day 28:
The fundus was visible in all 8 animals. Another rabbit had developed focal ERMs. All 8 animals were sacrificed.
Table 3.15 Clinical observations in Control Group 3 animals (N=13).

<table>
<thead>
<tr>
<th>Animal code</th>
<th>Strain</th>
<th>Operative complications</th>
<th>Postop. VH</th>
<th>Other postoperative complications</th>
<th>Day sacrificed</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG3A</td>
<td>Dutch</td>
<td>Hole in ant cap</td>
<td>No</td>
<td>None</td>
<td>2</td>
</tr>
<tr>
<td>CG3B</td>
<td>Dutch</td>
<td>None</td>
<td>Yes, day 1</td>
<td>No view</td>
<td>2</td>
</tr>
<tr>
<td>CG3C</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>None</td>
<td>7</td>
</tr>
<tr>
<td>CG3D</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>None</td>
<td>14</td>
</tr>
<tr>
<td>CG3E</td>
<td>Dutch</td>
<td>Anterior chamber haemorrhage</td>
<td>No</td>
<td>Focal ERM, day 7</td>
<td>14</td>
</tr>
<tr>
<td>CG3F</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>None</td>
<td>28</td>
</tr>
<tr>
<td>CG3G</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>None</td>
<td>28</td>
</tr>
<tr>
<td>CG3H</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>None</td>
<td>28</td>
</tr>
<tr>
<td>CG3I</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>RD day 14</td>
<td>28</td>
</tr>
<tr>
<td>CG3J</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>Focal ERM, day 28</td>
<td>28</td>
</tr>
<tr>
<td>CG3K</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>Focal ERM, day 21</td>
<td>28</td>
</tr>
<tr>
<td>CG3L</td>
<td>Dutch</td>
<td>None</td>
<td>No</td>
<td>None</td>
<td>28</td>
</tr>
<tr>
<td>CG3M</td>
<td>Dutch</td>
<td>Anterior chamber haemorrhage</td>
<td>No</td>
<td>None</td>
<td>28</td>
</tr>
</tbody>
</table>

NB  Three animals developed endophthalmitis - not included.

Postop VH  = postoperative vitreous haemorrhage
ant cap    = anterior lens capsule
3.15.4 Experimental Group A (N = 16):

In this group of animals retinotomies, created below the medullary rays, were patched using PVdF and octylcyanoacrylate. 8 were Albino and 8 were Dutch rabbits. Clinical findings are summarised in Tables 3.16-8.

Peroperative findings:
A hole was made in the anterior lens capsule in 1 rabbit. In 10 animals retinal patching proceeded without complications. In 6 (37.5%) animals additional, small breaks in posterior retina were made while manipulating the substrate or delivering the adhesive. Two rabbits had sclerotomy related breaks.

Findings after air/fluid exchange:
Air/fluid exchange was performed in 14 animals, 9 of whom had uncomplicated patching, but afterwards new retinal breaks were observed in 5 (55.6%), usually at the edge of the adhesive. In one animal the patch almost tore off. One animal developed dense vitreous haemorrhage. When iatrogenic retinal breaks had been made while patching (6 animals), these appeared larger after air/fluid exchange but this was not easy to judge because of the change in retinal image size when viewed with the indirect ophthalmoscope compared to using corneal contact lens.

Because of complications following air/fluid exchange the procedure was not performed in 2 animals. The consequences are described below.

Retinal pallor under and around the octylcyanoacrylate adhesive became obvious within a few hours of applying the patch (Figure 3.25).

Findings during the first week:
Three more animals developed dense vitreous haemorrhage. The view of the retina was good in just 12 animals. All showed marked pallor and oedema of the retina beneath and immediately adjacent to the adhesive (Figure 3.26). The pallor was present on day 1, progressed over the next 24-48 hours and then remained stable. New retinal breaks, usually in necrotic retina, or at the border of healthy and necrotic retina, developed in 4 animals, on days 3, 5, 6 and 6 (Figure 3.27). Most holes enlarged during the first postoperative week (Figure 3.28).

The 2 animals that did not have air/fluid exchange developed more intense retinal necrosis than other animals; the retinae disintegrated and the patches fell off on days 2 and 3. The patch also fell off in the rabbit where huge rips followed air/fluid exchange. During the first week 6 animals were sacrificed (3 with vitreous
haemorrhage, 1 with extensive retinal breaks after air/fluid exchange and the 2 animals that did not have air/fluid exchange).

**Findings on day 7:**
Ten animals were examined on day 7. One had dense vitreous haemorrhage. At the end of the first week RD was present in 6 animals (2 total, 4 subtotal RDs). All these animals had retinal breaks, either made during patching, or secondary to retinal necrosis, or developing after air/fluid exchange. In 3 animals the retina was flat. This included one animal with a small retinal break next to the patch. No eyes showed ERM formation. Two more animals were sacrificed on day 7 - both had RDs.

**Findings on day 14:**
8 animals were examined at this time point; 1 had dense vitreous haemorrhage. The retinal pallor was less pronounced in all eyes and affected retina appeared atrophic. No new retinal breaks were detected. Previous retinal breaks had enlarged in some animals. Three animals had total RD, 1 had a subtotal RD but the area of retina containing the patch was flat. One animal with a total RD had developed generalised ERMs and the animal with subtotal RD had developed focal ERMs. In 3 animals the retina was flat and in 2 animals the patch was still adherent. The retina of one animal had spontaneously reattached, and the patch, which was attached to a thin layer of detached cortical vitreous, had separated. This animal presumably had an incomplete vitrectomy, and the patch had been applied to a thin layer of cortical vitreous.

In all Dutch rabbits the RPE in the region where the patch had been applied developed a pigmentary reaction, which started on day 7, but which was more apparent on day 14. The animal with the dense vitreous haemorrhage was sacrificed.

**Findings at 21 days:**
7 animals were examined at this time point. The findings were essentially unchanged. One more animal with total RD had developed generalised ERMs.

**Findings at 28 days:**
7 animals were examined at this time point. In 2 animals the retina was flat, the patch in place and no ERMs had developed (Figure 3.29). The retina in the animal where the patch had separated remained flat. One animal had a subtotal RD, but the area of retina containing the patch was flat. Three animals had total retinal detachment. All the animals were sacrificed.
Table 3.16 Clinical observations in Experimental Group A animals (N=16).

Operative complications and early postoperative findings:

<table>
<thead>
<tr>
<th>Animal code</th>
<th>Strain</th>
<th>Operative complications</th>
<th>After A/F exchange</th>
<th>Postop VH</th>
<th>Retinal necrosis</th>
<th>New breaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXPA1</td>
<td>Albino</td>
<td>Retinal holes</td>
<td>Not done</td>
<td>No</td>
<td>Yes ++</td>
<td>Patch fell off</td>
</tr>
<tr>
<td>EXPA2</td>
<td>Dutch</td>
<td>Retinal hole</td>
<td>Vit. haem</td>
<td>Per-op</td>
<td>No view</td>
<td>No view</td>
</tr>
<tr>
<td>EXPA3</td>
<td>Albino</td>
<td>None</td>
<td>Huge tears</td>
<td>No</td>
<td>Yes</td>
<td>Patch fell off</td>
</tr>
<tr>
<td>EXPA4</td>
<td>Albino</td>
<td>None</td>
<td>Not done</td>
<td>No</td>
<td>Yes ++</td>
<td>Patch fell off</td>
</tr>
<tr>
<td>EXPA5</td>
<td>Albino</td>
<td>Retinal hole</td>
<td>None</td>
<td>Yes</td>
<td>No view</td>
<td>No view</td>
</tr>
<tr>
<td>EXPA6</td>
<td>Dutch</td>
<td>Retinal holes</td>
<td>?holes larger</td>
<td>Yes</td>
<td>No view</td>
<td>No view</td>
</tr>
<tr>
<td>EXPA7</td>
<td>Albino</td>
<td>Ant cap hole</td>
<td>None</td>
<td>No</td>
<td>Yes</td>
<td>Yes, day 3</td>
</tr>
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<td>Yes</td>
<td>No</td>
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<td>Retinal hole</td>
<td>None</td>
<td>Yes</td>
<td>No view</td>
<td>No view</td>
</tr>
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<td>EXPA10</td>
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<td>None</td>
<td>Retinal tears</td>
<td>No</td>
<td>Yes</td>
<td>Yes, day 5</td>
</tr>
<tr>
<td>EXPA11</td>
<td>Albino</td>
<td>None</td>
<td>None</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>EXPA12</td>
<td>Dutch</td>
<td>None</td>
<td>None</td>
<td>No</td>
<td>Yes</td>
<td>Yes, day 6</td>
</tr>
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<td>EXPA13</td>
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<td>Retinal holes</td>
<td>?holes larger</td>
<td>No</td>
<td>Yes</td>
<td>Yes, day 6</td>
</tr>
<tr>
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<td>None</td>
<td>Retinal tears</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>EXPA15</td>
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<td>None</td>
<td>Retinal tears</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
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<td>EXPA16</td>
<td>Dutch</td>
<td>None</td>
<td>None</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

A/F = air/fluid
Postop VH = postoperative vitreous haemorrhage
Per-op = per-operative
ant cap = anterior lens capsule
Table 3.17 Clinical observations in Experimental Group A animals (N=16).

Early postoperative findings:

<table>
<thead>
<tr>
<th>Animal code</th>
<th>Day sacrificed</th>
<th>RD onset (day postop)</th>
<th>RD extent on day 7</th>
<th>Focal ERM, day 7</th>
<th>General ERM, day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXPA1</td>
<td>1</td>
<td>Sacrificed</td>
<td>Sacrificed</td>
<td>Sacrificed</td>
<td>Sacrificed</td>
</tr>
<tr>
<td>EXPA2</td>
<td>1</td>
<td>Sacrificed</td>
<td>Sacrificed</td>
<td>Sacrificed</td>
<td>Sacrificed</td>
</tr>
<tr>
<td>EXPA3</td>
<td>2</td>
<td>1</td>
<td>Sacrificed</td>
<td>Sacrificed</td>
<td>Sacrificed</td>
</tr>
<tr>
<td>EXPA4</td>
<td>2</td>
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<td>Sacrificed</td>
<td>Sacrificed</td>
</tr>
<tr>
<td>EXPA5</td>
<td>2</td>
<td>No view</td>
<td>Sacrificed</td>
<td>Sacrificed</td>
<td>Sacrificed</td>
</tr>
<tr>
<td>EXPA6</td>
<td>2</td>
<td>No view</td>
<td>Sacrificed</td>
<td>Sacrificed</td>
<td>Sacrificed</td>
</tr>
<tr>
<td>EXPA7</td>
<td>7</td>
<td>1</td>
<td>1/2</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>EXPA8</td>
<td>7</td>
<td>1</td>
<td>1/4</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>EXPA9</td>
<td>14</td>
<td>No view</td>
<td>No view</td>
<td>No view</td>
<td>No view</td>
</tr>
<tr>
<td>EXPA10</td>
<td>28</td>
<td>2</td>
<td>1/2</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
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<td>28</td>
<td>None</td>
<td>Flat</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
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<td>1</td>
<td>Total</td>
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<tr>
<td>EXPA13</td>
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<td>None</td>
<td>None</td>
</tr>
<tr>
<td>EXPA14</td>
<td>28</td>
<td>4</td>
<td>3/4</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>EXPA15</td>
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<td>14</td>
<td>Flat</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>EXPA16</td>
<td>28</td>
<td>None</td>
<td>Flat</td>
<td>None</td>
<td>None</td>
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</table>
Table 3.18 Clinical observations in Experimental Group A animals

Findings at follow up;

<table>
<thead>
<tr>
<th>Animal code</th>
<th>Findings on day 14</th>
<th>Findings on day 21</th>
<th>Findings on day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXPA9</td>
<td>No view - vit. haem.</td>
<td>Sacrificed</td>
<td>Sacrificed</td>
</tr>
<tr>
<td>EXPA10</td>
<td>Total RD No ERMs</td>
<td>Total RD No ERMs</td>
<td>Total RD No ERMs</td>
</tr>
<tr>
<td>EXPA11</td>
<td>Flat retina Patch adherent</td>
<td>Flat retina Patch adherent</td>
<td>Flat retina Patch adherent</td>
</tr>
<tr>
<td>EXPA12</td>
<td>PVD Retina reattached Patch separated</td>
<td>Flat retina Patch separated</td>
<td>Flat retina Patch separated</td>
</tr>
<tr>
<td>EXPA13</td>
<td>Total RD Generalised ERMs</td>
<td>Total RD Generalised ERMs</td>
<td>Total RD Generalised ERMs</td>
</tr>
<tr>
<td>EXPA14</td>
<td>Total RD</td>
<td>Total RD Generalised ERMs</td>
<td>Total RD Generalised ERMs</td>
</tr>
<tr>
<td>EXPA15</td>
<td>Subtotal RD Area of patch flat Focal ERMs</td>
<td>Subtotal RD Focal ERMs</td>
<td>Subtotal RD Generalised ERMs</td>
</tr>
<tr>
<td>EXPA16</td>
<td>Flat retina Patch adherent</td>
<td>Flat retina Patch adherent</td>
<td>Flat retina Patch adherent</td>
</tr>
</tbody>
</table>
Figure 3.25 Fundus photograph of Experimental Group A eye 3 hours after applying a retinal patch made from PVdF (P, coloured blue with indelible pen) and octylcyanoacrylate (O)(Albino rabbit)

The adhesive is visible, and mild pallor of the retina has developed under the adhesive
Figure 3.26 Fundus photograph of same Experimental Group A animal as Fig. 3.26
24 hours after applying the retinal patch

The retinal necrosis has become more obvious
Figure 3.27 Fundus photograph of Experimental Group A eye 3 days after applying the retinal patch

Small retinal breaks (B) have developed in necrotic retina at the edge of the patch
The retinal holes have enlarged
Figure 3.29 Fundus photograph of Experimental Group A eye 28 days after applying the retinal patch. Same animal as in Fig 3.25 and 3.26.

