<u>Title</u> : *"An Evaluation of Gas Permeable Contact Lens Polymers and the Effects on the Corneal Endothelium of Long Term PMMA Contact Lens Wear "*

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Submitted to the University of Glasgow for the award of the degree of PhD

Tennent Institute of Ophthalmology, December 1993.

ABSTRACT

Purpose: Long term hard contact lens wear may produce corneal changes, the most significant of which are those in the corneal endothelium. A proposed link with hypoxia being the main cause of these changes is fairly strong, and therefore the purpose of this thesis was twofold. To determine;

1. If prolonged hard contact lens wear has deleterious and potentially dangerous effects on the endothelium and

2. If the newer generation of oxygen permeable polymers demonstrated enhanced mechanical and physical properties to those of non-permeable poly-methyl-methacrylate.

Methods: A method of polarography was developed as an electro-chemical means of measuring the diffusion of oxygen through various contact lens materials (n=8). Apparatus was devised to measure stress/strain curves on RGP material samples (n=12) and relate their mechanical properties to oxygen permeability values. High oxygen permeable lenses were fitted to astigmatic corneas to determine the degree of lens flexure and the corresponding induced astigmatism. Contact specular microscopy of the corneal endothelium was carried out on long term PMMA lens wearers (n=57) and a group of normal controls (n=45). Morphometry, using image analysis of the photomicrographs, allowed calculation of the mean cell area, cell density, coefficient of variation in cell area, and the skewness and kurtosis of the cell population of both groups of subjects. Central corneal thickness was measured in both groups of subjects.

Results: Polarography was found to be a repeatable method of measuring oxygen permeability in a range of GP materials of varying oxygen permeability. All RGP materials tested, demonstrated significantly greater oxygen permeability than PMMA but less than that suggested by the manufacturers. The Young's Modulus of materials (n=7) varied significantly across the range tested and there was a significant correlation between oxygen permeability and flexibility (r=-.98). The flexing of high Dk lenses on astigmatic eyes was significant, but the degree was unpredictable. Specular microscopy revealed significantly greater polymegethism (t test, $p<0.05$) in the group of lens wearers (COV=0.4) than the control group (COV=0.28) and an increased skewness index, although no significant difference was found in the parameters of mean cell area, cell density, or central corneal thickness between the two groups of subjects.

Conclusions: This project has shown that it is possible to obtain reliable results of oxygen permeability and flexibility of materials, and that increases in lens thickness to offset lens flexure will significantly decrease the oxygen permeability. The results from specular microscopy of the corneal endothelium, obtained from a unique group of lens wearers (n=57) are important to our understanding of the effects of long term (>10 years) contact lens wear. Although no individual cornea showed evidence of decompensation, the widespread endothelial changes observed suggest that this might be a risk following intra-ocular surgery.

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Preface/Acknowledgement

I would like to acknowledge the following groups for their financial support of the overall study, without which it would not have been possible to complete the project.

Bausch and Lomb, London.
Scotlens, Linlithgow.
Visual Research Trust, Glasgow.
Greater Glasgow Health Board.

Also, to those contact lens practitioners who assisted by sending some of their patients with a complete contact lens history. This allowed a larger patient group to be obtained in the clinical part of the study.

Supervision.

I would especially like to thank **Professor Colin M Kirkness**, Head of Department of Ophthalmology, University of Glasgow for his advice, support and expertise over the last two and a half years of this project. The clinical staff in his department were also extremely helpful and in particular **Gordon Dutton and Bertil Damato** offered many helpful suggestions. I would also like to thank **Professor William R Lee,** for the numerous discussions on the morphology and morphometry of corneal endothelial cells and guidance on the thesis. Thanks are also due to **Professor Wallace Foulds** for giving me the initial opportunity to obtain research funding and facilities in the Tennent Institute of Ophthalmology.

All the specular microscopy photographic developing and printing was carried out by **Mr John McCormick** medical photographer, Western Infirmary and **Mrs Dorothy Aitken,** ocular pathology laboratory, Western Infirmary provided a great deal of information on the laboratory methods and investigations of the corneal endothelium.

In the early part of the study significant technical assistance was given by **Dr Ray Ansell** Dept. of Physical Sciences Glasgow Caledonian University (oxygen measurement) and **Mr Robin Gilmour** Dept. Vision Sciences, Glasgow Caledonian University (equipment design). Statistical help was provided by **Mr Angus McFadyen**, Dept. Mathematics, Glasgow Caledonian University.

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INTRODUCTION

Contact lenses offer vision correction in ametropia to millions of people throughout the world. On a more limited basis they are also used as therapeutic devices in a range of external ocular conditions. Contact lenses provide a better optical alternative to spectacles in certain clinical situations. For example in high myopia and aphakia, contact lenses are both optically and cosmetically preferred. In keratoconus or on occasions after corneal surgery, they may be the only means of providing good visual acuity by correcting irregular corneal astigmatism.

In 1990 it was estimated that there were approximately 35 million contact lens wearers world-wide, the largest market being the USA with an estimated number of wearers approaching 18 million (Bruce and Brennan 1990). In the United States 20-30% of lenses fitted are for extended wear. With recent developments in lens production and materials, the challenge for researchers and clinicians has been to maximise the combination of lens optics and design with the physiological requirements of the cornea to provide safe contact lens wear.

The interaction of a plastic lens with the tear film and the surrounding ocular structures means that it is a multi-disciplinary group effort to ensure that materials, lens care products, corneal metabolism and general ocular effects are fully understood. The most salient effect of lens wear on the cornea is the hypoxically induced reduction in the rate of metabolic activity of the corneal epithelium (Bruce and Brennan 1990) which in turn leads to morphological changes in the endothelium first observed by Schoessler (1981).

Recent reports have also suggested that rare but significant clinical problems associated with contact lens wear do exist and that some lens types are safer than others (Poggio et al. 1989, Dart 1988, MacRae et al. 1991, Heaven and Hutchinson 1993). These studies have concentrated on the incidence of corneal infection or ulceration in lens wearers determined by surveys of ophthalmologist referrals (Poggio et al. 1989) or by the proportion of contact lens related problems seen at a casualty eye department (Dart 1988, Heaven and Hutchinson 1993). A recent review of disease and risks associated with contact lenses has been published by Dart (1993).

The statistics quoted in the above studies however are sometimes difficult to relate to the general contact lens wearing population since limited data is provided, or is available, for the total population of contact lens wearers, the majority of whom are presumably successful wearers. It is clear however, that extended overnight wear of lenses does carry a higher risk of corneal infection (Weissman et al. 1984, Holden et al. 1985, MacRae et al. 1991) and with the current trend toward disposable soft lenses lending encouragement to long wearing times, careful consideration of the potential risks is required.

In most countries, disposable contact lenses have been advocated by their manufacturers to be worn on an extended wear basis. The usual regime suggested has been to wear lenses for either 6 or 13 consecutive days. The lenses are then removed on the last night and a fresh pair of lenses inserted the following day. It was expected that regular lens replacement in this manner would reduce the incidence of complications making overnight wear safer. In some respects this has proved to be the case as it has been shown that both the acute red eye response and the incidence of contact lens related giant papillary conjunctivitis have been reduced (Holden 1988, Grant et al. 1988, Hamburg et al. 1991, Abelson 1993).

Despite the advantages of disposability, recent evidence of potential complications with this modality of lens wear has emerged. Not surprisingly most recent studies have concluded that disposable lenses are best used on a daily basis and that the risk of corneal ulceration increases with the number of consecutive nights of lens wear. Assuming that there is a link between chronic hypoxia and corneal ulceration, the number of overnight periods of lens wear should be kept to a minimum. Disposable lenses still produce reduced levels of oxygen at the corneal surface and therefore they are likely to have hypoxic effects in each layer of the cornea (Holden 1988).

The purpose of this study was to consider some important basic properties of contact lenses and relate these to clinical findings, where possible from long term wear of lenses. Contact lens studies have often been conducted over short time spans and some conclusions relating to corneal effects are inevitably limited by this approach. Longer term studies are required to provide industry with the necessary feedback on the development of better contact lenses. The question of the safety of long term wear of contact lenses still needs to be answered and therefore, part of this study was designed to investigate chronic corneal changes occurring in a group of patients who had been wearing lenses for more than ten years.

PMMA corneal lenses were fitted in large numbers between 1950-1980. Although it is presumed that many patients have ceased lens wear there remains a substantial number of individuals who still wear these lenses. Most of these patients are in the 35-60 age band and form an important and interesting group since they provide the best available evidence to determine if long term contact lens wear, and hence chronic hypoxia, produces any harmful effects on the cornea and associated structures.

Although these subjects cannot act as their own control in an experimental situation (measurement before and after lens fitting), they can be compared to a healthy group of age matched controls to determine both clinically and statistically significant differences. Ideally, subjects wearing one lens only, would provide the best control and although such wearers exist, they are not in sufficient numbers for statistical analysis. Also the trend clinically seems to be, that for every new contact lens wearer there is one dropping out. The reason for this apparent trend towards self limitation in the overall duration of contact lens wear needs to be established.

The central theme of this thesis is the role of atmospheric oxygen in maintaining normal corneal physiology in contact lens wear. Important factors to be considered included (1) the development of new gas permeable hard lens materials; (2) the passage of oxygen through lens materials; (3) the effect of varying the thickness of lenses on oxygen transmissibility; (4) corneal thickness increase due to oedema with lens wear, and (5) the effects of chronic hypoxia on corneal endothelial cell morphology.

There has been a proliferation of new hard gas permeable materials as alternatives to PMMA over the last 15 years with tremendous commercial drive to obtain a claimed increase in oxygen permeability. This has been in response to the general awareness of the need to develop safe plastics allowing a sufficient concentration of oxygen to be maintained at the corneal surface. The laboratory studies will look at the ability of some of these newer plastics to transmit oxygen relative to PMMA and also consider other important properties linked to oxygen permeability.

The bulk of the clinical studies within the project will concentrate on the morphology of the corneal endothelium. As a monolayer of cells essential to the maintenance of normal corneal physiology, it remains a crucial question to determine the long term effects from chronic hypoxia due to contact lens wear on this layer.

The aims of this project were therefore:

1. To determine that, unlike PMMA, newer hard lens polymers transmit oxygen, and to develop a method of measuring oxygen permeability which was consistent and accurate. This will be dealt with in Chapter 3.2.

2. To determine if the flexibility of a polymer increased as the oxygen permeability increased. This will be dealt with Chapter 4.

3. To assess the 'in vivo' optical effects (induced and residual astigmatism) of high Dk hard gas permeable lens flexure. This will be dealt with in Chapter 5.

4. To determine the effects of long term PMMA hard lens wear on the morphology of the corneal endothelium. These effects would specifically relate to possible endothelial cell loss or marked variations in cell size and shape which may influence corneal function, and thus have implications for all contact lens wearers. This will be dealt with in Chapter 6.

Basic and clinical research must continue to develop better and safer contact lenses as their use becomes more widespread, and to educate users in appropriate wear and care of lenses. Ultimately, the daily disposable lens involving no care system would overcome many of the present problems with soft contact lenses and could well be a reality in the very near future.

SECTION 1

CONTACT LENS MATERIALS AND THEIR CLINICAL EFFECTS.

Chapter 1. Materials Review.

1.1 Poly-methyl-methacrylate

It is now over one hundred years since the first description in the scientific literature of the concept of a lens in contact with the eye. It was not until the 1950's and 60's however that plastic corneal contact lenses were fitted in larger numbers and for 20 years poly-methyl-methacrylate (PMMA) was the only contact lens material commercially available. PMMA began to replace glass in the 1940's because of its toughness, optical properties and physiological inactivity. Although allowing little oxygen to reach the surface of the cornea it was generally considered to provide good refractive correction with few long term complications. It was not therefore a purpose designed polymer and it did not have any serious competitor until hydrogels emerged some years later.

PMMA is a thermoplastic belonging to a group of polymers which are capable of being shaped or moulded under the application of heat and pressure, but are rigid at room temperature. Although they may show some flexibility, it is the rigidity of PMMA which both produces initial discomfort but yet allows lenses to be fitted so as to ensure a tear flow beneath the lens.

1.1.1. Incidence of PMMA lenses

Today a substantial number of hard PMMA lenses are still fitted in many countries including Great Britain where, between 1989 and 1991, approximately 3% of all contact lenses manufactured were in PMMA. This was in spite of the knowledge that long term effects of PMMA mainly as a result of hypoxia, cannot be eliminated by the physical fit of the lens (Holden 1988).

The 1992 figures (ACLM, Optometry Today, March 8th 1993), although based upon lens costs, showed the trend that the number of PMMA lenses used was dropping but that approximately 2% of all lenses sold were PMMA (Fig.1). It has been demonstrated very clearly that chronic hypoxia induced by PMMA lens wear has long lasting and apparently irreversible effects on the cornea (Holden et al. 1985) although the physiological functioning of the cornea as a result of chronic hypoxia remains an area of dispute in the literature.

Corneal decompensation resulting in chronic oedema as a result of cosmetic contact lens wear has never, to my knowledge, been reported in the literature.

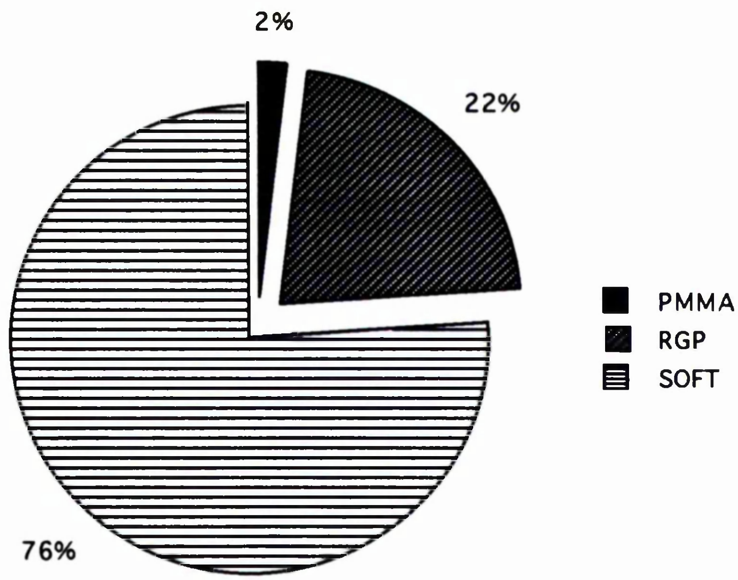


FIG.1

Proportion of the various lenses used in the UK(1992). These figures are based on the actual sales figures but are a good guide to the approximate relative proportion of lenses used.

1.2. Hard gas permeable material developments

It is important to consider that the ideal contact lens material will have a balance of properties each of which has individual clinical significance. The properties of flexibility, wettability, durability, machinability and deposit resistance are all important to the overall success of a material (Yokota et al. 1993) but clearly the oxygen transmission of contact lenses is of great significance.

Over the last 10-15 years a great deal of industry money and effort has gone into developing new hard plastics which are gas (oxygen) permeable (Tighe and Kishi 1988) and have the necessary optical and mechanical properties required to produce alternative lenses to PMMA and if possible be suitable for extended or continuous wear (Isaacson 1988, 1989).

1.2.1. Siloxy-methacrylates

A major advance in the development of gas permeable rigid contact lens materials is found in the work of Gaylord (1978). This resulted in various hard gas permeable co-polymers being manufactured and evaluated, predominantly based on siloxy methacrylates to obtain the necessary oxygen permeability. These materials, the chemistry of which was already known, contain branched dimethyl siloxane substituents on a methacrylate stem, combining the high oxygen permeability of silicone rubber and the convenience of free radical polymerisation. However despite the increase in oxygen permeability, it has been noted that these RGP materials are considerably more susceptible to deposits than PMMA (Fowler 1987) and consequently, lenses require more cleaning to prevent the build up of protein.

Improved oxygen permeability in the siloxymethacrylate materials has been achieved by increasing the proportion of the 'tris monomer' rather than utilising a supposedly superior siloxy monomer described in the large number of post Gaylord patents. The 'tris' monomer (tri-methyl-siloxy-methacryl-oxypropylsilane), which is a siloxy derivative of the meythacrylate monomer was the subject of patent disputes in the late 1980's (Tighe and Kishi 1988, Tighe 1989 in Stone and Phillips).

Although Gaylord's work (1978) marks the beginning of the use of siloxymethacrylates as contact lens materials, the underlying chemical concepts had been previously known. This was the aspect of the patents that led to them being upheld in court cases in the late 1980's. The Gaylord patent (1978) was ruled valid because the use of siloxymethacrylates to produce oxygen permeable contact lens materials was a solution to a problem that others had unsuccessfully tackled.

This supports the view that the 'tris' monomer is the only siloxy monomer of commercial significance and that increased oxygen permeability of siloxymethacrylate materials has been achieved by increasing the proportion of the 'tris' monomer (Tighe 1989, in Stone and Phillips).

The other more recent rigid gas permeable materials have been fluoroalkyl methacrylates which although being mentioned in the early Gaylord patents as far back as 1978, were not commercially developed until much more recently. Virtually all of the gas permeable materials now used for contact lens manufacture are specially designed polymers having no other commercial applications.

1.2.2. Fluoro-polymers

Hard lenses have therefore evolved from non-permeable PMMA, to oxygen permeable silicone acrylates and more recently to fluoro-silicone acrylates. The addition of fluorine does not contribute significantly to the actual oxygen permeability but tends to offset the hydrophobic nature of the silicone (Tighe and Mishi 1988).

Newer non silicone containing fluoro-polymers, having high oxygen permeability, have been described in the literature (Isaacson 1988, 1989) but as yet have not been extensively clinically evaluated although they do show some indications of being clinically successful. These materials use the concept of a long flexible oxygen permeable chain with polymerizable acrylate or methacrylate groups at each end (Keates et al. 1984).