The retina is flat and the patch in place. The retinal necrosis is no longer apparent.
3.15.5 Experimental Group B (N = 15):

In this group of 15 animals retinal breaks were repaired with a patch made from PVdF and Tisseel. Two of the animals were Albino rabbits and the remainder Dutch. Clinical findings are summarised in Tables 3.19-21.

**Peroperative findings:**
There were no anterior segment complications. In 10 animals applying the retinal patch proceeded without difficulty (Figure 3.30). Iatrogenic retinal holes, either in posterior retina, or sclerotomy related, were made in 3 animals. One animal developed a haemorrhagic retinal detachment of one quadrant and in one animal the patch did not completely cover the retinal break.

**Findings after air/fluid exchange:**
The patch was found to have separated from the retina in 2 animals. The patch was reapplied at the second attempt in 1 animal, but a third attempt was required in the other. In 3 animals the area of retina containing the patch had elevated after air/fluid exchange, but in all cases the patch remained stuck to the retina, and no retinal tears were caused in these or any other animals. In a further animal the patch had partially separated from the retinal surface. One more eye had developed a subtotal RD.

**Findings during the first week**
Two animals had dense vitreous haemorrhage on day 1 and another developed vitreous haemorrhage on day 5. In 12 rabbits the view of the posterior pole was good and 10 were followed for one week. Five rabbits had developed subtotal RD during the first postoperative week, and the detachment became total in the animal developing a localised RD during surgery.

The 500 lU strength Tisseel was only used in 1 animal. This was the only eye to develop focal ERMs during the first postoperative week. Despite the addition of aprotonin the fibrin clot underwent lysis in all eyes. The fibrin developed a fluffy appearance and eventually disappeared. In some animals this process was discernible after only two days and was complete in all eyes by 14 days (mean 6.7 days, SD +/- 2.9)(Figure 3.31 and 3.32). In one animal the clot had lysed by 6 days, and the patch had fallen off by day 7 and was floating in the mid vitreous cavity. This animal developed a subtotal retinal detachment, starting on day 1, but this spontaneously reattached on day 7. Five animals were sacrificed during the first week (2 with uncomplicated patching and the 3 animals with dense vitreous haemorrhage).
Findings at 14 days:
10 animals were examined at this time point; 1 had dense vitreous haemorrhage. In the rabbit patched using the 500 IU strength Tisseel the posterior pole was not visible because the anterior chamber was full of fibrin, and the vitreous very hazy. In 8 animals the view was good. The retina was flat in 4 animals but detached in the remainder, either subtotally (2 animals) or totally (2 animals). Focal ERMs had developed in 3 more eyes, and generalised ERMs in a further 3. Two of the latter eyes had retinal detachment. The retinal patch was found floating in the vitreous cavity in 1 more eye (Figure 3.33). Two animals were sacrificed at this time point - 1 with dense vitreous haemorrhage, and the animal where the stronger Tisseel preparation had been used.

Findings on day 21:
8 animals were examined at this time point. The retina remained flat in 3 animals but was detached in the other 5 eyes (3 totally, 2 subtotally)(Figure 3.34 and 3.35). Two of the eyes with flat retinae had developed generalised ERMs. Focal ERMs had developed in a further 2 rabbits (one in flat and the other in detached retina) and generalised ERMs in 2 more rabbits. The focal ERMs were usually found in relation to the medullary rays. The generalised ERMs extended throughout the vitreous cavity, often producing marked traction on the medullary rays and peripheral retina.

The blood vessels of the medullary rays were dilated and tortuous, and this was particularly marked in eyes with retinal detachment. The area of the patch was not visible in all eyes with retinal detachment. In several eyes the substrate had separated from the retinal surface and was visible in the mid vitreous cavity.

Findings on day 28:
8 animals were examined at this time point. The most striking thing about these animals was that they had all developed generalised ERMs, often with pronounced traction, particularly on the medullary rays. Despite this 3 retinas remained flat, while 1 animal had a subtotal RD and the other 4 eyes had total RD. In 3 of the latter the retina had become funnelled, and in one large retinal breaks were visible. All the animals were sacrificed.
Table 3.19. Clinical observations in Experimental Group B animals (N=15)

Operative complications and early postoperative findings:

<table>
<thead>
<tr>
<th>Animal code</th>
<th>Strain</th>
<th>Strength Tisseel</th>
<th>Operative complications</th>
<th>After A/F exchange</th>
<th>Postop. VH</th>
<th>Clot lysis Day gone</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXPB1</td>
<td>Dutch</td>
<td>4 IU</td>
<td>None</td>
<td>None</td>
<td>No</td>
<td>ND sacrificed</td>
</tr>
<tr>
<td>EXPB2</td>
<td>Dutch</td>
<td>4 IU</td>
<td>Inadequate patch</td>
<td>None</td>
<td>No</td>
<td>ND sacrificed</td>
</tr>
<tr>
<td>EXPB3</td>
<td>Dutch</td>
<td>4 IU</td>
<td>Retinal hole</td>
<td>None</td>
<td>No</td>
<td>6</td>
</tr>
<tr>
<td>EXPB4</td>
<td>Dutch</td>
<td>4 IU</td>
<td>Retinal hole</td>
<td>None</td>
<td>No</td>
<td>6</td>
</tr>
<tr>
<td>EXPB5</td>
<td>Dutch</td>
<td>4 IU</td>
<td>None</td>
<td>Patch lifted</td>
<td>Yes, day 1</td>
<td>No view</td>
</tr>
<tr>
<td>EXPB6</td>
<td>Albino</td>
<td>500 IU</td>
<td>Retinal holes</td>
<td>Patch lifted</td>
<td>No</td>
<td>ND sacrificed</td>
</tr>
<tr>
<td>EXPB7</td>
<td>Albino</td>
<td>4 IU</td>
<td>None</td>
<td>None</td>
<td>Yes, day 1</td>
<td>No view</td>
</tr>
<tr>
<td>EXPB8</td>
<td>Dutch</td>
<td>4 IU</td>
<td>Retinal holes</td>
<td>Patch came off x2</td>
<td>No</td>
<td>14</td>
</tr>
<tr>
<td>EXPB9</td>
<td>Dutch</td>
<td>4 IU</td>
<td>Retinal holes</td>
<td>Patch came off x1</td>
<td>No</td>
<td>6</td>
</tr>
<tr>
<td>EXPB10</td>
<td>Dutch</td>
<td>4 IU</td>
<td>None</td>
<td>None</td>
<td>No</td>
<td>7</td>
</tr>
<tr>
<td>EXPB11</td>
<td>Dutch</td>
<td>4 IU</td>
<td>None</td>
<td>Patch partially off</td>
<td>Slight, Day 3</td>
<td>7</td>
</tr>
<tr>
<td>EXPB12</td>
<td>Dutch</td>
<td>4 IU</td>
<td>None</td>
<td>None</td>
<td>No</td>
<td>4</td>
</tr>
<tr>
<td>EXPB13</td>
<td>Dutch</td>
<td>4 IU</td>
<td>None</td>
<td>None</td>
<td>No</td>
<td>4</td>
</tr>
<tr>
<td>EXPB14</td>
<td>Dutch</td>
<td>4 IU</td>
<td>None</td>
<td>Patch partially off</td>
<td>No</td>
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<td>Dutch</td>
<td>4 IU</td>
<td>None</td>
<td>None</td>
<td>No</td>
<td>5</td>
</tr>
</tbody>
</table>

One rabbit developed endophthalmitis - not included.

A/F = air/fluid
Postop. VH = postoperative vitreous haemorrhage
Table 3.20 Clinical findings in Experimental Group B animals

Early postoperative findings:

<table>
<thead>
<tr>
<th>Animal code</th>
<th>Day sacrificed</th>
<th>RD onset (day postop)</th>
<th>RD extent on day 7</th>
<th>Focal ERMs day 7</th>
<th>General ERMs, day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXPB1</td>
<td>2</td>
<td>Flat on day 2</td>
<td>Sacrificed</td>
<td>Sacrificed</td>
<td>Sacrificed</td>
</tr>
<tr>
<td>EXPB2</td>
<td>2</td>
<td>Flat on day 2</td>
<td>Sacrificed</td>
<td>Sacrificed</td>
<td>Sacrificed</td>
</tr>
<tr>
<td>EXPB3</td>
<td>7</td>
<td>1</td>
<td>Reattached</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>EXPB4</td>
<td>7</td>
<td>No view</td>
<td>No view</td>
<td>No view</td>
<td>No view</td>
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<tr>
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<td>7</td>
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<td>No view</td>
<td>No view</td>
<td>No view</td>
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<tr>
<td>EXPB6</td>
<td>14</td>
<td>1</td>
<td>3/4</td>
<td>Yes</td>
<td>None</td>
</tr>
<tr>
<td>EXPB7</td>
<td>14</td>
<td>No view</td>
<td>No view</td>
<td>No view</td>
<td>No view</td>
</tr>
<tr>
<td>EXPB8</td>
<td>28</td>
<td>1</td>
<td>Total</td>
<td>None</td>
<td>None</td>
</tr>
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<td>EXPB9</td>
<td>28</td>
<td>14</td>
<td>Flat</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>EXPB10</td>
<td>28</td>
<td>14</td>
<td>Flat</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
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<td>28</td>
<td>1</td>
<td>1/4</td>
<td>None</td>
<td>None</td>
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</tr>
<tr>
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<td>None</td>
<td>Flat</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
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<td>1</td>
<td>Flat</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>EXPB15</td>
<td>28</td>
<td>21</td>
<td>Flat</td>
<td>None</td>
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</tbody>
</table>
Table 3.21 Clinical observations in Experimental Group B animals

Findings at follow up:

<table>
<thead>
<tr>
<th>Animal code</th>
<th>Findings on day 14</th>
<th>Findings on day 21</th>
<th>Findings on day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXPB6</td>
<td>Fibrin in AC</td>
<td>Sacrificed day 14</td>
<td>Sacrificed day 14</td>
</tr>
<tr>
<td></td>
<td>No view post. segment</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sacrificed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EXPB7</td>
<td>Vitreous haemorrhage</td>
<td>Sacrificed day 14</td>
<td>Sacrificed day 14</td>
</tr>
<tr>
<td></td>
<td>No view post. segment</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sacrificed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EXPB8</td>
<td>Total RD</td>
<td>Total RD</td>
<td>Total RD</td>
</tr>
<tr>
<td></td>
<td>No ERMs</td>
<td>Focal ERM s</td>
<td>Generalised ERM s</td>
</tr>
<tr>
<td>EXPB9</td>
<td>Total RD</td>
<td>Total RD</td>
<td>Total RD</td>
</tr>
<tr>
<td></td>
<td>Generalised ERM s</td>
<td>Generalised ERM s</td>
<td>Generalised ERM s</td>
</tr>
<tr>
<td>EXPB10</td>
<td>Subtotal RD</td>
<td>Subtotal RD</td>
<td>Total RD</td>
</tr>
<tr>
<td></td>
<td>Generalised ERM s</td>
<td>Generalised ERM s</td>
<td>Generalised ERM s</td>
</tr>
<tr>
<td>EXPB11</td>
<td>Subtotal RD</td>
<td>Subtotal RD</td>
<td>Subtotal RD</td>
</tr>
<tr>
<td></td>
<td>Focal ERM s</td>
<td>Generalised ERM s</td>
<td>Generalised ERM s</td>
</tr>
<tr>
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<td>Flat retina</td>
<td>Flat retina</td>
<td>Flat retina</td>
</tr>
<tr>
<td></td>
<td>No ERM s</td>
<td>Focal ERM s</td>
<td>Generalised ERM s</td>
</tr>
<tr>
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<td>Flat retina</td>
<td>Flat retina</td>
</tr>
<tr>
<td></td>
<td>Focal ERM s</td>
<td>Generalised ERM s</td>
<td>Generalised ERM s</td>
</tr>
<tr>
<td>EXPB14</td>
<td>Flat retina</td>
<td>Flat retina</td>
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<tr>
<td></td>
<td>Focal ERM s</td>
<td>Generalised ERM s</td>
<td>Generalised ERM s</td>
</tr>
<tr>
<td>EXPB15</td>
<td>Flat retina</td>
<td>Total RD</td>
<td>Total RD</td>
</tr>
<tr>
<td></td>
<td>Generalised ERM s</td>
<td>Generalised ERM s</td>
<td>Generalised ERM s</td>
</tr>
</tbody>
</table>

AC = anterior chamber
Figure 3.30 Fundus photograph of Experimental Group B eye immediately after applying a retinal patch using Tisseel (T) and PVdF (Dutch rabbit)

The adhesive has spread widely
Figure 3.31 Fundus photograph of Experimental Group B eye. Same eye as in Fig 3.30, 3 days after applying the patch

The fibrin adhesive (Tisseel) is showing signs of lysis
Figure 3.32 Fundus photograph of Experimental Group B eye. Same eye as in Fig 3.30, 4 days after applying the patch

The Tisseel adhesive has undergone fibrinolysis and is barely discernible
Figure 3.33 Macrophotograph of Experimental Group B eye sacrificed 21 days after applying a retinal patch made from Tisseel and PVdF

The Tisseel fibrin adhesive has undergone fibrinolysis, the patch has separated from the surface of the retina and the retina has totally detached
ERM formation has led to traction/rhegmatogenous retinal detachment, with the development of large retinal breaks.
Figure 3.35 Fundus photograph of Experimental Group B eye 21 days after applying the patch

ERM formation has led to traction/rhegmatogenous retinal detachment. The vasculature of the medullary rays has become pronounced.
3.15.6 Experimental Group C:

In the two of the three eyes patched using autologous fibrin postoperative clinical details were obtainable. Both eyes had developed marked generalised ERM formation by 4 weeks, with total retinal detachment in one animal.