It has long been recognised that silicone elastomers could have great potential as contact lens materials due to their very high oxygen permeability (Dk) but to date, in spite of a vast amount of research and financial investment, the fitting and wearing of silicone elastomer lenses has been unsuccessful due to their hydrophobic surfaces. These elastomers consist of alternating silicone and oxygen atoms with carbon and hydrogen containing radicals pendant to the backbone. The silicone elastomer is a highly flexible rubber like material and is neither a hydrogel nor a rigid plastic. It can therefore be considered as a hybrid. Whether the inherent problems of silicone elastomers can be overcome remains a challenge for the polymer chemist and the lens designer.

The most successful modifications of the silicone formulations produced the silicone acrylates. These are copolymers of methyl-methacrylate and alkyl-siloxanyl-methacrylate with a carbon-carbon repeating structure. Silicone is present in side chain radical structures and other monomers are added to allow both hardness and wettability. These are now commonly used for rigid (RGP) lenses.

Although increasing the proportion of silicone improved the oxygen transmissibility, it also made lenses subject to the same surface problems as the elastomers. Typically the silicone acrylates used today have oxygen permeability diffusion coefficient (Dk) values between 10-50 Dk units which is insufficient to allow extended wear of lenses but suitable for most daily wear situations, particularly in the thinner minus powers. The units of oxygen permeability are usually quoted as Dk x 10^{-11} (cm^2/sec)(ml O_2/ml x mmHg). These units will be fully explained in Chapter 3 when discussing the measurement of oxygen transmissibility of contact lenses.

1.2.3. Fluoro-silicone acrylates

The addition of fluorinated monomers to silicone acrylate materials has resulted in the fluoro-silicone acrylates. These had some success in clinical trials as early as 1975 (Miller 1975) but were never developed commercially until much later.

This form of RGP contact lens material is the most wettable and resistant to tear film deposition (McLaughlin 1989). The proportion of fluorine in different fluoro-silicone acrylate polymers varies, the total amount being restricted by the miscibility of monomer components as well as the balance of lens properties attainable. These polymers have slightly increased oxygen permeability and improved surface characteristics, relative to the silicone acrylates which contain large amounts of silicone (McLaughlin 1989).

The deposit resistance of different fluoro-silicone acrylate materials in which the fluorine content was varied was not found to be concentration dependent for fluorine levels greater than 20% (Tomlinson et al. 1991). The silicone acrylates and fluoro-silicone acrylates form the largest group of commercially available gas permeable materials.

In the fluorocarbon materials, fluorine is copolymerized with a small amount of methyl-methacrylate for increased tensile strength, and n-vinyl-pyrrolidone to improve wettability. The fluorine is incorporated into the carbon backbone of the polymer as polyperfluoroether. The fluorine which replaces hydrogen ions comprises 40-50% of the polymer by weight, an amount 3-10 times more than in the fluoro-silicone acrylates. The fluorocarbon materials have low surface energy giving a high resistance to deposits. Generally both the fluorosilicone acrylates and the fluorocarbons provide a higher level of usable oxygen transmissibility than currently available hydrogel materials (Keates et al. 1984).

Again, early mention of these polymers was made in patents, the most significant being filed in 1967 and assigned to Du Pont in 1970 (Girard et al. 1970). It describes the advantages of contact lenses prepared from polymers derived from perfluoroalkylethyl methacrylates and in particular the low refractive index (1.39) and the high oxygen permeability. However difficulties in conventional lens production meant that no commercial development occurred at that time.

Of great importance is the fact that gas permeable non water bearing lenses are more resistant to fungal or bacterial invasion than are hydrogel lenses. Consequently, research into developing new polymers with high oxygen permeability, but still maintaining the other.important properties of hard and soft lenses, continues to be a high priority within industry.

1.3. Hydrogels

A major breakthrough in lens materials was made in the early 1970's when Wichterle, a Czechoslovakian chemist working in Prague, developed the first soft lens from a hydrogel material which had been first described in 1960 (Wichterle and Lim 1960).

The simplest hydrogel is poly 2-hydroxyetheyl methacrylate known as polyHEMA. Its basic structure is very similar to that of poly-methyl-methacrylate the most significant difference being that every unit of the poly-HEMA chain contains a hydroxyl group. In the absence of water both PMMA and polyHEMA are hard, clear and glassy. When in contact with water the materials behave quite differently. Whereas PMMA remains hard and glassy, the hydroxyl groups in polyHEMA take up water converting it to a clear flexible elastomeric gel.

The properties of the hydrogel are influenced by the structure of the polymer network and by the water which acts like a conventional plasticiser. The water also acts as a bridge between the natural and the synthetic systems giving greater biocompatability and creates a semi-permeable membrane allowing transport of oxygen and water soluble metabolites through the polymer matrix.

These features allow a low degree of oxygen permeability but a significantly enhanced comfort over hard lenses. As a result longer daily wearing times were possible and overnight extended wear became a much discussed concept. This was further advanced with the development of higher water content hydrogels allowing greater oxygen transmissibility and subsequently towards the current trend of disposable soft lenses.

Increasing the water content of a hydrogel was the next technical development to occur. The distinction of the first patent publication involving the use of a polyvinyl pyrrolidone (PVP) backbone onto which 2-hydroxyethyl methacrylate was grafted was in October 1968 (Leeds, patent published 1970) and shortly afterwards a series of patents described a similar process involving the use of polyvinyl pyrrolidone into which vinyl pyrrolidone monomer was grafted. The use of other monomers such as lauryl methacrylate and cyclohexyl methacrylate is also described and water contents as high as 87% have been claimed (Steckler, patent published 1970).

Brennan et al. (1987a) have demonstrated the effect of increasing lens water content on the overall oxygen transmissibility through a soft lens. Whilst decreasing lens thickness does increase oxygen transmissibility, increasing water content has a greater effect since oxygen transmissibility increases exponentially with increasing water content, whereas its relationship with thickness is linear. The overall aim has been to try to design lenses which achieve a high degree of oxygen transmissibility to minimise corneal hypoxia when lenses are worn.

As highlighted previously, the limitation with hydrogels to date has been that their oxygen permeability is directly proportional to their water content and over the last 15 years, little development has taken place in the range of materials available for contact lens use despite the wider exploration of hydrogels in other biomedical applications.

A review published in 1980 (Pedley et al. 1980) showed that polyHEMA was the only hydrogel used to any significant extent other than in contact lenses. Since that time soft contact lens materials have advanced little in their chemical concepts, whereas the literature associated with biomedical applications has in general, advanced rapidly. The introduction of disposable soft lenses introduces both a new concept in mass production of lenses combined with in some cases, non polyHEMA based hydrogels.

Brief reference has been made to non hydrogel soft materials (Zantos 1990) but as yet no such material has been made available for commercial contact lens use. Attempts have been made to produce gas permeable hard lenses with hydrophilic surfaces, but clinically they have been found to be very similar to both silicone and fluoro-silicone acrylates (Fatt 1993).

Recent reports in the medical literature highlight the fact that soft lens wear, particularly on an extended wear basis, significantly increases the risk of microbial keratitis which is a sight threatening condition (Poggio et al. 1989, Schein et al. 1989). Even with the concept of disposable lenses, it is evident that all problems of contact lens wear have not been solved and research must address these problems. An additional significant variable is one of patient compliance which is known to be extremely variable (Sokol et al. 1990, Devonshire et al. 1993).

Therefore, although world-wide use of soft contact lenses is increasing in proportion to gas permeable, due mainly to comfort and ease of fitting, problems do still exist, and many authorities would still prefer to find improved gas permeable materials because of their greater safety demonstrated in recent microbiological studies.

There are difficulties in evaluating lens materials since it is a complex process of trying to quantify physical properties and assess longer term clinical performance. Many factors, including some that are not quantifiable, are involved and therefore, a careful analysis of the literature is necessary to provide information on the current state of lens and material technology.

In conclusion, the ideal contact lens material would have a high oxygen permeability but also maintain the balance of physical properties shown in Fig. 2. Clinically this still remains to be achieved. In fact PMMA meets a good number of these criteria, the significant exceptions being the lack of any oxygen permeability and the initial discomfort. It may be that current technology cannot produce a lens material which has a high gas transmission, is easily manufactured, is deposit resistant, stable in use and provides the comfort of a hydrogel. To minimise lid irritation and obtain maximum comfort, a lens needs to be soft and or flexible but as a result, optical correction of corneal astigmatism becomes more difficult due to lens flexure.

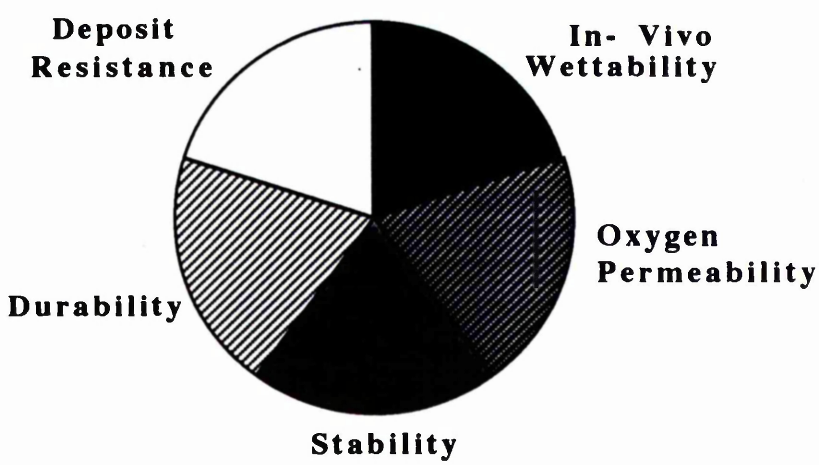


FIG. 2

Five of the most important contact lens material properties. In general a balance of all properties should be obtained.

Consideration of the properties of biological environments that are to be interfaced with synthetic polymers is very important. However making a synthetic similar to the cornea has proved to be difficult because the surface and bulk properties are governed separately by the epithelium and stroma and their natures are quite different. In spite of a better understanding of the chemistry of the corneal metabolism, producing a truly biomimetic surface is not an easy task. Research should continue the challenge to find the answer to these problems.

1.4. Mechanical Properties Of Contact Lenses

1.4.1. Introduction

The word polymer literally means "many parts". A polymeric solid material may be considered to be one that contains many chemically bonded parts or units, which themselves are bonded together to form a solid.

Plastics are a large and varied group of synthetic materials which are processed by forming or moulding into shape. Plastics can be divided into two classes, thermoplastic and thermosetting plastics, depending on how they are structurally chemically bonded. Thermoplastics, which include most of the polymers used for contact lenses, require heat to make them formable and after cooling, retain their shape they were formed into. Most thermoplastics consist of very long main chains of carbon atoms covalently bonded together. The long molecular chains are bonded to each other by secondary bonds.

Factors such as the basic comfort of a lens, the optical performance of a lens in cases of corneal astigmatism, and the lens base curve to cornea fitting relationship, have all been shown to relate to the mechanical properties of lens materials. These properties include the modulus, toughness, form stability and ease of manufacture. The fabrication of an article from a polymeric material in the bulk state involves deformation of the material by applied forces. Afterwards, the finished product is subjected to stresses and hence it is important to be aware of the mechanical properties of each material, and understand the basic principles underlying their response to such forces.

The mechanical properties of elastic solids can be described by Hookes Law, which states that applied stress is proportional to the resultant strain but is independent of the rate of strain. For liquids the corresponding statement is known as Newton's Law, with the stress now independent of the strain, but proportional to the rate of strain (Smith 1990). Both laws are valid only for small strains, and whilst it is essential that conditions involving large stresses leading to eventual mechanical failure be studied, it is also important to examine the response to small mechanical stresses.

In many cases a material may exhibit the characteristics of both a liquid and a solid, and is then said to be in a viscoelastic state. The response of polymers to mechanical stresses can vary widely and depends on the particular state the polymer is in at any given temperature. As a result of their chain like structures, polymers are not perfect elastic bodies and deformation is accompanied by a complex series of molecular re-arrangements.

Consequently, the mechanical behaviour of polymers is dominated by viscoelastic phenomenon in contrast to materials such as metal and glass where atomic adjustments under stress are more localised and limited.

1.4.2. Linear viscoelastic behaviour of amorphous polymers

A polymer can possess a wide range of material properties and of these the hardness, deformability, toughness and ultimate strength are amongst the most significant when considering contact lenses. Certain features such as a high rigidity modulus and impact strength combined with low creep characteristics are desirable in a polymer, if eventually, it is to be subjected to loading.

Unfortunately these are conflicting properties, as a polymer with a high modulus and low creep response does not absorb energy by deforming easily, and hence has poor impact strength. This means a compromise must be sought depending upon the use to which the polymer will be put, and this requires a knowledge of the mechanical response in detail. With contact lens polymers a low modulus will give a flexible lens assisting comfort, but may give poor lens parameter stability, and possible material creep, as a result of external forces on the eye or in handling.

Clearly the comfort of a lens is of major importance to successful contact lens wear, which may be one main reason why the soft lens share of the total world-wide contact lens market has significantly increased in the 1990's. This includes countries like the United Kingdom which has traditionally favoured the hard lens since its introduction and development in the 1950's.

Much of the recent research on contact lens materials has concentrated on the measurement of oxygen permeability and has tended to ignore mechanical properties such as flexibility. However the mechanical properties of materials and lenses are important as discussed in Chapter 2, but specifications of these properties are rarely provided. Apparatus for the mechanical testing of polymers is available for larger samples but rarely adaptable for contact lens samples (Tighe and Kishi 1988). The contact lens literature is very vague about the need for data on mechanical properties, or which ones are important, and consequently, new test methods need to be developed (Tighe and Kishi 1988).

The definition 'rigid gas permeable' (RGP) is now routinely used, but can be misleading. The use of the term rigid suggests or infers that such lenses will be resistant to bending or flexing which, as most contact lens practitioners will verify, is clearly not the case. These materials have changed significantly over the last twenty years since their introduction, and have developed into complex polymers designed specifically for the production of contact lenses.

Two of the main mechanical properties of gas permeable lens materials which influence lens performance, are the modulus of elasticity and the hardness of the plastic. Some of the consequences of these two properties, which may be evident clinically, are flexing of lenses on toric corneas, the awareness of a lens on insertion, and the scratch resistance of a lens. The mechanical properties of a material, in addition to its permeability and surface characteristics, are therefore crucial to the clinical performance of an RGP contact lens.

The mechanical properties also determine the dimensional stability of the lens, a point which has significance to both manufacturers and clinicians. It was taken into consideration recently by the International Organisation for Standardisation (ISO) in developing new standards for rigid contact lenses, covering classification and tolerances for manufacture. (ISO 8321-1, 1991; BS 7208 1992).

The revised standards recognise that most of the newer custom designed lens polymers are in fact more difficult for manufacturers to work with than PMMA. This is particularly so in the fact that the tolerance for the lens back optic zone radius has been doubled (now ±0.05mm), indicating that the dimensional stability of the group of RGP's is less than with PMMA. This point has undoubted clinical importance, in particular, to the verification, tolerances, and fitting of rigid lenses.

Hardness tests

Hardness is a measure of the resistance of a material to permanent deformation. This property can be measured by forcing an indentor into the surface of a material. The indentor material is normally made of a material much harder than the material being tested. An empirical hardness number is calculated based on the cross sectional area or depth of the impression.

The four common hardness tests used in engineering and materials testing are Brinell, Vickers, Knoop, and Rockwell. In these tests it is easier to measure a parallel sided contact lens blank than a curved finished lens but the data is difficult to interpret. The tests generally measure either the resistance of a material to indentation, or the resistance of a material to scratching, or measure the recovery from indentation.

Some hardness values for contact lens materials exist, but they are difficult to relate to actual lens performance, for example, the scratch resistance of a lens. Mechanical failure with PMMA and RGP contact lenses usually relates to their breakage, chipping, scratching, crazing or distortion. The surface energy required to fracture an amorphous brittle glassy polymeric material such as PMMA is about 1000 times greater than that which would be required if the fracture involved just the simple breaking of carbon-carbon bonds on a fracture plane.

Thus, glassy polymeric materials such as PMMA are much tougher than inorganic glasses. The extra energy required to fracture thermoplastics is much higher because distorted localised regions called crazes form before cracking occurs. A craze in a glassy thermoplastic is formed in a highly stressed region of the material, and consists of an alignment of molecular chains combined with a high density of interdispersed voids (Cowie 1991).

Contact lens practitioners will be familiar with the clinical presentation of this phenomenon which is sudden discomfort, blurring of vision, and an opaque stress pattern visible on examination of the PMMA or RGP lens. Checking the base curve of a lens in the radiuscope will confirm the distortion of a lens.

True crazing, usually as a result of patient mishandling (unintended bending or flexing of a lens) needs to be differentiated from the surface crazing referred to with some gas permeable lenses where surface breakdown or failure of the polymer occurs due to combination of lens manufacturing methods and deposits from the tear film. This has been explained by excessive heat during lens manufacture of certain materials. The interaction between the tears (and its constituents) and the polymer, is crucial to the long term mechanical performance of a lens.

The lathing techniques for gas permeable materials required to produce finished lenses, take into account the differences in hardness found between materials. These differences can be assessed by looking at the swarf characteristics of materials such as the continuous strands found with PMMA or the powder effect produced with most of the RGP materials when cutting with a diamond tool. The assessment of swarf characteristics is a well established practice in the engineering industry when dealing with metals and the same phenomenon of continuous or particle swarf exists.

In fact, the powder effect of the swarf when producing lenses from polymer buttons is greatest with the highest Dk materials commonly used, and lathe speeds and depth of cut with the diamond tool can be adjusted to make allowance for such factors. This means that careful polishing techniques are also required to eliminate possible 'chipping' effects on the surfaces and edges of a lens. From a manufacturing point of view, it would be ideal to have a gas permeable material with the machinability of PMMA. However with the current range of silicone and fluorosilicone acrylate materials, this remains a contradiction in terms.

The mechanical properties of hard gas permeable and soft hydrogel lenses are inherently different, and therefore need to be considered separately. Rigid gas permeable materials will be discussed in detail in Chapter 4, but some brief mention will be made here about hydrogels since none of the experimental work carried out in this project involved hydrogels.