3.16 STATISTICAL ANALYSIS OF DATA

3.16.1 Data analysed:

Data from clinical grading were available from 73 rabbits, but data from only 53 were analysed statistically. Thirteen rabbits from Control Group 1 were excluded as they had undergone a different surgical procedure (i.e. RD formation) and a further 7 were excluded because they had missing data due to dense vitreous haemorrhage which obscured fundus details.

Data on posterior segment inflammation was not analysed as it was not always possible to distinguish inflammatory cells from red blood cells, and it was difficult to grade posterior segment flare due to thickening of the anterior capsule.

Data were entered into a personal computer using Paradox. Data for anterior segment inflammation (ASI), ERM formation, the development of RD were entered for each experimental group at every available time point. In addition the strain of rabbit was recorded (albino or Dutch). Data were analysed through stratified analyses, using maximum likelihood and Mantel-Haenszel methods to estimate relative risks and relative incidence rates, and to test the statistical significance of the rates. Wherever possible the analysis took into account both surgical group as well as rabbit strain although for some analyses the numbers were too small to allow analysis by rabbit strain. The data were collated, formulated and analysed in such a way that a number of preliminary questions could be addressed:

1. Do albino and Dutch rabbits respond in the same way within each surgical group with respect to ASI, ERM formation and RD? If there was no significant difference data from the two strains could thereafter be analysed together.
2. Looking at the same criteria, do Control Group 2 eyes differ significantly from Control Group 3 eyes? If not then data from the two groups could be combined into a single Control Group.

Once these questions had been answered the data was analysed to ascertain whether there was any difference between the surgical groups with respect to
1. focal ERM formation (grade 2,3)
2. generalised ERM formation (grade 4,5)
3. RD of any extent (grade 2-5)
4. anterior segment inflammation (ASI score)

3.16.2 Analysis of data with respect to rabbit type:

Overall the incidence of focal ERM was significantly higher in albino rabbits than Dutch rabbits (p = 0.034)(Table 3.22), but when each group was analysed separately a significant difference was only reached comparing rabbit stains in Control Groups (p = 0.04). Numbers were too small to allow stratified analysis of data on generalised ERM formation and development of RD. Analysis of ASI showed that there was no significant difference between albino and Dutch rabbits (data not shown). In all subsequent analysis rabbit strain was not taken into consideration as this analysis demonstrated that there were reasonable levels of homogenicity.

3.16.3 Analysis of data comparing Control Groups:

Analysis of data on ASI, ERM formation and RD demonstrated that there was no significant difference between Control Groups 2 and 3 (data not shown). These groups have therefore been combined for subsequent analysis (Control Group).

3.16.4 Analysis of ERM Formation Data:

Analysis of data comparing the incidence of focal ERMs in the Experimental Groups to the incidence in the Control Group showed that EXPA did not differ significantly from Controls (p = 0.153)(Table 3.23), whereas EXPB had a significantly higher incidence of focal ERMs than Controls (p=0.019)(Table 3.24). Analysis of data comparing the incidence of focal ERMs between Experimental Groups showed that there was no significant difference (p = 0.52)(Table 3.25), except for albino rabbits (p = <0.001) but this may be explained by the small number of albino rabbits in the Experimental Groups.

Generalised ERM formation occurred significantly more often in both Experimental Groups compared with Controls (EXPA p = 0.014, EXPB p = >0.001)(Table 3.26), and EXPB eyes had a higher incidence of generalised ERM formation than EXPA eyes, but this did not reach significance (p = 0.066)(Table 3.27).
3.16.5 Analysis of Retinal Detachment Data:

Analysis of data of RD of any degree showed both Experimental Groups to have highly significantly increased rates compared to Control animals (EXPA p = >0.001, EXPB p = >0.001) (Table 3.28) with EXPA eyes having insignificantly higher rates than EXPB eyes (p = 0.068)(Table 3.29). All other degrees of RD were significantly higher in Experimental Groups than Controls (p values ranging from 0.006 - >0.001), but again there was no difference comparing EXPA with EXPB (p values ranging from 0.15-0.59) (Data not included).

3.16.6 Analysis of Anterior Segment Inflammation (ASI) Scores:

Data for rabbits followed for 7 days:
Mean ASI scores for animals with complete data for the first 7 postoperative days were calculated for each group and by rabbit type (Figure 3.36). The scores were not normally distributed and so analysis using ANOVA was not applicable.

Complete data:
ASI scores for each group at every available time point were entered into Paradox, combining data for albino and Dutch rabbits. Again, the scores were not normally distributed and analysis using ANOVA was not applicable. Transformation of the scores normalised the distribution but did not stabilise the variables adequately for analysis using parametric methods. Non-parametric tests were therefore used (the Kruskal-Wallis One-Way analysis of variance) which does not require data to be normally distributed, nor does this method require equal variance between comparison groups. Data from Control Groups 1 and 2, and Experimental Groups A and B were compared at time points 1,3,5,7,14,21 and 28 days. ASI scores for Albino and Dutch rabbits were combined as the only group where there were large enough numbers of each strain showed that they behaved very similarly in terms of magnitude of change, and change over time. Results of Kruskal-Wallis One-Way analysis of variance are shown in Table 3.30. The main findings are that Experimental Group B eyes had significantly more ASI than Control Group 2 eyes at every time point, and significantly more than Control Group 1 eyes on days 21 and 28. This is in contrast to Experimental Group A eyes which had similar levels of ASI compared to Control Group eyes, only having significantly more inflammation on days 21 (comparing both Control Groups) and day 28 (compared to Control Group 2). However, there was no significant difference in inflammation between Experimental Group A and Experimental Group B eyes at any time point.
Table 3.22 Stratified analysis to compare the incidence of focal ERM formation in Control and Experimental Groups A and B by rabbit type

<table>
<thead>
<tr>
<th>Stratum 1</th>
<th>Stratum 2</th>
<th>Stratum 3</th>
<th>Weighted av.</th>
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<tr>
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<td>Exp Group A</td>
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<tr>
<td>Albino</td>
<td>Dutch</td>
<td>Albino</td>
<td>Dutch</td>
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<td>Incidence*</td>
<td>46</td>
<td>0</td>
<td>0</td>
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<td>Rel Risk #</td>
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<td>0</td>
<td>5.4</td>
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<td>-</td>
<td>-</td>
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<td>M-H Chi Sq</td>
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<td>0.04</td>
<td>0.443</td>
<td>0.084</td>
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* = incidence per 1,000 days at risk  
# = comparing Dutch with albino

MH = Mantel-Haenszel Chi square  
95% CL = 95% confidence interval

Table 3.23. Stratified analysis to compare the incidence of focal ERM formation in albino and Dutch rabbits by experimental group (Controls and Experimental Group A)

<table>
<thead>
<tr>
<th>Stratum 1</th>
<th>Stratum 2</th>
<th>Weighted average</th>
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<td>Controls</td>
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<td>Albino</td>
<td>Dutch</td>
<td>Albino</td>
</tr>
<tr>
<td>Incidence*</td>
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<td>0</td>
</tr>
<tr>
<td>Rel Risk #</td>
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<td>1.34</td>
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<td>M-H Chi Sq</td>
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<tr>
<td>p value</td>
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<td>0.81</td>
</tr>
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</table>

* = incidence per 1,000 days at risk  
# = comparing Controls with Experimental Group A
Table 3.24 Stratified analysis to compare the incidence of focal ERM formation in albino and Dutch rabbits by experimental group (Controls and Experimental Group B)

<table>
<thead>
<tr>
<th>Stratum 1 Albino</th>
<th>Stratum 2 Dutch</th>
<th>Weighted average All</th>
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<td>Controls EXPB</td>
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<td>46.0</td>
<td>142.9</td>
</tr>
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<td>Rel Risk #</td>
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<td>4.76</td>
</tr>
<tr>
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<td>0.39-24.84</td>
<td>0.92-24.54</td>
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<tr>
<td>M-H Chi Sq</td>
<td>1.27</td>
<td>4.25</td>
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<td>p value</td>
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<td>0.039</td>
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* = incidence per 1,000 days at risk  
# = comparing controls with Experimental Group B

Table 3.25 Stratified analysis to compare the incidence of focal ERM formation in albino and Dutch rabbits by experimental group (Experimental Groups A and B)

<table>
<thead>
<tr>
<th>Stratum 1 Albino</th>
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<th>Weighted average All</th>
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</thead>
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<td>EXPA EXPB</td>
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<td>142.9</td>
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<tr>
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<td>3.55</td>
</tr>
<tr>
<td>95% CL</td>
<td>-</td>
<td>0.41-30.34</td>
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<td>M-H Chi Sq</td>
<td>11.29</td>
<td>1.52</td>
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<tr>
<td>p value</td>
<td>&lt;0.001</td>
<td>0.22</td>
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</table>

* = incidence per 1,000 days at risk  
# = comparing Experimental Group A with B
Table 3.26 Incidence of generalised ERM formation: comparison of Control Group eyes with Experimental Group A and B eyes

<table>
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<th></th>
<th>Controls</th>
<th>Exp Group A</th>
<th>Exp Group B</th>
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<tbody>
<tr>
<td>Incidence *</td>
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<td>37.74</td>
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<tr>
<td>Rel Risk #</td>
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<td>Infinite</td>
<td></td>
</tr>
<tr>
<td>95% CL</td>
<td>-</td>
<td>-</td>
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<tr>
<td>M-H Chi Sq</td>
<td>6.07</td>
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<tr>
<td>p value</td>
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</table>

* = incidence/1,000 days at risk
# = comparing controls with Experimental Groups A and B

NB Not stratified by rabbit type because numbers too small

Table 3.27 Incidence of generalised ERM formation: comparison of Experimental Group A with Experimental Group B

<table>
<thead>
<tr>
<th></th>
<th>Exp Group A</th>
<th>Exp Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk *</td>
<td>9.80</td>
<td>37.74</td>
</tr>
<tr>
<td>Rel Risk</td>
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<td>95% CL</td>
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<td>M-H Chi Sq</td>
<td>3.37</td>
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<tr>
<td>p value</td>
<td>0.066</td>
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</tbody>
</table>

* = incidence/1,000 days at risk

NB Not stratified by rabbit type because numbers too small
Table 3.28 Incidence of retinal detachment of any extent: comparison of Control Group eyes with Experimental Group A and Experimental Group B eyes

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Exp Group A</th>
<th>Exp Group B</th>
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<tbody>
<tr>
<td>Risk *</td>
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<tr>
<td>Rel Risk</td>
<td>46.94</td>
<td>19.84</td>
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<tr>
<td>95% CL</td>
<td>10.1 - 217.2</td>
<td>4.2 - 93.4</td>
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<tr>
<td>M-H Chi Sq</td>
<td>73.57</td>
<td>28.62</td>
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<tr>
<td>p value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
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</tbody>
</table>

* = incidence/1,000 days at risk

NB Not stratified by rabbit type because numbers too small

Table 3.29 Incidence of retinal detachment of any extent: comparison between Experimental Group A and B eyes

<table>
<thead>
<tr>
<th></th>
<th>Exp Group A</th>
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<tr>
<td>Risk *</td>
<td>155.17</td>
<td>65.57</td>
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<tr>
<td>Rel Risk</td>
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<tr>
<td>95% CL</td>
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<td>M-H Chi Sq</td>
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<tr>
<td>p value</td>
<td>0.068</td>
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</table>

* = incidence/1,000 days at risk

NB Not stratified by rabbit type because numbers too small
Figure 3.36 Plot of mean ASI scores for animals with complete data for the first 7 days by experimental group and rabbit type.
Table 3.30 Analysis of anterior segment inflammation using Kruskal-Wallis One-Way analysis of variance.

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<td>13 16</td>
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<tr>
<td></td>
<td></td>
<td>R1-R2</td>
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<td>R1-R2</td>
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<td></td>
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<td>R1-R2</td>
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<td>R1-R2</td>
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<td>&lt;0.05</td>
</tr>
</tbody>
</table>

N = Number of animals in the comparison groups at each time point
R1-R2 = Difference between rank scores of the comparison groups
Crit. Diff = Critical difference. If R1-R2 is > this value the difference is significant (at p <0.05).
3.17 HISTOLOGICAL EXAMINATION OF TISSUES

Animals in each surgical group were sacrificed at 2, 7, 14 and 28 days and tissues prepared for light microscopy, or electron microscopy (SEM or TEM). Animals were also sacrificed after cryotherapy and gas compression vitrectomy.

3.17.1 Following Cryotherapy:

The presence of a chorioretinal scar was confirmed histologically as treated areas showed atrophy of all retinal layers with fibroglial proliferation and infiltration by heavily pigmented cells. (Figure 3.37). The adhesive nature of the scar was demonstrated in eyes with total retinal detachment as the retina in cryotherapy treated areas remained attached.

3.17.2 Following Gas Compression Vitrectomy:

Scanning electron microscopy of the posterior segment of a normal globe showed a layer of condensed vitreous over the whole retinal surface, with particularly thick gel over the optic nerve head and medullary rays. Following gas compression vitrectomy the smooth surface of the ILL, free of vitreous, could be seen. However vitreous was still adherent over the medullary rays and optic nerve head (Figure 3.38).

Light microscopy of eyes two weeks after gas injection and thick sections from superior and inferior retina showed no abnormality in the retina, choroid or optic nerve.
Figure 3.37 Light micrograph of peripheral retina two weeks after cryotherapy.

A chorioretinal adhesive scar is present (H and E, Magnification x 450).
Figure 3.38 Scanning electron micrographs of the posterior pole of a rabbit eye after gas compression vitrectomy

A Posterior pole showing medullary ray (MR), curled up retina (R), and frill of vitreous (V) attached to the edge of the medullary ray (MR) (Mag x32)
B The internal limiting lamina of the retina is clear of cortical vitreous (Mag x 1.4K)
3.17.3 Control Group 1:

**Light Microscopy at 2 days:**

One animal was sacrificed after 2 days (CG1A). The cornea, iris and ciliary body were normal, with a few polymorphonuclear leucocytes (PMNs) and macrophages adjacent to the drainage angle. The anterior lens capsule was clean centrally, but anterior epithelial cells were present between the capsular remnants peripherally. Tags of vitreous were present over the ciliary body and at the optic nerve head. The retina was normal apart from the area where it had been detached. Here the retina was oedematous throughout its thickness with loss of photoreceptor OS and oedema of the inner retinal layers. Fine haemorrhage and pigment laden cells, probably RPE cells, were present in the subretinal space beneath the area of induced RD. The RPE was normal apart from under the area of RD where the monolayer seemed to have been interrupted. Photoreceptor OS could be seen adherent to the apical surface of some RPE cells. The choroid was normal. Inflammatory cells (monocytes and macrophages with and without cytoplasmic melanin) were present on the surface of the retina inferiorly. A few non pigmented macrophages were observed on the outer retinal surface, in the area of RD.

**Light microscopy at 5 days:**

One animal died at this time point (CG1B). Apart from fine haemorrhage in the posterior segment the abnormal findings were again restricted to the area of RD (Figure 3.39). Here the retina was locally disorganised and folded, with loss of photoreceptor OS. Heavily pigmented cells had invaded the retina. Beneath the area of RD the monolayer of RPE was discontinuous and some RPE cells had migrated into the subretinal space.

**Light Microscopy at 7 Days:**

Two eyes were examined by light microscopy at this time point (CG1C and D). In both eyes anterior segment findings were essentially as above, apart from increased numbers of anterior epithelial cells on the lens capsule. In one eye the area of RD showed oedematous, thickened retina with loss of photoreceptors. In the second eye the retina had reattached and the affected region demonstrated localised atrophy of all layers, with disorganisation and loss of photoreceptors and outer nuclear layer. The retina was invaded by heavily pigmented cells and demonstrated mild preretinal and subretinal gliosis with the formation of superficial folds. The RPE was normal part from the area where the retina had been detached; here the RPE demonstrated patchy hypertrophy, proliferation and atrophy. A few PMNs, pigment laden macrophages, lymphocytes and the occasional plasma cell were present in the vitreous remnant overlying the ciliary processes.
Electron microscopy at 7 days:
The area of the RD was processed for TEM and the remainder of the posterior segment for SEM (CG1E). In this animal the localised RD had almost completely reattatched (Figure 3.40). Inner retinal layers were essentially normal, apart from prominent Muller cells. Fewer nuclei than normal were present in the outer nuclear layer, and photoreceptor OS were shortened and oedematous. Heavily pigmented cells were present in the potential subretinal space. The RPE demonstrated areas of proliferation and areas of atrophy.

Scanning electron microscopy showed a residual frill of vitreous attached to the medullary rays, which extended towards the periphery of the retina (Figure 3.41).

Light Microscopy at 14 days:
The globe of one animal (CG1G), which developed recurrent RD 7 days after vitreo-lensectomy was processed for light microscopy. The section passed through the area of the retinotomy and original RD. Here the retina was markedly disorganised and the RPE atrophic (Figure 3.42). Pigment laden cells were visible between the edges of the retinotomy. The remainder of the retina showed features of recent RD i.e. loss of photoreceptor OS.

Light microscopy at 28 days:
Two globes were prepared for light microscopy (CG1J and K) and specimens from a further rabbit (CG1H) processed for TEM, and thick sections examined. In the first two eyes the cornea, iris and ciliary body were normal, with no inflammatory infiltrate. In both eyes anterior epithelial cells had proliferated and formed peripheral "lentoid" structures. In one eye the capsule was thrown up into folds centrally by proliferating epithelial cells. In both eyes the vitreous had been completely removed inferiorly, but tags were still attached at the optic disc. The retina, RPE and choroid were normal, apart from the region where the retina had previously been detached. Here the retina was atrophic throughout its layers with loss of photoreceptor OS, and the RPE hypertrophic. The retina was invaded by pigment laden cells and there was evidence of localised subretinal gliosis (Figure 3.43). A few inflammatory cells, mainly lymphocytes with a few pigment laden macrophages and plasma cells were found on the inner retinal surface inferiorly. The choroid in both eyes was normal.

Sections of retina taken from the area of RD in animal CG1H showed thinning of all retinal layers, with loss of nuclei from the outer nuclear layer, and complete loss of photoreceptor OS (Figure 3.44). Pigmented cells had invaded the outer retinal layers, and the RPE cell monolayer was discontinuous.
Figure 3.39 Light micrograph of Control Group 1 animal 5 days after vitreolensectomy and creation of a localised retinal detachment.

The retina is locally disorganised, with loss of photoreceptor outer segments (H and E, Magnification x 225).
Figure 3.40 Light micrograph of thick sections Control Group 1 animal 7 days after vitreolensectomy and creation of a localised retinal detachment

A The area of detachment shows loss of photoreceptor outer segments and proliferation of RPE cells, some of which have migrated into the subretinal space (Toluine blue, Magnification x 270). B Area of spontaneous retinal reattachment (Toluine blue, Magnification x 450).
Figure 3.41 Scanning electron micrograph of Control Group 1 animal 7 days after vitreolensectomy and creation of a localised retinal detachment

The internal limiting lamina of the retina (ILL) is clear of vitreous, but a frill of vitreous (V) is adherent to the edge of the medullary rays (MR) (Mag x 27).
Figure 3.42 Light micrograph of Control Group 1 animal 14 days after vitreolensectomy and creation of a localised retinal detachment.

The section passes through the retinal break, showing pigment laden cells between the edges of the retinotomy (H and E, Magnification x 180).
Figure 3.43 Light micrograph of Control Group 1 animal 28 days after vitreolensectomy and creation of a localised retinal detachment.

Fine subretinal membranes have developed in the area of the localised detachment, causing folding of the retina (H and E, Magnification x 275).
Figure 3.44 Light micrograph of thick sections of Control Group 1 animal 28 days after vitreolensectomy and creation of a localised retinal detachment.

In the area where the retina was previously detached there are fewer nuclei than normal in the outer nuclear layer (ONL), photoreceptor outer segments are missing and the outer retina has been invaded by pigment laden cells (Toluine blue, Magnification x 450).
3.17.4 Control Group 2:

**Light Microscopy at 2 days:**
One globe was processed for light microscopy at this time point (CG2A). Anterior segment structures were essentially normal apart from some fibrin in the anterior chamber and on the iris surface. The anterior capsule was free of epithelial cells centrally, but a layer of cells was present peripherally. The vitreous had been removed over inferior retina but tags remained adherent to superior retina, and at the optic nerve and medullary rays. The retina, choroid and RPE were normal. Inflammatory cells were not present in the anterior chamber, but occasional macrophages were observed invading the vitreous remnant from the ciliary processes.

**Electron Microscopy at 5 days:**
One animal was sacrificed at this time point (CG2B) and the globe sectioned. One half was processed for SEM and anterior and posterior segments structures of the other half (including superior and inferior retina) processed for TEM and thick sections examined. SEM showed the ILL to be clear of vitreous apart from over the medullary rays. Here a fine meshwork of vitreous fibres was visible, with many red blood corpuscles on the surface of the vitreous. No inflammatory cells were seen. Thick sections of the ciliary body, drainage angle and cornea showed no abnormality. A few inflammatory cells were observed in the drainage angle and invading the vitreous remnant. Posterior segment structures, including the optic nerve head, retina, RPE were all normal.

**Electron microscopy at 21 days:**
One globe (CG2D) was processed for SEM and TEM (as above), and thick sections examined. There was no observed differences compared to the animal described above.

**Light Microscopy at 28 days:**
Three eyes were examined histologically at this time point (CG2J,K and N). One animal had developed focal ERMs (CG2J). All demonstrated similar findings. Anterior segment structures were normal. The anterior chamber of one eye contained a few scattered inflammatory cells, mainly lymphocytes. In all eyes lens epithelial cells had proliferated to form "lentoid" structures peripherally, with wrinkling and fibrosis of the central part of the lens capsule. The vitreous remnant, in the region of the ciliary body, was invaded by a few small inflammatory cells, mainly lymphocytes. Histologically the retina, choroid and RPE in each eye was normal.
3.17.5 Control Group 3:

**Light microscopy at 2 days:**
One globe was processed for light microscopy (CG3A). The cornea, iris and ciliary body were normal and the anterior capsule clean. Fine fibrin strands were evident in the anterior and posterior chambers. Very few inflammatory cells were present. The retina was normal except immediately adjacent to retinal break where it was oedematous throughout its thickness. The RPE and choroid were normal.

**Electron microscopy at 2 days:**
The posterior segment of one globe was processed for SEM (CG3B). The ILL was free of vitreous, and clumps of red blood corpuscles, small, round cells (probably blood derived lymphocytes) and other larger cells, with extensive filipodia and the topographic features associated with macrophages, were visible on the surface of the retina (Figure 3.45).

**Light microscopy at 14 days:**
Two globes were prepared for light microscopy (CG3D and E). Animal CG3E had developed focal ERMs. The anterior and posterior segments were essentially normal. One or two inflammatory cells were seen in the inferior vitreous remnant.

**Light microscopy at 28 days:**
Four eyes were examined (CG3F, H, I and L). In all three eyes without RD anterior segment structures were normal, no inflammatory cells were present, and "lentoid" structures had developed to varying degrees. One animal had a total RD (CG3I), and the anterior chamber of this animal contained a meshwork of fine fibrin.

The three eyes with flat retinas showed similar features. The choroid, RPE, and retina away from the site of the break were normal. In one eye retina adjacent to the retinotomy site was atrophic, gliotic and infiltrated by heavily pigmented cells. In another eye the section passed through the area of the retinotomy (Figure 3.46), while in the third eye there was a localised area of retinal folding. Very few inflammatory cells were present.

The retina in the fourth eye was subtotally detached and thrown up into deep folds by fibrocellular proliferations. The detached retina was oedematous, with loss of photoreceptor OS. The RPE showed generalised scalloping, while the choroid was normal. Macrophages, some pigment laden, were seen on the outer surface of detached retina. Inflammatory cells, mainly pigmented macrophages, lymphocytes and plasma cells were present on the inner retinal surface, but only inferiorly.
Figure 3.45 Scanning electron micrographs of Control Group 3 eye 2 days after vitreolensectomy and creation of a retinal break

A (Mag x 6.5K) and B Red blood corpuscles and inflammatory cells are present on the internal limiting lamina of the retina (Mag x 1.2K)
Figure 3.46 Light micrograph of Control Group 3 animal 28 days after vitreolensectomy and creation of a retinal break.

The section passes through the retinal break. The retina is disorganised locally but normal elsewhere (H and E, Magnification x 90).
3.17.6 Experimental Group A:

Light microscopy at 2 days:
Three globes were processed for light microscopy (EXPA3,5 and 6) and one processed for TEM and thick sections examined (EXPA4). In each eye the cornea, iris and ciliary body were normal. Fine haemorrhage was present in the anterior chamber of one eye, with fibrin in the posterior chamber. A few inflammatory cells, mainly PMNs and macrophages, were present in the anterior chamber and the drainage angle. The anterior capsule was free of epithelial cells, apart from at the poles, where epithelial cells were visible.