1.4.3. Hydrogel materials

Hydrogels are inherently different in their mechanical properties to rigid gas permeables. In their dehydrated state, they are hard and brittle. Low water content hydrogels such as pHEMA are harder and more brittle than their higher water content counterparts in the dehydrated state.

When hydrated in water, all hydrogels become soft and rubber like with a low tensile and tear strength. The lack of mechanical strength is the main limitation to the life of a soft lens. The low modulus combined with the soft material, explains the comfort of such lenses, but does mean that any corneal astigmatism is transferred through the lens as a result of the 'wrap' factor, and that such lenses are more prone to damage from handling.

Some independent studies have been done on the mechanical properties of gas permeable contact lens polymers (Yokota et al. 1992), but little published work is available on the measurement of hydrogels used for contact lens production. Test methods for hydrogels are poorly defined, mainly because the materials need to be in their hydrated state, for results to be meaningful (Tighe 1989).

Therefore contact lens manufacturers' literature has to be searched to obtain any information on materials, and even then, it tends to have unspecified methodology and lacks a standardised format. To give some indication of differences between materials, the two main properties of tensile strength and rigidity modulus are given in Table1 (over).

The effect of water content of hydrogels can be clearly seen from Table 2 which shows the typical values of tensile strength and elongation to break. The material elasticity increases and strength decreases with increasing water content. The data in Tables 1 and 2 have been compiled from a range of manufacturers technical publications.

	PMMA	Silicone Elastomer	pHEMA
Tensile strength	50×10^7	10×10^7	0.5×10^7
Rigidity modulus	1000×10^7	8×10^7	5×10^7

TABLE 1

A comparison of the mechanical properties of three different types of contact lens materials (The units are dynes/cm^2).

	Hema(38%)	60%	73%
Tensile strength	6.50	4.6	3.6 kgm/cm^2
Elongation to break	110%	155%	260%

TABLE 2

Some mechanical properties of hydrogels to show the effect of the increase in water content.

1.4.4. Production methods

Production methods for soft lenses include, lathe cut, spin cast, static cast, and melt pressed. As with gas permeable materials, lathe cutting involves the need to consider the mechanical properties of materials. Low water content materials tend to be harder and therefore more difficult to cut and polish, whereas higher water content materials are softer and are easier to cut and polish.

Therefore practitioners need to consider the tensile modulus and elongation to break of all hydrogels, and it is recommended that manufacturers determine these values experimentally to allow data to be quoted in technical specifications for materials. The test method should also be known, and the units of measurement should be relevant to contact lens materials.

Following a period of about 10 years where little change has been seen in new hydrogel development, there is good reason to hope that the next decade will see new innovation.

As pointed out by Tighe and Mishi (1988) and Tighe (1989), the general understanding of biomaterials has been advancing, whilst no new techniques for testing polymers have been developed. Most of the present methods are inappropriate to current requirements and it is important to ensure that the situation is remedied. The mechanical properties of contact lens materials are important to the industry, the practitioner, and to the successful wearing of contact lenses.

1.5. Problems in standardisation

As previously indicated there has been a continual emergence of new lens materials (mainly hard gas permeable) over the last decade and consequently a great deal of confusion has arisen regarding the extent of differences between materials. Legal cases have also been brought against infringement of patent rights in the United States of America. The outcome of these legal cases was that the early patents describing silicone acrylates were deemed to be sufficiently new and different, and consequently, subsequent materials in the same grouping must infringe patent rights.

The effect that this had, other than the obvious financial considerations, was to force material development along a different line, particularly in respect to the fluoro-silicone acrylates.

1.5.1. British Standards.

In an attempt to rectify and clarify the situation and as an aid to both industry and the contact lens profession, new British standards have recently been introduced for hard and gas permeable contact lenses, BS 7208 (parts 1,2,and 3) having already been accepted as International standards. They are in three parts relating to specification, classification, and methods of testing. As indicated in the foreword in the literature of the new standards, the need for a standardised method of classifying the materials from which contact lenses are made, has been apparent for many years.

Polymers are more difficult to identify than other chemical compounds that have unique formulae and can be identified accordingly. However relatively few components are used in the different polymers currently used for contact lens production, and the standard was drafted to enable the classification of these materials into groups containing common monomer units. Also the standard describes a procedure for labelling the material with certain physical properties (eg. oxygen permeability) which are known to have a very significant effect upon the physiology of the eye when wearing a contact lens.

1.5.2. Focon and Filcon Materials.

Materials are divided into two groups which are Focon for hard or rigid materials and Filcon for soft lens materials. The classification is based on the two most important features of lenses in use, that is the water content of a soft lens and the oxygen permeability of gas permeable materials. Examples of each should illustrate the intention of the nomenclature within the standards.

(a) Filcon 1a (38)

'Filcon' indicates a soft lens, '1' is the sub group for polyHEMA, 'a' indicates that the material does not contain any significant quantity of non ionisble component, and '38' is the water content (%).

(b) Focon 5

'Focon' indicates a hard gas permeable material, and '5' a copolymer of one or more alkyl methacrylates and/or siloxanyl methacrylates, plus other water active monomers, cross linking agents and at least 5% by weight of a fluoroalkyl methacrylate or other fluorine containing monomers. The oxygen permeability (Dk) is greater than 20.

In what has been a confusing array of manufacturers materials, claims and counter claims, it will hopefully become clarified by the introduction of these standards, as all concerned within the contact lens industry become familiar with the scope of the standards.

1.6. Identification of contact lens polymers

As mentioned previously it is difficult to determine which material a finished contact lens has been manufactured from particularly in the current range of gas permeables. Even to differentiate a PMMA lens from a gas permeable lens is often difficult. Practitioners must rely on lens production laboratories providing accurate information on the materials from which lenses are made.

As indicated in the previous section, the major difference between hydrogels relates to the water content of the material. However this is now relatively easily identified by the recent introduction of a simple instrument to measure the refractive index of a lens in the hydrated state (Efron and Brennan 1985).

There is a direct relationship between the refractive index and water content of a soft lens, and consequently identification of this clinically important parameter is easily determined by currently available custom designed instrumentation. There are relatively few different soft lens materials used, and therefore, it is possible to categorise them according to their water content, which in turn is based upon their chemical composition. The typical range in water content used clinically is 38% to 75%.

The question of hard gas permeable identification is much more difficult. Due to the proliferation of materials over the last 15 years, many vary only slightly in chemical composition, making it impossible from clinical observation, to differentiate one material from another when in finished lens form.

1.6.1. Infra red Spectroscopy for indentification of lens constituents

In conjunction with the Department of Physical Sciences at Glasgow Caledonian University, it was found that using laboratory methods of analysis such as computerised infra red spectroscopy, the major chemical components of a material, or more specifically the bonding between components, could be identified and the material classified into one of the broad headings of PMMA, silicone acrylates, fluoro-silicone acrylates or polyperfluoro-ethers. It is very difficult to identify the smaller differences between materials within a group although clinically this may have little significance.

The method of diffuse reflectance infrared spectrometry has been known for many years, but a recent addition of fourier transform infrared spectrometry has created a powerful tool for the analytical chemist. The bulk properties of a material can be assessed by directing infrared light on to the sample where it penetrates it before being scattered, and collected by a detection system. An absorption spectrum can then be displayed, indicating the various wavelength peaks corresponding to specific elements, and the associated bonding between elements.

The most practical suggestion relating to hard gas permeable materials, has been that a library of all the materials available could be developed from infra red spectroscopy (H Andrew, Dept. Physical Sciences, Glasgow Polytechnic 1991, personal communication). Each infra red spectra is in effect a chemical 'finger print' of the material, and the complete library could be stored in the instruments computer data base.

Any material or lens submitted for identification, could then be analysed and compared to the 'library ' and a list of the 3 or 4 most likely materials in order of probability could be identified. Alternatively, if it was necessary to identify only the presence of the siloxane bond to determine that a material was in general a gas permeable polymer rather than PMMA, the instrumentation could be simplified to examine only the relevant part of the infra red spectra.

1.7. Conclusions.

In summary therefore, by simple clinical measurement the water content of a hydrogel can be identified, and hence a material placed into one of the categories of low water content (Hema 38-40%), mid water content (PVP/HEMA 55-65%) or high water content (PVP 70% and above).

With hard gas permeable lenses, there is no similar clinical test to determine which specific material has been used to produce a lens. Chemical analysis by means of infra red spectroscopy, will allow the major chemical elements of a polymer to be identified and therefore broad classification into material groups is possible. Samples for testing can either be in the form of a lens or a 'button' of material. However, simple tests and procedures need to be developed to allow easier laboratory identification of lens materials.

Tighe and Kishi (1988) in their review of gas permeable materials commercially available at that time, present data on chemical type, oxygen permeability, contact wetting angle, hardness, and refractive index. They do point out that other than on hardness, little information was available for mechanical properties.

Chapter 2. Literature Review

The Effects of Contact Lenses on the Cornea.

2.1 Introduction

Our understanding of how and why contact lenses affect the eye has been advanced considerably over the last ten years. Corneal pathophysiology during contact lens wear may be hypoxic, hypercapnic, allergic, toxic, mechanical or osmotic in origin (Bruce and Brennan 1990). Of these, the most common aetiological factors are hypoxia and hypercapnia (CO_2 build up).