In two eyes the posterior segment was full of haemorrhage. The section passed through the area of adhesive in two eyes and through retina adjacent to the adhesive in the other globe. The findings were similar in the three eyes. The superior retina appeared normal, as did the peripheral inferior retina. The area of retina where the adhesive had been applied however, showed gross changes. The inner retina was thrown up into fine folds and the retina beneath and adjacent to the adhesive was completely disorganised with full thickness coagulative necrosis (Figure 3.47). Nuclei of the outer nuclear layer where pyknotic and the photoreceptors oedematous. Retina adjacent to the adhesive was oedematous throughout its thickness, with vacuolation of all layers (Figure 3.48). These changes became less pronounced further away from the adhesive, while peripheral retina appeared normal.

The RPE was normal apart from immediately beneath the retina where the adhesive had been applied. Here many cells had separated from Bruch's membrane, some had rounded up and others were vacuolated (Figure 3.49). Some of the cells had ruptured, releasing melanin granules into the subretinal space. In both eyes red blood cells were found between damaged RPE cells, and the choroid was locally haemorrhagic. The posterior segment contained haemorrhage, fibrin, vitreous remnants and a few inflammatory cells. The latter were mainly macrophages, seen mainly in the vitreous remnant, in relation to the ciliary processes. Very few inflammatory cells were present in the subretinal space or choroid.

EXPA4. Retina adjacent to the retinal patch showed similar changes to those described above. The retina was thrown up into folds and was oedematous, with loss of the outer nuclear layer and photoreceptor outer segments. In places the retina appeared totally disorganised. Residual nuclei in the inner and outer nuclear
layers were pyknotic. Underlying RPE cells were flattened and thinned. Peripheral retina and RPE were normal.

**Light microscopy at 7 days:**
One globe was processed for light microscopy (EXPA8). This rabbit developed peri-adhesive retinal breaks with localised retinal detachment. The section passed through the retinal break. The peripheral retina appeared normal but retina on either side of the break showed areas of oedema and atrophy in other areas. The inner retina had prominent Muller cell nuclei. The outer nuclear layer was much thinner than normal and the nuclei of remaining cells were pyknotic. There was loss of photoreceptor outer segments. The retina on one side of the break was folded, with evidence of a localised fibrocellular epiretinal membrane (Figure 3.50). The RPE underlying attached retina in the superior part of the eye was normal. RPE under detached retina demonstrated scalloping, with cells migrating into the subretinal space. The vitreous remnant contained inflammatory cells, mainly macrophages, lymphocytes and plasma cells. Macrophages were present on the outer retinal surface of detached retina. The choroid was haemorrhagic in the region where the patch had been applied, showed minimal invasion by inflammatory cells and was normal elsewhere.

Another globe was processed for TEM, and thick sections were prepared (EXPA7). This rabbit had developed new retinal breaks in necrotic retina adjacent to the patch, with retinal detachment. Retina immediately adjacent to the patch was atrophic, with loss of nuclei from the outer and inner nuclear layers. The ILL was finely folded and the ganglion cell layer showed prominent Muller cell nuclei. The external limiting lamina was intact, but there was loss of photoreceptor outer segments. The underlying RPE cells were absent in places and elsewhere some of the cells were vacuolated.

**Light microscopy at 14 days:**
One globe was processed for light microscopy (EXPA9). Dense vitreous haemorrhage prevented fundus examination. The section passed through the retinal patch, which appeared be adherent to the retina (Figure 3.51). The anterior and posterior chambers were full of fibrin, and the posterior segment full of haemorrhage. Occasional inflammatory cells, mainly macrophages, were visible on the endothelium and in the drainage angle. Staining with methyl green pyronin (MG/PY) demonstrated that some of these cells were plasma cells. The superior retina was detached, but as the morphology of the retina was normal the detachment was probably a processing artefact. Inferiorly, retina in the extreme periphery was normal but elsewhere marked changes were apparent. Two large
retinal holes had developed in thinned, atrophic retina on either side of the patch. There was a fairly clear demarcation between atrophic retina and normal, peripheral retina. Retina beneath and adjacent to the patch was folded, gliotic and disorganised. In the immediate vicinity of the patch, within atrophic retina, large cells with foamy, pigmented cytoplasm were observed (Figure 3.52). In the superior part of the globe the RPE and choroid were normal. Inferiorly, particularly under the patch, RPE cells had separated from Bruch’s membrane and could be seen in the subretinal space (Figure 3.53). Many pigment laden cells, either macrophages or RPE, cells could be seen in the subretinal space. Subretinal haemorrhage was present under the patch, and the choroid was locally haemorrhagic. Very few inflammatory cells were present, either in relation to the retina or invading the vitreous remnant or in the choroid. MG/PY staining showed very occasional plasma cells in the subretinal space. A fine, transvitreal fibrocellular membrane was observed, passing from the lens capsule to the optic nerve head.

**Light microscopy at 28 days:**
Microscopy was available from four eyes at this time point. One eye (EXPA16) had an attached patch and flat retina. The other eyes all had total retinal detachment (EXPA10, 13 and 14) which had been present for at least two weeks.

**EXPA16.** Fine strands of partially detached cortical vitreous were visible overlying the retina in places. Where the cortical gel was adherent to the retina the ILL appeared thinned. Occasional inflammatory cells (macrophages, occasionally pigment laden, and plasma cells) were visible on the ILL, particularly inferiorly. The choroid appeared normal, as was the RPE. Despite repeated sectionning specimens of the area of the retinal patch were not obtained.

**EXPA13.** In this rabbit the retina was totally detached, with a closed funnel (Figure 3.54). There were areas of localised retinal folding, which were caused by fine, subretinal membranes (Figure 3.55). Photoreceptor outer segments had been lost throughout the retina. In inferior retina a retinal break was present. Retina adjacent to the break was markedly atrophic, with loss of the outer nuclear layer. Macrophages were observed on the outer retinal surface, but apart from this the eye showed very little signs of inflammation. The choroid appeared normal throughout. RPE cells were seen invading the subretinal space.

**EXPA10.** In this rabbit the findings were similar. In inferior retina there was an area where the retina was markedly atrophic, with loss of photoreceptor outer segments and the outer nuclear layer. This area of retina was thrown up into many, small
folds by a thin subretinal fibroglial membrane. Once again, signs of inflammation were minimal.

EXPA14. The findings in this animal were similar to those described above.

**Electron microscopy at 28 days:**
Scanning electron microscopy was performed on animals EXPA11 and 15, and thick sections were prepared for animal EXPA12. Scanning electron microscopy showed that the patches had remained adherent to the retina (Figure 3.56). The surface of the octylcyanoacrylate adhesive was highly convoluted (Figure 3.57). In animal EXPA15 round cells, probably macrophages, were present on the ILL, and in places the ILL was raised into small mounds, presumably by underlying glial cells (Figure 3.58). In animal EXPA11 oedematous photoreceptor OS and cells were present on the surface of the PVdF substrate. The majority of the cells were spread, with short and long filipodia (Figure 3.59).

Scanning electron microscopy of the one eye showed a narrow frill of vitreous attached to the margins of the medullary rays. Small, rounded cells, presumably inflammatory cells, were visible on vitreous frill and overlying the vascular complex of the medullary rays.

Thick sections from animal EXPA12 were prepared from retina and RPE adjacent to the area where the patch had been applied and from peripheral retina and RPE. Retina adjacent to where the patch had been applied showed changes very similar to those described above. Sections of peripheral retina showed fibrocellular ERMs (Figure 3.60). The underlying RPE was abnormal, as many cells vacuolated. Large, vacuolated RPE cells were observed in the subretinal space (Figure 3.61). Peripheral retina showed changes typical of RD i.e. loss of photoreceptor OSs. Peripheral RPE cells were rounded and exhibited a scallop shell like appearance ("scalloping").
Figure 3.47 Light micrographs of Experimental Group A animal 2 days after application of a patch using PVdF and octylcyanoacrylate

A The retina has been thrown up into folds, and retina beneath the octylcyanoacrylate adhesive (O) shows full thickness coagulative necrosis (H and E; Magnification x 90). B Higher power of the same area of retinal necrosis (H and E; Magnification x 270)
Figure 3.48 Light micrograph of Experimental Group A animal 2 days after application of a patch using PVdF and octylcyanoacrylate.

The area of retina adjacent to the adhesive is oedematous, with piknotic nuclei in the outer nuclear layer (H and E; Magnification x 200).
Figure 3.49 Light micrograph of Experimental Group A animal 2 days after application of a patch using PVdF and octylcyanoacrylate.

Retinal pigment epithelium beneath the octylcyanoacrylate adhesive. Some cells have ruptured, others have separated from Bruch's membrane, and others are vacuolated (H and E; Magnification x 450).
Figure 3.50 Light micrographs of Experimental Group A animal 7 days after application of a patch using PVdF and octylcyanoacrylate

A Section passes through a retinal break which developed adjacent to the patch. An area of focal, preretinal ERMs (E) has produced localised folds (H and E; Magnification x 75). B Atrophic retina adjacent to the retinal patch. (H and E, Magnification x 225)
Figure 3.51 Light micrograph of Experimental Group A animal 14 days after application of a patch using PVdF and octylcyanoacrylate.

The section passes through the area where the retinal patch was applied. The retina in the vicinity of the patch is disorganised (H and E; Magnification x 100).
Figure 3.52 Light micrograph of Experimental Group A animal 14 days after application of a patch using PVdF and octylcyanoacrylate.

High power of retina at the edge of the patch. Pigment laden foam cells can be seen (FC) (H and E; Magnification x 400).
Figure 3.53 Light micrograph of Experimental Group A rabbit 14 days after application of a patch using PVdF and octylcyanoacrylate.

Choroid and RPE beneath the patch. The RPE monolayer is attenuated, and pigment laden macrophages are visible in the subretinal space (M). (H and E; Magnification x 400).
Figure 3.54 Light micrograph of Experimental Group A rabbit 28 days after application of a patch using PVdF and octylcyanoacrylate.

This rabbit developed a total, funnelled detachment (H and E; Magnification x 5).
Figure 3.55 Light micrograph of Experimental Group A rabbit 28 days after application of a patch using PVdF and octylcyanoacrylate.

Fine subretinal membranes have resulted in retinal folds (H and E; Magnification x 450).
Figure 3.56 Scanning electron micrograph of a retinal patch in an Experimental Group A rabbit 28 days after application (Mag x 40)

The PVdF substrate (S) was applied to the retina with octylcyanoacrylate (O). The patch has elevated from the retina in places, due to artefacts during processing.
Figure 3.57 Scanning electron micrograph of the surface of the octylcyanoacrylate adhesive used to secure a patch in an Experimental Group A rabbit - 28 days after application (Mag x 350)

The octylcyanoacrylate adhesive (O) has a convoluted appearance and is still adherent to the surface of the retina
Figure 3.58 Scanning electron micrograph of the retinal surface of an Experimental Group A rabbit 28 days after application of a patch using octylcyanoacrylate (Mag x 1K)

Small, rounded cells are present on the surface of the ILL. The ILL exhibits elevations and radiating folds produced by retinal glial cells.
Figure 3.59 Scanning electron micrographs of the surface of a retinal patch in an Experimental Group A rabbit 28 days after application

A (Mag x 2.7K) B Red blood cells (R), macrophages (M) and oedematous photoreceptor outer segments (OS) on the surface of the PVdF substrate (Mag x 3.7K)
Figure 3.60 Light micrograph of thick sections retina of an Experimental Group A rabbit 28 days after application of a patch using PVdF and octylcyanoacrylate.

A highly cellular ERM (E) is visible, growing on the internal limiting lamina of the retina (ILL) (Toluine blue, Magnification x 900).

Figure 3.61 Light micrograph of thick sections the RPE beneath the area of a retinal patch of an Experimental Group A rabbit 28 days after application.

The retinal pigment epithelium demonstrates "scalloping", and RPE cells separated from the monolayer and can be seen in the subretinal space. (Toluine blue; Magnification x 900).
3.17.6 Experimental Group B:

**Light microscopy at 2 days:**
One eye was processed (EXPB2). In this animal the retinal break was not completely repaired at the time of surgery. The section passed through the area of the retinal patch, but not the retinotomy. The section shows the Tisseel adhesive and the PVdF substrate, but the two appeared to have separated. The anterior and posterior chambers were full of feathery fibrin. Inflammatory cells, mainly PMNs, macrophages and plasma cells were found in the anterior chamber, principally in the drainage angle (Figure 3.62). Many more inflammatory cells were present in the inferior drainage angle than in the superior angle. Inflammatory cells, again, mainly macrophages, PMNs and pigment laden cells were present in the vitreous remnant, particularly inferiorly and in the vitreous remnant overlying the optic disc. Inflammatory cells were also seen on the ILL. A thin layer of Tisseel adhesive was present preretinally (Figure 3.63), extending up to the edge of the optic disc. The adhesive could also be seen in the subretinal space; presumably having passed through the retinotomy. The preretinal fibrin had a feathery appearance, particularly at the edges whereas the fibrin in the subretinal space appeared more dense. Very few inflammatory cells were seen in relation to the preretinal fibrin, which was in contrast to the subretinal clot. Here macrophages formed an almost confluent cell monolayer on the surface of the fibrin adhesive (Figure 3.64). Many of these cells were heavily pigmented, with an appearance similar to RPE cells. The inner surface of the substrate was covered by wispy fibrin and a monolayer of pigment laden macrophages. The retina in the vicinity of the patch as well as elsewhere was normal. The choroid underlying the patch was haemorrhagic. This eye demonstrated a much more vigorous inflammatory reaction than other eyes described so far. Pigment laden and non pigmented macrophages, plasma cells and PMNs were found in abundance in the inferior drainage angle, in the vitreous remnant and on the surface of the inferior retina.