It is now known that the oxygen demands of the cornea are high (Holden and Mertz 1984, Holden et al. 1984), and that carbon dioxide accumulates causing corneal acidosis to occur with most current contact lenses (Efron and Ang 1990), especially with extended wear (Bonnanno and Polse 1987b). Since the normal cornea is avascular, the oxygen required by the epithelium for essential metabolism is obtained by diffusion from the air when the eye is open and from the tarsal conjunctiva when the eye is closed.

Smelser and Ozanics first demonstrated in 1952, that oxygen deprivation due to PMMA hard lenses leads to structural and optical changes in the cornea. Davson (1955) showed that normal corneal thickness could be maintained only if there was metabolic activity in the corneal cells. It was then reasoned that hypoxia caused by an impermeable PMMA hard contact lens, could cause a breakdown in corneal physiology, the end result being corneal oedema. Oxygen uptake at the anterior corneal surface was then quantified by Hill and Fatt (1963).

A review of the literature since that time, of the effects of hypoxia on the cornea as a result of contact lens wear, reveals that changes have been reported to occur in each of the individual layers (Holden 1988). These effects have been shown to be greater with extended wear but can also be seen in daily wear of lenses.

Contact lenses have a wide range of predictable effects on the eye including tear turnover (Kok et al. 1992, Ichijima et al. 1992a), oxygen availability, corneal metabolism (Tsubota and Laing 1992, Ichijima et al. 1992b), epithelial and endothelial morphology and corneal sensitivity (Fatt 1978, Millodot and O'Leary 1980, Bruce and Brennan 1990).

Most of these changes are reversible after discontinuation of lens wear with the notable exception of endothelial polymegethism (MacRae et al. 1986).

The more common and significant findings are reviewed in this chapter. They involve both cellular and physiological changes within the cornea. Some of these changes have been, and are still regularly observed clinically using slit lamp microscopy on contact lens wearers, whereas others are experimental findings and observations from 'in vitro' animal studies, using either the cat or monkey cornea as the model.

2.2. Corneal Anatomy

2.2.1. The Epithelium

The corneal epithelium is on average, 50 microns thick. The main cell types are basal, wing and surface cells with some melanocytes, polymorphs, dendrites and Langerhans cells (Hogan et al. 1971, Mayer 1984). Type 1V collagen has been identified in the epithelial basement membrane (Marshall et al. 1993). In the open eye the partial pressure of oxygen in the epithelium is 155 mmHg, the main source being from the atmosphere. In the closed eye the partial pressure of oxygen drops to 55 mmHg, the main supply being the tarsal conjunctiva (Ruben 1988).

The complex arrangement of interpacking of the epithelial cell layers accounts for the barrier function of these cells in protecting the corneal stroma from foreign bodies and invading organisms. The epithelium contributes to the stability of the tear film (Dilly 1985), and obtains its nutrition from the tears, the aqueous humour and the limbal capillaries. It has a very high metabolic rate because of its regenerative properties (Wilson and Fatt 1980).

2.2.2. Epithelial oxygen requirements : the open and closed eye

Recently in vivo assessment of epithelial activity during hypoxia has become possible with the technique of redox fluorometry. This technique measures the metabolic state of the cells by analysis of corneal epithelial auto-fluorescence to ultraviolet light. Evaluation of the fluorescence of mitochondrial oxidised flavoprotein in the in vivo rabbit eye, shows epithelial metabolic rate to be closely linked to oxygen availability (Tsubota and Laing 1992).

It is also important to note that the closed eye environment produces high accumulations of cellular debris. Fullard and Wilson (1986) described a novel technique of a non contact corneal irrigation chamber to collect eyewash samples after periods of either closed eye only, or closed eye and contact lens wear. This technique washes the central 8mm of the anterior surface of the cornea. They found high levels of both epithelial and inflammatory cells indicating the more challenging physiological environment associated with contact lens wear particularly in the extended wear modality (Wilson et al. 1989).

When the eye washings were performed during the day, an average of 19 epithelial cells were collected. On awaking after sleep an average of 120 epithelial cells were collected. However with inflammatory cells, eye washing during the day typically yields an average of 9 cells (leukocytes), whereas after prolonged eye closure such as sleep, 6500 inflammatory cells were typical, suggesting that the closed eye is almost in a sub clinical inflammatory state. Recent work on the changing balance of tear proteins in the open and closed eye seems to confirm this conclusion (Sach et al. 1993).

A reduction in epithelial metabolic rate affects every aspect of epithelial physiology including mitotic rate, cellular junctional integrity and the cellular reserves of glycogen (Tsubota and Laing 1992). Some interesting epithelial features have been observed both clinically and experimentally as a direct consequence of contact lens wear (Zantos 1983, Tsubota and Yasmida 1992).

2.2.3. Epithelial thinning

In what has become known as the Gothenburg study, Holden and co-workers (1985) assessed 27 subjects who had worn high water content soft lenses unilaterally in extended wear for 5 years. The value of this study was that all subjects in only wearing one lens could act as their own experimental control. The significant finding was that the epithelial thickness of the lens wearing eye was reduced by 6% returning to normal within one week of cessation of lens wear.

The epithelial thinning observed with wear of low oxygen transmissible lenses (PMMA and thick hydrogels) is most likely due to a reduction in the number of superficial cells and a flattening of the underlying cells, which has subsequently been shown to occur in monkeys after only a few days of wear.

2.2.4. Microcysts

These were first reported by Ruben et al. (1976) for daily wear of soft lenses, and by Zantos and Holden (1978) for extended wear of soft lenses. They are small (15-20 μm in diameter), round, circumscribed, transparent epithelial inclusions showing reversed illumination (Greenberg and Hill 1973).

They represent a delayed epithelial response since they take 2-3 months to show after beginning contact lens wear and 3 months to clear after cessation of lens wear (Holden et al. 1985).

Clinically it has been suggested that if less than 50 microcysts are present, they can be regarded as being within normal physiological limits (Zantos 1983). Greater than this amount should be regarded as an indication of more severe physiological stress, perhaps requiring cessation of lens wear or at least an increased oxygen transmissibility in the lenses.

One interesting feature of microcysts is that they are refractile objects in that they possess a different refractive index than the surrounding cornea. Refractile objects have either a reversed or unreversed illumination appearance on slit lamp microscopic examination. Zantos (1983) has shown that epithelial microcysts have reversed illumination, unlike vacuoles and bullae in the cornea which always show unreversed illumination.

They appear to form near the basement layer and gradually move toward the anterior surface of the epithelium when they will stain with fluorescein. Kenyon et al. (1986), suggest discontinuation of lens wear if the microcysts stain with fluorescein. Following the discontinuation of lens wear there is a burst of activity, with the number of microcysts increasing for a short period before reducing over a period of 1 to 3 months.

Clearly the presence of microcysts has been mainly associated with extended wear of soft lenses and their pattern of development must provide information on epithelial metabolic rate and cellular turnover. Their exact cause and clinical significance is still open to debate, and further research is necessary to establish the morphology, aetiology, and pathology of microcysts.

2.2.5. Reduction of epithelial adhesion

Experiments on the cat and monkey cornea have shown that extended wear of low oxygen transmissible lenses produces a decrease in both the number and thickness of all cell layers and the development of abnormally shaped basal cells in the epithelium (Madigan 1987). The most important finding however, was a reduced epithelial adhesion caused by a decrease in hemidesmosome synthesis.

Madigan (1987) reported from her experiments with cats that it was always easier to strip the epithelium from the underlying basement membrane in those eyes which had worn thick soft lenses on a continuous basis for up to 15 months. Further experiments by Madigan and Holden (1992) have found the reduced epithelial adhesion to correlate with reduced hemidesmosome density in the cat cornea while wearing thick high water content soft lenses for up to 4 months.

The reduction in hemidesmosome density has been attributed to both loss of basal cell shape and chronic epithelial hypoxia, but interestingly, the role of oedema in producing a loss of epithelial adhesion appeared minimal. Although mainly associated with extended or continuous wear, these results nevertheless indicate that the epithelial cells are more prone to damage following contact lens wear.

This has been confirmed by O'Leary and Millodot (1981), who found that epithelial fragility was greater as a result of contact lens wear. They measured fragility using a Cochet Bonnet aesthesiometer, the threshold of fragility being the minimum pressure exerted by a nylon thread to produce epithelial damage, as shown by fluorescein staining. It is difficult however to correlate observed fluorescein staining with the actual cellular damage produced by such a stimulus.

2.2.6. Corneal epithelial staining

Superficial punctate keratitis (SPK) appears in several different forms associated with contact lens wear. It occurs as pits in the epithelial surface due to damage to groups of cells. This can happen without discomfort to the patient, since the superficial epithelium is not innervated. As a result, the barrier function of the epithelium is disrupted placing the cornea at risk from penetration by micro-organisms.

Although hypoxia is a frequent aetiologic factor, SPK may also occur for many other reasons and in many different configurations. Epithelial punctate staining due to solution toxicity is often more diffuse than staining produced by hypoxia, and is characteristically associated with symptoms of stinging or burning at the time of lens insertion. If SPK is associated with corneal infiltrates then solution hypersensitivity may be the causative factor.