**Light microscopy at 7 days:**
Three animals were sacrificed at this time point and tissue prepared for light microscopy (EXPB3,4,5). Two eyes developed dense vitreous haemorrhage during the first week after patching (EXPB4 and 5) and in the third eye PVD led to separation of the retinal patch and reattachment of the retina (EXPB3).

EXPB3. The anterior chamber of this animal contained a fine fibrin deposit, with minimal inflammatory reaction. The superior retina showed signs of retinal detachment i.e. loss of photoreceptor outer segments. Elsewhere the retina was normal. The section passed through the area where the patch had been applied
The fibrin clot had almost completely lysed - only a fine, feathery clot remained. The choroid and RPE were normal throughout the eye. Inflammatory cells, but not many in number, were found in the subretinal space superiorly, and in the vitreous remnant, and on the surface of the retina. The majority of these cells were pigment laden macrophages.

EXPB4. The histological findings in this animal did not differ significantly from the animal described above. The section passed through retina adjacent to the patch. Here the retina was atrophic, with loss of the outer retinal layers. Peripheral retina inferiorly showed signs of retinal detachment. Elsewhere the retina was normal. Macrophages, some pigment laden, were visible in the subretinal space beneath the area where the patch had been applied (Figure 3.66), otherwise there was minimal inflammatory reaction and the choroid and RPE appeared normal.

EXPB5. In this animal the anterior chamber contained a fine fibrin mesh, and the posterior chamber and subretinal space were full of haemorrhage and fibrin. A localised area of inferior retina was disorganised, which presumably was where the retinal patch had been applied. Elsewhere the retina appeared normal. The choroid appeared normal apart from a localised area where the choroid was densely packed with lymphocytes and plasma cells. Macrophages were found in abundance in the subretinal space, particularly under the area where the retinal patch had been applied. A few inflammatory cells, mainly plasma cells were present in the inferior drainage angle.

Light microscopy at 14 days:
Tissues from two animals were prepared for light microscopy at this time point. In one (EXPB6) patching had been undertaken using the 500 IU strength thrombin preparation. At 14 days the eye showed a vigorous inflammatory reaction, with obscuration of fundus details. In the other animal (EXPB7) vitreous haemorrhage obscured the view of the fundus.

EXPB6. The section passed through the area where the retinal patch had been applied (Figure 3.67). A large clot of the Tisseel fibrin adhesive was still present on the surface of the retina inferiorly. Unlike the fibrin adhesive in the animals described above the fibrin clot in this animal appeared dense and was surrounded and invaded by macrophages. The retina was totally detached, atrophic and folded, with loss of the outer retinal layers. These changes were not localised to the area of the fibrin adhesive but were found throughout the retina. The most striking finding in this globe was the intense inflammatory infiltrate, principally by plasma cells, which involved tissues of the anterior and posterior segments (Figure 3.68).
The drainage angle, iris, ciliary body and processes, choroid, vitreous remnant and the preretinal and subretinal space were all heavily infiltrated. Strands of condensed vitreous, infiltrated by inflammatory cells were visible, extending from the vitreous base posteriorly to the optic nerve head, and anteriorly to the anterior capsule.

EXPB7. In this animal the retina was totally detached, with fibrin in the preretinal and subretinal space. A large retinal break was present immediately above the disc. The section passed through the area where the patch had been applied, passing through a remnant of the fibrin adhesive. The PVdF substrate had separated from the surface of the retina and had settled in the most dependent part of the globe, in the subretinal space (Figure 3.69). The substrate was surrounded by cells, mainly macrophages, many of which appeared to have settled on the surface of the substrate. The retina beneath the fibrin adhesive was folded and disorganised. Elsewhere the retina showed signs of long standing and more recent retinal detachment. Cellular membranes extended from the vitreous base to the capsule remnant, to the optic nerve head and to peripheral retina. Again, these membranes seemed to be the result of condensation and contraction of strands of vitreous. This globe also showed intense inflammatory infiltrate, but not to the same degree as animal EXPB6. Sections were stained with MG/PY which confirmed that the majority of the inflammatory cells were plasma cells.

Light microscopy at 28 days:
Three globes were processed for light microscopy at this time point (EXPB 8,9 and 15). All had total retinal detachment with generalised ERMs.

EXPB8. In this eye the retina was markedly atrophic, with large retinal holes. The retina was thrown up into fine folds by thin, subretinal membranes. Condensations of vitreous extended from the vitreous base to the capsule remnant and appeared to have become incorporated in anterior epithelial cells which had proliferated on the capsular remnant (Figure 3.70). The choroid and subretinal space were heavily infiltrated by inflammatory cells (principally lymphocytes and plasma cells), and giant cells were also present on the outer retinal surface and in the subretinal space (Figure 3.69). Retinal pigment epithelial cells were also observed in the subretinal space and heavily pigmented cells were also found on the vitreous condensations.

EXPB9. In this eye had a total, funnelled RD. A retinal break was present in inferior retina, adjacent to the optic nerve head. Inferior retina was atrophic, with loss of the outer nuclear layer. As in the previous eye the retina was folded by thin subretinal
membranes. Peripheral, inferior retina had been pulled anteriorly by vitreous condensations which extended from the vitreous base to the anterior capsule, to the optic nerve head. In this eye the inflammatory infiltrate, which was less intense than in EXPB8, was found principally in the inferior choroid. Again, RPE cells had migrated into the subretinal space. Giant cells were not observed.

EXPB15. The findings in this eye were similar to those in EXPB9. Staining with MG/PY confirmed that the choroidal infiltrate was with a mixed population of inflammatory cells, some of which were plasma cells. Peripheral RPE, and two specimens of retina were processed from another animal for TEM (EXPB11) and thick sections examined. This animal had RD at the time of sacrifice, and generalised ERMs were present. RPE cells were "scalloped", and some had migrated into the subretinal space. The retina showed features typical of long-standing RD i.e. loss of photoreceptor outer segments. In one specimen of retina preretinal membranes had developed, producing contracture of the ILL (Figure 3.71). The preretinal membranes were highly cellular, and many of the cells had pigment granules in their cytoplasm. Other cells had a spindloid morphology, with the appearance of fibroblasts.

Electron microscopy at 28 days:
One globe was processed for SEM (EXPB 10). In this animal the retina was detached at the time of sacrifice, and generalised ERMs were present. SEM of this globe showed the PVdF substrate lying on the retina inferiorly, having separated from the retina. Few details of the retinal surface or of the surface of the PVdF substrate were visible as all structures were covered by a thin layer of fibrin.

3.17.7 Experimental Group C:
The eye of one animal, sacrificed 7 days after patching using autologous fibrin, was processed. A fine meshwork of fibrin was present in the anterior and posterior segments, and subretinal space. The fibrin adhesive could not be discerned. Inferior and superior retina were thrown up into large, full thickness folds. Peripheral inferior retina appeared normal, whereas in the posterior pole the retina was markedly oedematous. Superior retina was atrophic and showed signs of RD. The RPE appeared normal, and there was very little inflammatory response apart from a few macrophages invading the subretinal space.
Figure 3.62 Light micrograph of the drainage angle of an Experimental Group B rabbit, 2 days after application of a patch using Tisseel as the adhesive.

Inflammatory cells are present in abundance in the drainage angle (D, Descemets membrane) (H and E, Magnification x 225).
Figure 3.63 (see previous page) Light micrograph of retinal patch in an Experimental Group B rabbit, 2 days after application of a patch using Tisseel as the adhesive (H and E, Magnification x 100)

Very little Tisseel adhesive is seen in the preretinal space and it has a feathery appearance. The adhesive (T) has spread into the subretinal space. The retina is normal.
Figure 3.64 Light micrograph of the retina in the vicinity of a patch applied using Tisseel in an Experimental Group B rabbit, 2 days after application.

The Tisseel adhesive (T) has spread into the subretinal space through the retinotomy. Macrophages and cells with heavily pigmented cytoplasm are present on the surface of the Tisseel (H and E, Magnification x 400).
Figure 3.65  Light micrograph of a section through the Tisseel adhesive in an Experimental Group B rabbit, 7 days after application of the patch.

The Tisseel adhesive (T) has a fine, feathery appearance. The retina and underlying RPE are normal (H and E, Magnification x 27).
Figure 3.66 Light micrograph of the RPE beneath a retinal patch applied using Tisseel in an Experimental Group B rabbit, 7 days after application of the patch.

Abundant macrophages are present in the subretinal space. The RPE appears healthy but some cells are rounded up in this region (H and E, Magnification x 790).
Figure 3.67 Light micrographs of the Tisseel adhesive (500 IU strength) in an Experimental Group B rabbit, 14 days after application of the patch.

A A large clot of Tisseel adhesive is present on the surface of the retina (T). The choroid (C) is heavily infiltrated by inflammatory cells (H and E, Magnification x 36).
B Abundant macrophages are visible on the surface of the Tisseel adhesive (H and E, Magnification x 225).
Figure 3.68 Light micrographs of the drainage angle (A) and ciliary body (B) in an Experimental Group B rabbit, 14 days after application of a patch using Tisseel (500 IU strength).

A The drainage angle and anterior chamber are heavily infiltrated by inflammatory cells, rich in plasma cells (H and E, Magnification x 225). B The ciliary body and peripheral vitreous also show an intense inflammatory reaction (H and E, Magnification x 180)
Figure 3.69 Light micrographs of the choroid and PVdF substrate (A) and peripheral retina (B) of an Experimental Group B rabbit, 14 after application of a retinal patch using Tisseel.

A The choroid is heavily infiltrated by inflammatory cells, which have also settled on the PVdF substrate (S) (H and E, Magnification x 90). B Vitreous membranes, formed by condensation of strands of residual vitreous, producing vitreoretinal traction (H and E, Magnification x 90).
Figure 3.70 Light micrographs of peripheral retina (A) and choroid and subretinal space (B) in an Experimental Group B rabbit, 28 days after application of a patch using Tisseel.

A Vitreous condensations extending from the vitreous base to the anterior capsular remnant (AC). These membranes have produced vitreoretinal traction (H and E, Magnification x 36). B The choroid is infiltrated by inflammatory cells, and a giant cell is visible in the subretinal space (H and E, Magnification x 360).
Figure 3.71 Light micrographs of thick sections of the retina of an Experimental Group B rabbit 28 days after application of a patch using Tisseel.

A The retina show signs of long-standing detachment i.e. loss of photoreceptor outer segments (arrow). Preretinal epiretinal membranes are present (E)(Toluine blue, Magnification x 72). B The preretinal membranes (E) are highly cellular. Many cells have pigment laden cytoplasm (Toluine blue, Magnification x 450).
The discussion has been divided into several parts. In the first instance the findings of the in vitro investigations are discussed and the reasons given why certain materials were selected for use in the animal model.

Retinal patching in the animal model is then discussed, including the technical and practical problems associated with the technique, and the complications encountered. The clinical outcome in the Control Group eyes and Experimental Groups are discussed, together with the histological findings.

Finally, in the light of the findings of this study, it is considered whether retinal patching has a part to play in the management of selected RDs, and possibilities for future developments of the technique are also raised.

A) IN VITRO EVALUATION OF ADHESIVES

4.1 BOVINE EYE CUP EXPERIMENTS:

The initial experiments, undertaken using bovine eye cups, demonstrated that only a few of the adhesives available adhered to bovine retina and that none adhered to the retinal surface of eye cups filled with media. The latter finding meant that the adhesives chosen for evaluation in an animal model had to be delivered in an air filled eye; a disadvantage that will be discussed later.

All three N-butylcyanoacrylate compounds (Histoacryl, avacryl and nexacryl) and octylcyanoacrylate adhered to dried bovine retina indicating that the anionic sites present on the surface of the ILL were able to initiate polymerisation. The addition of lophendylate did not inhibit this process. Histoacryl was chosen for further investigation rather than the other N-butylcyanoacrylates because it is readily available, its coloration makes it easy to visualise and it is currently used in the management of other ophthalmic conditions i.e. for repairing corneal perforations.

The only fibrin preparation that consistently adhered to bovine retina was the Tisseel preparation; the others formed clots that did not have adhesive qualities. When fibrinogen is converted to fibrin by thrombin soluble, monomeric fibrin is formed. In the presence of activated Factor XIII fibrin fibrils cross link to form insoluble fibrin, which stabilises the clot. The balance of components in the Tisseel
preparation have presumably been manipulated so as to produce a clot which has adhesive qualities, a feature not found with the other fibrin preparations.

One of the mussel proteins also adhered to bovine retina, but unfortunately insufficient volumes were available for further evaluation either in vitro or in vivo.

4.2 TISSUE CULTURE AND ORGAN CULTURE TOXICITY STUDIES:

The overall findings of the tissue culture experiments indicate that the cyanoacrylate group of adhesives are toxic to eye derived cells, whether the cells are grown on plastic or on glass. No toxicity was seen with the Tisseel fibrin preparation.

The organ culture studies also showed that the cyanoacrylates are toxic to ocular tissues.

4.2.1 Cyanoacrylates:

In the first experiment Histoacryl was compared with octlycyanoacrylate. The findings of this experiment confirmed those of other authors, showing that the higher cyanoacrylate analogues are less toxic than lower analogues(163,217). Our study also showed that cells surrounding Histoacryl continued to die for several days after the adhesive had been added, suggesting that toxic components continued to be released after polymerisation had taken place. Glial cells were found to be more susceptible than RPE cells.

There are several explanations for the cytotoxicity observed with the cyanoacrylate group of adhesives. Firstly, heat is generated during polymerisation and this is related to the rate of polymerisation, which occurs more rapidly in the lower analogues(160). This may be one factor accounting for the greater toxicity seen with Histoacryl and may explain the zone of "fixed" cells surrounding this preparation.

In the study by Nesburn it was found that toxicity was reduced if the cell monolayers to which the cyanoacrylates had been added were rinsed several times before refeeding and incubating(163). This suggests that soluble, toxic compounds are released during polymerisation, or that soluble, toxic impurities are present. However, the study by DeRenzis demonstrated that polymerised cyanoacrylates are also toxic to cells in tissue culture. He added discs containing unpolymerised and polymerised cyanoacrylates to cell monolayers and found no appreciable
difference in toxicity(217). This adds weight to the argument that toxic compounds are released after polymerisation is complete. Cyanoacrylates are degraded by a process of hydrolysis, releasing formaldehyde and cyanoacetates. Again, this process has been shown to occur more readily in the lower analogues both in vitro(162) and in vivo (218). This may account for the greater and delayed toxicity seen in our study with Histoacryl.

Over a period of time healthy cells from the periphery of the flask invaded the cell free area surrounding the drops of adhesive. Cells were also observed migrating onto the surface of the octylcyanoacrylate adhesive. This suggests, that after a period of time, polymerised cyanoacrylates no longer release toxins, or if they do, they diffuse into the media and do not reach concentrations high enough to inhibit cell migration or proliferation. Diffusion of toxic products would not readily occur if the adhesive were used within tissues, but would if they were used in a fluid filled medium, such as the posterior segment of the eye after vitrectomy. As far as use in detachment surgery is concerned therefore, toxicity related to polymerisation is probably the major factor to consider. This was not taken into account by authors who evaluated the effects of different adhesives on neurite outgrowth from embryonic retina in organ culture (219). In these experiments portions of embryonic retina were placed on top of a drop of Histoacryl that had polymerised 18 hours earlier. The portions of retina were bathed in Matrogel and placed on top of the Histoacryl. The retinal tissue did not therefore come into direct contact with the adhesive. Even so, the authors found that Histoacryl inhibited neurite outgrowth when compared to controls specimens that were not placed on Histoacryl.

In the second experiment the effect of adding the retarding agent lophendylate was investigated as it was anticipated that delivery of a cyanoacrylate adhesive in the animal model would be easier if polymerisation could be slowed. Here the results were unexpected, as the addition of lophendylate increased the toxic effects of octylcyanoacrylate when added to glia grown on plastic, although it was not toxic itself. It was anticipated that the reverse would be true as, owing to dilution, only 1 μL of octylcyanoacrylate was used (whereas 2 μL applications were used previously). The third experiment threw some light on this. In this experiment glia were grown on glass and the addition of lophendylate made no significant difference to the area of cell death when mixed with octylcyanoacrylate. This suggests that a chemical interaction between the cyanoacrylate/lophendylate mixture and the plastic of the tissue culture flasks was taking place in the earlier experiment. This study also confirmed the greater toxicity of Histoacryl compared to octylcyanoacrylate and demonstrated the anticipated reduction of toxicity when lophendylate was mixed with Histoacryl. It also demonstrates that the toxicity
observed in the earlier experiments could not have been due to a chemical interaction between Histoacryl or octylcyanoacrylate and the plastic of tissue culture flasks.

Despite evidence of toxicity octylcyanoacrylate was chosen for evaluation in the animal model of retinal patching because of its lower toxicity in tissue culture compared to Histoacryl. The limited organ culture studies also confirmed that the cyanoacrylate adhesives are toxic to ocular tissues, causing full thickness necrosis in bovine retina and inducing morphological changes in RPE cells adjacent to the adhesive. These changes were observed within a few hours of application of the adhesive, suggesting again that toxicity is, in part at least, related to polymerisation. Attempts to quantify tissue toxicity in limited organ culture were unfortunately unsuccessful.

4.2.2 Tisseel:

When fibrin was added to cells in tissue culture no toxicity was observed; indeed glial cells readily grew onto and invaded the clot. When Tisseel was added to the media overlying RPE cells the cells again readily invaded the clot, growing along the fibrin fibrils, taking on a spindloid morphology.

Fibrin plays a vital, initial role in the wound healing process, by acting as a scaffold for the cells involved in tissue repair, such as inflammatory cells and fibroblasts. Fibrin and fibrin degradation peptides have been shown to be chemoattractant for leucocytes, PMNs, and monocytes (220) and are also chemoattractant for fibroblasts (221). An in vitro study by Vidaurri-Leal showed that when human RPE cells were overlaid with a fibrin clot they assumed a fibroblastic, spindloid morphology and invaded the clot(222). This finding was confirmed in our study using bovine RPE. Our studies were not designed to investigate whether fibrin is chemoattractant for RPE and glial cells, but they do demonstrate that fibrin is an attractive substrate for these cell types.

4.3 SELECTION OF ADHESIVES FOR IN VIVO EVALUATION:

Two adhesives were selected for evaluation in the animal model as we wished to compare synthetic and biological materials from the technical, clinical and biological points of view. Octylcyanoacrylate was selected from the other cyanoacrylates on the grounds of lower toxicity. Octylcyanoacrylate was used alone, and not mixed with lophendylate, for two reasons. Firstly, the in vitro experiments undertaken using glia grown on glass suggested that the addition of
lophendylate to octylcyanoacrylate does not significantly reduce its toxicity and, secondly, adding another variable would have meant having more experimental groups, with even smaller numbers in each group. Tisseel was used as it was the only biological adhesive that consistently adhered to bovine retina as well as being non toxic to eye derived cells.

N-Butylcyanoacrylate has been used for retinopexy in both experimental animals and humans, but octylcyanoacrylate has not previously been evaluated. Several different fibrin preparations have been used clinically and experimentally and in most instances the adhesive was injected into the subretinal space. Under these circumstances the clot would be in a confined space; we wished to evaluate fibrin in a different setting i.e. by delivering it onto the retinal surface knowing, however, from other authors that the clot was likely to lyse. We wished to evaluate different delivery systems, whether the clot would remain adherent to the retinal surface, how long it would take to lyse at this site, and what effect fibrin degradation products might have on the inflammatory response and the development of epiretinal membranes.

Unfortunately both the chosen adhesives had to be delivered at an air-tissue interface, which in the animal model, meant application after fluid/air exchange.

B) IN VITRO EVALUATION OF SUBSTRATES

4.4 BOVINE EYE CUP EXPERIMENTS:

A wide range of biological and synthetic materials were available at the beginning of the study for evaluation as patching substrates. The first experiment eliminated those materials that could not be introduced through a standard 20G sclerotomy using intraocular forceps, leaving 10 membranes for biocompatibility testing. Other materials could have been introduced if the sclerotomy had been enlarged, but there were several reasons why this was not done; firstly, in a clinical setting this would give rise to the loss of an air-tight or water-tight seal, making control of intraocular pressure difficult; secondly, haemorrhage might result and thirdly, enlarging the sclerotomy would encourage incarceration of basal vitreous gel. When the latter occurs there is an increased risk of sclerotomy related retinal tears, and incarcerated vitreous can act as a scaffold for fibrovascular ingrowth. This can result in anterior loop traction and late vitreous haemorrhage.
4.5 TISSUE CULTURE BIOCOMPATIBILITY STUDIES:

The biocompatibility studies involved the investigation of cell settlement on the synthetic membranes, and cell growth on the biological membranes. Some of the synthetic materials, such as PVdF, readily supported cell settlement whereas others did not. Glial cell settlement rates were higher than RPE settlement rates. Whether a cell in tissue culture will settle on, and attach, to a substrate is determined by many factors including the type of cell used (i.e. whether it is attachment dependent or not), the composition of the media and the nature of the substrate. Some cells, such as Hela cells, are not substrate dependent and grow in suspension in tissue culture, but both glia and RPE are attachment dependent cells. The initial attachment of cells to a substrate is thought, in part, to be mediated biochemically, with fibronectin linking cell membrane receptors to molecules on the surface of the substrate(223). A 140 kDa surface glycoprotein has been identified as a major membrane receptor for fibronectin in mammalian cells in tissue culture and it is thought that this receptor may act as a transmembrane link between extracellular fibronectin and cytoskeletal components in the cell cytoplasm (224). Fibronectin adsorbed onto the surface of the substrate would therefore anchor the cell to the surface. More permanent, cytoskeletonally mediated attachment plaques develop later(225). The nature of the substrate is also important; cell membranes have a weak net negative charge and cells will not settle and grow on negatively charged surfaces, nor on hydrophobic materials. In our study the cells were initially added to 1 ml of NCS overlying the all test substrates before feeding with media. NCS not only has the effect of neutralising typsin, but is also a source of fibronectin. The conclusions that can be drawn from our study, therefore, are that the surface of some but not all of the substrates were unsuitable for cell settlement presumably because they were hydrophobic, had the wrong charge or because fibronectin was not adsorbed onto the surface of the substrate.

All the biological membranes investigated supported the growth of bovine RPE cells. These findings were anticipated, as bovine anterior lens capsule and bovine Descemets membrane are modified basement membranes for lens epithelial cells and corneal endothelial cells respectively. Chick allantoic membrane is composed of eululin, a form of elastin fibre, and it was anticipated that cells would also grow on this matrix.

Considerable morphological variation was observed depending upon which substrate the RPE cells were growing. The morphology and behavioural characteristics of cells in tissue culture are known to be influenced by the nature of
the extracellular matrix or substrate on which they are seeded, although the mechanisms responsible are not fully understood. In some way the number, nature, site and type of attachments to the extracellular matrix influences intracellular mechanisms, possibly via cell membrane receptor linkage with cytoplasmic cytoskeletal elements. This can result in morphological changes, alterations in growth and locomotary characteristics and modification of tertiary cell function.

4.6 SELECTION OF SUBSTRATES FOR IN VIVO EVALUATION:

Two substrates were selected for evaluation in the animal model of retinal patching; PVdF and propylene. None of the biological membranes were selected, mainly on the grounds of practicality as they are difficult to prepare, store and sterilise. PVdF and propylene were selected from the other synthetic materials as both could be introduced through a standard 20G sclerotomy and they were at either end of the spectrum with regard to biocompatibility. A biocompatible substrate, PVdF, was selected to determine whether a retinal patch made from this material would support a localised wound healing process and the other membrane, propylene, was selected for comparison. Both materials are commercially available and are easy to sterilise and store. When placed on the retinal surface of an air filled eye both membranes were very difficult to see because they are transparent. It might be possible to include coloration in the manufacture of the materials so as to avoid using a felt pen, as was necessary in this study.

C) IN VITRO EVALUATION OF RETINAL PATCHING

4.7 BOVINE EYE CUP EXPERIMENTS:

The findings of these experiments showed that all the substrates that could be introduced into the posterior segment of bovine eyes remained adherent to bovine retina using the "successful" adhesives, suggesting that any combination would remain adherent in vivo.

From a practical point of view evaluating the method of delivery of the patching materials was useful. Placing the substrate over the break to be repaired and then delivering the adhesive to the edges of the substrate was found to be the easiest and most controlled way of applying the patch. A better seal was achieved using this rather than the other methods of application, and the adhesive was less likely to spread through the retinal break. In the animal model the same procedure was adopted.
D) IN VIVO EVALUATION OF RETINAL PATCHING

4.8 CHOICE OF ANIMAL:

The aims of the in vivo investigations were to assess the technical and practical aspects of retinal patching, to monitor operative complications and to evaluate the outcome both clinically and histologically. Retinal patching has not been investigated previously by other authors in rabbits or other species, although certain procedures used in developing the model have, such as gas compression vitrectomy.

The major factors that determined the choice of animal were practical and financial. The rabbit eye differs from the human eye anatomically and physiologically, particularly with respect to its vigorous response to injury and tendency to haemorrhage(226). The rabbit eye is, however, large enough to allow surgical procedures to be performed. The rabbit has also been used extensively in PVR research(86-7,109-112,209,227-8), and has been used as a model of retinal detachment(229-231). This has allowed the findings of this study to be compared with those of other authors.

Although not prominent or mobile the rabbit eye can be prolapsed without damage, which makes access to the posterior segment possible. Rabbits are docile, relatively easy to anaesthetise, and are easy to handle and examine, which reduces the possibility of ocular injury.