Diffuse punctate keratitis must be differentiated from a number of other corneal changes including coarse punctate erosions, three and nine staining, and foreign body tracks. It is important to remember that soft lens wear can produce or be associated with corneal staining and that it is not simply a mechanical effect, related more to the wearing of a hard lens disrupting epithelial cells. Epidermal growth factors (EGF) are substances with biological activity enhancing the growth and differentiation of the corneal epithelium and are some of the newer pharmaceutical agents which may play a part in contact lens wear in the future.

Pastor et al. (1992) found that in a double blind placebo controlled study, the mean epithelial healing time was significantly enhanced in a group treated with EGF compared with a placebo group, and that the number of epithelial defects completely healed after a specific time period was greater in the EGF treated group. It is possible that topical EGF may be of significant help in treating persistent corneal epithelial defects in contact lens wearers.

2.3. Microbial Keratitis

There has been a great deal of debate about this condition in the contact lens literature over the last 2-3 years. Microbial keratitis is an uncommon but serious complication of contact lens wear. It is an acute disease and can cause visual loss due to corneal scarring. Recent studies suggest keratitis incidence to be 20.9 per 10,000 extended soft lens wearers per year and 4.1 per 10,000 daily wear soft contact lens wearers per year (Poggio et al. 1989). Clearly there is an increased incidence of corneal ulceration associated with extended wear of soft lenses.

The study which engendered tremendous publicity in the lay press (Poggio et al. 1989), by associating a higher risk of microbial keratitis with extended wear of soft lenses, relied to some extent upon information obtained from patients about the type of lens worn and therefore has significant limitations.

However Poggio's group have followed up with newer epidemiological studies, quantifying the incidence and risk factors for corneal infections with the various lens wear modalities. Their results (Poggio and Abelson 1993) confirm that the incidence of corneal ulcers among disposable extended wear lens users did not differ significantly from the incidence found with conventional extended wear, but was significantly higher than the rate for conventional daily wear.

Dart et al. (1991) investigated risk factors in contact lens related ulcerative keratitis in a case controlled study and found that amongst EWSCL users lens hygiene and compliance were not significant factors. They found that a longer cycle time (> 6 days) was associated with ulcerative keratitis. Buehler et al. (1992) again in a case controlled study in the United States, found that daily wear soft lens users had the lowest risk of developing ulcerative keratitis. Relative to this, disposable soft lens users had a 19.4x greater risk and surprisingly this was greater than with conventional extended wear.

However, both Beuler et al. (1992) and Poggio et al. (1993) point out that wearing times between lens removal is greater with disposables than with conventional extended wear lenses. Such studies should address both the actual lens wearing schedule and patient compliance. It is also evident that such studies need to culture organisms to ensure the presence of microbial keratitis and not simply assume positive findings (Kirkness et al. 1993).

Since lens hygiene factors could be assumed to be the main contribution to microbial keratitis, the introduction of disposable lenses should have largely resolved the problem. However the study performed at Moorfields Eye Hospital in London by Dart et al. (1991) found poor lens hygiene to be associated with daily wear soft contact lens users only.

Numerous other authors have documented corneal microcysts, infiltrates, and ulcers with disposable lenses. In the light of this, the safety of extended wear of contact lenses, including disposable, needs careful reviewing. Bacterial adherence to lenses and subsequent bacterial colonisation of the lens surface during wear may explain keratitis that occurs in wearers without lens material contamination and in those using disposable extended wear lenses.

The higher incidence of microbial keratitis with extended wear compared to daily wear is attributed in part to corneal hypoxia and the subsequent epithelial disruption. Infection associated with RGP contact lens wear occurs less frequently. The most commonly associated pathogen with contact lens wear is pseudomonas aeruginosa.

To assess the role of bacterial adherence in the pathogenesis of pseudomonas infections, Fleiszig et al. (1992) obtained superficial corneal epithelial cells and leukocytes from 10 patients who used soft contact lenses on an extended wear basis. The mean number of bacteria adhering to epithelial cells was significantly greater in the lens wearing eyes relative to healthy controls. It is also interesting to note that at least one study has found that bacterial adherence to disposable soft lenses is greater when the lenses are new, and less after a period of wear suggesting the importance of a biofilm on the lens in order to minimise bacterial invasion of the lens surface (Boles et al. 1992).

Further study is necessary to establish the role of adherence and bacterial biofilm formation in the pathogenesis of keratitis and in the contamination of lens materials. It is important to remember that the risk of microbial keratitis associated with daily wear of hard gas permeable lenses is extremely low. The cohort studies conducted to date, have mainly concentrated on describing the relative risk of different modes of contact lens wear in relation to microbial keratitis. Until the total number of contact lens wearers is ascertained, the majority of whom are successful, then figures from such studies can be misleading in their clinical significance.

Three major aetiological factors giving rise to ocular compromise with contact lens wear are probably overnight wear, protein deposition, and patient non-compliance. In the meantime, case controlled studies need to be done until agreement on the relative risk of lens wear modalities is reached. Appropriate instructions can then be given to patients wearing lenses.

2.4. The Stroma

2.4.1. Stromal anatomy

The stroma represents more than 90% of the total corneal thickness being on average 440 microns thick. The most anterior aspect is Bowman's layer which is made up of a tightly interdigitating arrangement of collagen fibrils which are very resistant to trauma, foreign bodies, and bacteria (Hogan et al. 1971). Types I, III, V and V1 collagen have been identified in the stroma. A complete review of ocular collagens including those in the cornea, has recently been published (Marshall et al. 1993).

The main cell type in the stroma is the keratocyte. The mid stroma has a partial oxygen pressure of 95 mmHg being supplied mainly from the atmosphere. This drops to 44 mmHg in the closed eye, with the tarsal vessels being the main oxygen supply (Freeman 1972).

The bundles of collagen fibrils are arranged in lamellae. Within each bundle the collagen fibrils are parallel to each other but the lamellae traverse each other at almost right angles. This grid like arrangement of lamellae acts as a diffraction grating in which the light rays are usually separated from each other by less than one wavelength of light and interfere with scattered light by cancelling or eliminating it by destructive interference (Hogan et al. 1971).

The bundles are separated from each other by a mucopolysaccharide ground substance that is very hydrophilic. The adsorption of fluid keeps the bundles of collagen separated by a set distance. However if the distance changes with the accumulation of fluid, the transparency is reduced. If a scar results in a contraction of the collagen fibrils in an attempt at healing, opacification of the area also results.

2.4.2. Stromal physiology

It would appear that the contribution of the stroma to the maintenance of normal corneal physiology and function has received limited attention from researchers. However the physiological changes occurring within the stroma and their effects on the endothelium have recently been evaluated by a series of detailed and carefully controlled experiments (Bonnanno and Polse 1987a, 1987b, 1987c). The earlier 'classic' experiments of Klyce (1981) showed that lactate diffuses to the stroma as a result of increased production due to hypoxia. An osmotic imbalance is created, leading in turn to increased corneal hydration.

2.4.3. The effects of pH

Stromal pH has been shown to be altered by both hypoxia and hypercapnia. These conditions respectively cause the accumulation of lactate and bicarbonate in the corneal stroma, leading to an acidic shift in stromal pH. The hypothesis that lowered pH reduces corneal function, seems tenable however, since data from 'in vitro' animal and human studies have shown several cellular processes to be pH dependent.

It has been shown that reduced pH can cause endothelial oedema and junctional breakdown (Gonnering et al. 1979), a decrease in endothelial Na+ / K+- ATPase activity (Whikehart et al. 1987), a reduced net sodium flux (Green et al. 1986), a diminished trans endothelial potential and fluid flux (Fischbarg et al. 1974), and a reduced epithelial Cl- transport (Fischer and Wiederholt 1978).

Holden et al. (1985) also showed that with brief exposures to CO_2 and therefore presumed stromal acidosis, transient morphological changes occurred in the corneal endothelium. Therefore the work of Bonnanno and Polse (1987a, 1987b, 1987c), and Cohen et al. (1992), on stromal pH and its effect on corneal hydration control, is important to our understanding of corneal physiology.

The pH of the stroma is reported to be 7.54±0.01 under open eye conditions decreasing to 7.39±0.01 with eye closure (Bonnanno and Polse 1987a). These researchers determined that a thick low water content soft lens worn with the eye open, decreased stromal pH to 7.15±0.04. Since the lens would have a very low oxygen transmissibility (Dk/t), this finding indicates the lower extreme to which stromal pH would change with open eye wear.

The experimental data obtained from the method of fluorophotometry indicates that it is a reliable technique and relatively non invasive. Further experiments need to be done using this method to look more closely at stromal changes particularly in long term wear of contact lenses.

The induction of stromal acidosis as a result of hypoxia and hypercapnia has both short and long term ramifications, the most significant being the compromise of endothelial function and therefore, corneal hydration control. This will be referred to later in the discussion of the results obtained from specular microscopy of the endothelium in long term contact lens wearers (Chapter 6).