It was anticipated that several animals would be used in developing the model and, because of the nature of the study, several different experimental groups were required. Rabbits are cheaper than many other animals with similar sized globes and are less expensive to maintain. The cheaper price meant that greater numbers could be used, which allowed statistical analysis of clinical data as well as histological study at different time points.

4.9 NUMBER OF ANIMALS USED:

Because the rabbit eye is anatomically and physiologically different from the human eye the surgical sequence and techniques used had to be modified in order to prepare the eye for retinal patching, as described in Chapter 2. In the human eye vitrectomy (without lensectomy) and application of a retinal patch could, in theory, all be performed during the same operation. In this study using rabbits, three operations were required, each under general aesthetic and each taking between
half an hour and two hours to perform. In addition at least two weeks had to elapse between the first three procedures and there was no technical support or surgical assistant. These factors had a strong bearing on the numbers of animals that could be operated on, limiting the number of animals in each group. This has influenced the choice of statistical analysis of data, which will be discussed where relevant.

4.10 TIMING OF CLINICAL EXAMINATIONS:

Decisions regarding the grading system, timing of observations and the maximum length of follow up were made whilst developing the animal model. The grading system used for anterior and posterior segment inflammation was a modification of that used by other investigators (209), whilst that used for posterior segment changes evolved with experience. Following vitreolensectomy several animals developed focal ERMs in flat retina, usually confined to the medullary rays. These were graded as mild or moderate. Other animals developed generalised ERMs, with transvitreal and preretinal membranes and these too were graded as mild or moderate. The distinction between mild and moderate were made by clinical judgement and it could be argued that the distinctions were arbitrary. However, all observations were made by the same individual and are therefore consistent. The extent of RD was graded so that localised detachments could be distinguished from more extensive detachments.

Observations were made on days 1-7, 4, 21 and 28. Early observations were made to record changes occurring during the postoperative period, and later observations to record the consequences of those changes. 28 days was selected for the maximum period of follow up, again based on experience. During this period of follow up many eyes developed RD and epiretinal membrane formation. Analysis of data at different time points allowed comparison between the different study groups.

4.11 THE ANIMAL MODEL:

Development of the animal model, including steps taken in overcoming some of the difficulties, has already been described (Chapter 2.13). This model proved satisfactory in that cryotherapy induced good chorioretinal adhesion and gas compression vitrectomy caused either pre-operative PVD or aided per-operative PVD. After vitreo-lensectomy patching materials could be delivered onto the retinal surface of an air filled eye. However, some problems that are particular to the rabbit remained.
The problem of peri and postoperative fibrin formation was circumvented by using low molecular weight heparin in the infusion fluid. This compound has a better antithrombotic / bleeding ratio than standard heparin, and in vitro has been found to reduce contraction of fibroblasts and proliferation of RPE cells(232). Use of low molecular weight heparin in this study may therefore have increased the rate of perioperative vitreous haemorrhage and reduced the incidence of postoperative epiretinal membrane formation. It was, however, used for every animal in each study group.

Passing instruments through cryotherapy treated peripheral retina avoided RD formation in most animals, but undetected sclerotomy related breaks or vitreoretinal traction were probably the cause of the unexpected RDs in Control Groups eyes (5%) and may have contributed to the higher incidence in the Experimental Groups, particularly when the detachment did not appear to arise secondary to the repaired retinotomy. The rabbit eye is not very mobile and detailed examination of the retinal periphery is difficult. In a study by van der Zee using rabbits mechanical vitrectomy (without prior gas compression vitrectomy) was complicated by rhegmatogenous RD in 10% of 170 animals and traction RD secondary to fibrovascular ingrowth in 16% of the remainder(233). The authors comment that the anterior location of their sclerotomies may have contributed to these findings.

The rabbit retina is very thin and tears much more readily than human retina. Inadvertent tears were created in 6 animals while removing preretinal haemorrhage with the flute needle, and while manipulating patching materials.

It is not possible to remove the vitreous where it is adherent over the medullary rays and residual vitreous probably acted as a scaffold for the focal ERMs observed at these sites.

In the rabbit it proved necessary to leave the anterior capsule intact so that fluid/air exchange would not cause pupillary miosis and anterior epithelial cells were removed as extensively as possible to prevent capsule opacification. Slight wrinkling of the capsule and reflections off the surface of the air bubble sometimes made visualisation of the posterior pole difficult.

Visualisation of the posterior segment was particularly difficult in albino rabbits' eyes because of reflections from the choroid, but much easier in Dutch rabbits. A change of species was made half way through the study. Statistical analysis of data comparing the two rabbit types with respect to retinal hole formation, development of RD, and the development of focal or generalised epiretinal membrane formation.
showed little difference between the two species. The development of focal epiretinal membrane formation was higher however in albino rabbits compared to Dutch rabbits in Control Group 2 eyes and Control Group 3 eyes. This is not easy to explain, but may have been due to a surgical "learning curve effect", as albino rabbits were operated on earlier in the study than Dutch rabbits.

Inadvertent breaks made in the anterior lens capsule and zonular rupture usually led to intense pupillary miosis with loss of visualisation of the posterior pole. The view was usually good enough, however, to perform procedures necessary for Control Group eyes. If, at the outset of the operation the intention had been to patch, and the view became inadequate, the procedure was restricted to that of one of the control groups. These complications occurred in 9 rabbits and explains the significantly higher incidence of anterior segment complications in Control Group eyes (Table 3.11).

4.12 SURGICAL PROCEDURES AND TECHNIQUES:

4.12.1 Observations following Cryotherapy:

Histological examination of cryotherapy sites confirmed the clinical finding of chorioretinal adhesion.

4.12.2 Observations following Gas Compression Vitrectomy:

This technique proved an effective and relatively safe method of inducing PVD, as has been found by other authors(234). No eyes developed shallowing of the anterior chamber or raised intraocular pressure on the day of maximum gas expansion. Histological examination of eyes showed no evidence of retinal damage despite the short period of acutely elevated intraocular pressure immediately after gas injection. Two eyes did however, develop RD two weeks following the procedure, but in both instances vitreous was lost at the time of gas injection.

4.13 OBSERVATIONS IN CONTROL GROUPS EYES

The rationale for having Control Group 1, where localised RDs were created, was to document the natural history of the detachment, with a view to finding out whether retinal patching promoted retinal reattachment. However, we were surprised to find that the area of RD spontaneously reattached, and that this took place too quickly to provide a statistical window. In non-vitrectomised rabbit eyes Foulds found that RDs produced by injecting hyaluronidase into the vitreous
overlying a retinal break resulted in RD that also spontaneously reattached. The only way this author found to produce RDs that persisted was to mechanically disrupt the vitreous overlying the retinal break in addition to injection of hyaluronidase. Foulds postulated that in the first model of RD formed vitreous plugged the retinal break, preventing recruitment of further subretinal fluid. In the second model he argued that disruption of the vitreous by mechanical and biochemical action prevented the retinal break from becoming plugged and so the RD persisted (230). Ohkuma was also able to produce long lasting RDs in the rabbit eye by aspiration of vitreous alone, but many of these eyes developed secondary retinal breaks (229). In our model of RD in vitrectomised eyes it is difficult to explain why the detachments spontaneously reattached, but one possible explanation is that post-operative fibrin may have plugged the retinal break. Low molecular weight heparin was used in the infusion fluid in all eyes and this prevented the formation of per-operative fibrin. However, it may not have persisted long enough in the posterior segment to prevent fibrin formation during the postoperative period.

In Control Group 1 eyes the RPE underneath the area of RD was hypertrophic. This clinical finding was confirmed histologically and is a phenomena that has previously been noted models of RD in the rabbit (235).

In Control Groups 1 and 3 a localised wound healing response was seen at the edge of the retinal break, with localised proliferation of glial cells causing folding of the edge of the break. This glial response has been reported by other authors (230, 236-7). There was also evidence of localised damage to the underlying RPE.

In all Control Group eyes very little inflammation was observed clinically, and few inflammatory cells were seen in histological specimens.

Eyes developing RD had changes in the retina and RPE that have been described by other authors i.e. loss of photoreceptor OS and scalloping and migration of RPE cells into the subretinal space (231).

4.14 OBSERVATIONS FOLLOWING RETINAL PATCHING USING OCTYLCYANOACRYLATE

One of the major problems encountered after applying patches using octylcyanocrylate was the development of new breaks at the edge of the patch during air/fluid exchange. This probably occurred because after polymerisation cyanoacrylates become solid and any movement of the patch induced by fluid entering the eye would result in shearing forces at the edge of the adhesive. If the air/fluid
exchange could be done in a slow and controlled manner this complication would be minimised.

Coagulative necrosis of the retina in the immediate vicinity of the octyl-cyanoacrylate adhesive was a notable feature in all eyes and often led to the development of additional retinal breaks. These findings have been reported by McCuen who commented that the rabbit retina may be more sensitive to the toxic effects of cyanoacrylates than the primate retina(174). He also noted that the changes were dose dependent and commented that they may also be caused by impurities in the adhesive(173). McCuen also reported changes in the RPE underling the area of adhesive (175). The formation of tears at the edge of the patch could be prevented by performing air/fluid exchange in a controlled manner, and would be less likely in human eyes which have greater scleral ridigity. The degree and area of coagulative necrosis could also be reduced if purified adhesive of medical grade were used, and small applications made.

Only two eyes patched using octylcyanoacrylate and followed for four weeks did not develop RD, and in one eye the retina reattached after the patch separated. None of these eyes developed ERMs. By the end of the study period generalised ERMs had developed in three of the four eyes with RD. Retinal detachment preceded the development of generalised ERMs in all eyes where these formed, irrespective of the length of follow up. Histologically ERMs have many features in common with healing wounds in other tissues; they contain macrophages, fibroblast-like cells and an extracellular matrix comprised of collagens and fibronectin. The sequence of events in eyes patched using octylcyanoacrylate would seem to suggest that ERM formation occurred as a result of RD rather than as a consequence of the octylcyanoacrylate itself.

4.15 OBSERVATIONS FOLLOWING RETINAL PATCHING USING TISSEEL

The findings in this Experimental Group confirmed those of other authors who have used fibrin in retinopexy. In all eyes the fibrin adhesive underwent fibrinolysis, and there was no evidence of tissue toxicity.

The most notable feature is this Experimental Group however, was the histological evidence of an intense inflammatory response, characterised by early infiltration of the anterior and posterior segments by macrophages and PMNs, with later recruitment of plasma cells and lymphocytes. ERM formation was also a notable feature in this Experimental Group. The sequence of events in these animals differed from Experimental Group A animals, as ERMs often developed before RD,
suggesting that the fibrin adhesive (or breakdown products) had some part to play in the pathogenesis of the epiretinal membranes.

There is a body of evidence to suggest that inflammation plays an important role in the pathogenesis of ERMs and that the development of contractile membranes can be likened to the healing phase of the inflammatory response (139,209,238). Macrophages, which have been consistently reported in histological studies, are thought to play a major role, orchestrating events and modifying cellular behaviour via inflammatory mediators(239-41). In Experimental Group B animals the fibrin adhesive rapidly underwent fibrinolysis, presumably releasing fibrin degradation peptides, which are known to be chemoattractants for inflammatory cells. However, in this study clinical grading of signs of anterior segment inflammation did not show that eyes patched with Tisseel had more inflammation than those patched with octylcyanoacrylate and so there is only histological evidence to support the notion that Experimental Group B eyes had more ERMs because of an increased inflammatory reaction. In this study grading of posterior segment inflammatory signs was unsatisfactory, but if this could have been done in a reliable way, a difference between the Experimental Groups may have been demonstrated.

Immunogenic mechanisms may also have contributed to the intense inflammation seen in the Experimental Group B animals, as the Tisseel adhesive contains human and bovine components which would be immunogenic to the rabbit. Immunogenic responses may explain the intense plasma cell response seen in many animals.

It was not possible to prepare fibrin with adequate adhesive properties using autologous sources of fibrinogen and thrombin, but if this were possible the inflammatory response may be muted, and ERM formation consequently reduced. However, even autologous fibrin is not benign, as postoperative intraocular fibrin formation following vitrectomy carries a poor prognosis, with an increased incidence of pupil block glaucoma and PVR. Attempts have been made to induce fibrinolysis using intraocular tissue plasminogen activator (tPA) following vitrectomy, with encouraging results (242-3). However, the use of tPA is associated with an increased incidence of intraocular haemorrhage and it is only recommended for use if other anti-inflammatory agents fail.
4.16 SUMMARY

The findings of the animal work undertaken in this study show that there are still many problems to be overcome in translating what is a good idea (retinal patching) into a practical reality. The two main areas of difficulty relate to delivery of the materials to the area of retina to be repaired, and the lack of suitable patching materials. The major limiting factor at present is the lack of a non-toxic, permanent adhesive which is easy to deliver and which does not stimulate an inflammatory response. If a suitable adhesive were to become available many of the more technical problems probably could be solved. The rabbit is not an ideal animal model for posterior segment work, and this represents a barrier to future research. More suitable animal models, such as the pig or primates are very expensive which might limit further in vivo research into retinal patching.
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