Since all contact lenses impede oxygen flow to the cornea to some extent, stromal oedema is a frequent occurrence with contact lens wear and is responsible for many of the corneal changes observed. The degree of corneal oedema may be qualitatively monitored from the biomicroscopic appearance of stromal striae and posterior stromal folds as well as from changes in corneal curvature. Bergmanson and Chu (1982) found greater oedema in the posterior stroma with contact lens wear suggesting that the endothelium is the major entry site of fluid.

2.4.4. Stromal swelling

However other studies have demonstrated that the posterior stroma has a greater capacity to swell than the anterior stroma, and this may give rise to the striate corneal lines seen during contact lens wear. This is most likely to be a buckling of the posterior stroma and Decemet's membrane and directly due to the swelling effect of the oedema. Cristol et al. (1992) found that in a comparison of stromal oedema induced from the anterior and posterior surfaces, it was 3.65x faster through the posterior than the anterior surface in the rabbit. The difference in the human was even greater, being 13.1x as fast through the posterior surface. These results must have some significance to anterior eye surgeons when considering the response of the cornea to trauma.

Maximising the concentration of oxygen behind a contact lens must therefore have beneficial effects to the cornea, and this has been confirmed by the experiments with silicone elastomer lenses which show zero corneal swelling following wear of these highly oxygen transmissible lenses (La Hood et al. 1988).

2.4.5. Effects on corneal refraction

As the stroma forms the physical bulk of the corneal tissue, its role in providing the support necessary for optical regularity at the epithelial surface is important. Long term wearers of PMMA hard lenses may develop irregular astigmatism and distortion of the central and peripheral cornea. Reports suggest that up to 6 dioptres of astigmatism may be induced and since it does not always resolve when lens wear is ceased, the condition has been termed "corneal warpage syndrome" (Wilson et al. 1990). The phenomenon of corneal warpage is not well understood. It has been attributed to a dual mechanical and hypoxic aetiology and has great significance with respect to determining the associated structural changes that may be present in the stroma.

Clinically significant changes in corneal topography can be associated with daily or extended wear of rigid or soft contact lenses. Corneal warpage was a term originally used by Hartstein (1965), to describe those contact lens induced changes in corneal topography that are generally reversible, although it has also been suggested that they could be permanent, subsequently giving rise to keratoconus (Phillips 1990). The changes have not been associated with corneal oedema.

Whether corneal warpage could be a permanent change induced by long term contact lens wear would need to be more carefully studied. It might happen that a long term hard lens wearer could develop keratoconus irrespective of the contact lens wear. Subsequently it could then be mistakenly suggested that the keratoconus was due to the wearing of contact lenses.

Patients with corneal distortion are normally asymptomatic when wearing contact lenses but find that vision is reduced when changing to spectacles, a symptom that has been termed 'spectacle blur'. The diagnostic finding in such patients is distortion of the keratometer mires but more recently the condition has been investigated using the new computer topographic corneal modelling systems (Wilson et al. 1990, Montenegro et al. 1993). Using these systems, small areas of corneal irregularity can be detected. Although it has generally been found that PMMA lenses produce the greatest corneal distortion, poorly fitting gas permeable and soft lenses may also produce this condition.

Holden and co-workers (1985) found that a small but significant amount of stromal thinning had occurred in a group of unilateral contact lens wearers after 5 years of extended wear. They hypothesised that chronic corneal oedema causes an alteration of function in the stromal keratocytes.

Consequently the production of collagen, glycoproteins, and proteoglycans is reduced, less stromal tissue is produced, and stromal thinning results. It has also been proposed that the action of lactate, which accumulates in the stroma under hypoxic conditions may lead to some dissolution of stromal tissue and alterations in proteoglycan synthesis as a result of anaerobic glycolysis, associated with contact lens wear (Cintron 1993).

Clinically it has been found that corneal warpage and spectacle blur are often best managed by refitting the patient with gas permeable lenses of high oxygen transmissibility (Kame et al. 1989).

2.4.6. Summary

Summarising therefore on the cycle leading to stromal oedema, the oxygen uptake of the cornea has individual subject variations but is between 2 and 5 $\mu l/cm^2/h$ (Ruben 1989). Most of this oxygen is related to cellular activity and the squamous epithelial cells take most of this uptake. Depletion of oxygen due to contact lens wear decreases the rate of cell reproduction (mitosis) and reduces the reserves of glycogen in the cells. The glycolytic anoxic cycles then become more active but produce less energy and decrease the nucleotide production of the basal cells.

The oxygenation cycles required to break down pyruvates and lactates to carbon dioxide and water are deficient and therefore corneal hypoxia produces increased lactates in the stroma and anterior chamber. The upset in the balance of ions reduces the outflow of water carrying molecules, such as bicarbonate and chlorine, and therefore the stroma becomes oedematous.

2.5. The Endothelium

2.5.1. Anatomy

The corneal endothelium is a single cell layer of neural crest origin and forms the most posterior layer of the cornea. It consists of approximately 350,000-500,000 mainly hexagonal cells with straight sided borders measuring 4-5μm wide, 3-5 μm in thickness and 7-10 μm in length and is in direct contact with the aqueous humour (Tuft and Coster 1990). The partial pressure of oxygen in the endothelium is 55 mmHg supplied from the aqueous (Ruben 1989). The closed eye partial pressure of oxygen is similar since the aqueous is the main oxygen supply. Some lymphocytes, but no collagen, are present in the endothelium (Mayer 1984, Tuft and Coster 1990) although the presence of lymphocytes, has since been disputed (Lee 1993).

Adjacent endothelial cells are joined to each other by cell junctions of the macula occludens and adherens type. The intercellular space near the anterior chamber is closed by a zonula occludens. The zonula occludentes have been reported to be discontinuous in the endothelium and may be a contributing factor to the permeability of this layer (Hirsch 1977).

Such an arrangement forms a barrier to molecules 10 μm or greater in diameter. The cytoplasm contains an abundance of rod shaped mitochondria and the nuclei are round and flattened in the anterior posterior axis (Hogan et al. 1971).

2.5.2. Physiology : barrier and pump functions

The integrity of this layer is crucial for maintenance of normal corneal transparency. It's main function is to keep the cornea in a relatively dehydrated state for optimum clarity and there is a direct correlation between the swelling pressure, hydration and the normal thickness of the cornea.

The natural tendency of fluids to follow the pressure gradient into the stroma is counteracted by an active pump mechanism which exists in the endothelium to eliminate fluid from the stroma. Clinically this is demonstrated in a number of pathological conditions of the cornea, for example in Fuchs' dystrophy when the endothelial cell density can drop to a level such that there are insufficient pump sites to control hydration (Tuft and Coster 1990). In this situation the cornea eventually becomes chronically oedematous.

Evidence for the pump function is also provided by the experiments conducted by Maurice (1972) in which he blocked the sodium potassium ion transport by ouabaine and found the barrier effect was insufficient to prevent corneal oedema. His experiment on isolated in vitro endothelium showed that this layer alone could move water at a rate consistent with in vivo corneal hydration.

Hodson and Miller(1976) proposed that the endothelium actively transports bicarbonate ions into the anterior chamber, and water tends to follow osmotically. However the validity of the HCO_3^- ion theory has recently been questioned (Doughty 1990) with the suggestion that the stroma is the site for the corneal hydration control.

The rate of fluid leak across the endothelium can be calculated using the ocular slit lamp or scanning fluorophotometer (McLaren and Brubaker 1986). The endothelial permeability to fluorescein increases with age and intraocular surgery (Leisgang 1991).

As long as the functional reserve of the endothelium is not exceeded, corneal transparency is maintained. Functional response in the endothelium has been extensively tested using an animal model (Geroski and Edelhauser 1984, 1993). The results of their experiments suggest a possible mechanism which, at least in the cat model, might contribute to the physiologic reserve of the endothelial monolayer.

As damaged cells are lost in endothelia having limited regenerative capacity, an intact monolayer is re-established by enlargement of the remaining cells. This would be expected to reduce ion and fluid leak and, thereby, effect an increase in the integrity of the endothelial barrier. With less leak, the demands on the endothelial pump would be reduced, resulting in a decrease in endothelial pump site density. This hypothesis, first proposed by Bourne and Brubaker (1983), suggests that a mechanism would provide an efficient means of preserving corneal transparency, since the energy demands of pumping imposed on the healed endothelium would be reduced.

Future wound healing studies will further define the cellular events of healing and functional recovery after wounding. Investigating the adaptive mechanisms which preserve corneal transparency despite significant cell loss, will further the understanding of the endothelial physiologic reserve.

2.5.3. Changes in the endothelium during life

Beginning at the eighth week of gestation, endothelial cells deposit Descemets membrane. This first appears as patches of lamellar material and forms a complete layer by sixteen weeks. Collagen is continuously deposited thereafter until the eighth month of gestation, by which time the membrane forms a layer approximately 3 microns thick (Tuft and Coster 1990).

In an interesting recent study on the prenatal and postnatal ultra structure of the cornea, Arne (1992), using scanning electron microscopy, found that in the prenatal period, cells were flat and cube shaped. Changes at the intercellular junctions were seen at 20 weeks of foetal life and, in two cases, two layers of cells were found in the peripheral cornea.

Klyce and Beuerman (1988) and Ozanios and Jacobiec (1990) have shown that the corneal endothelium is formed by two layers of cuboidal cells but the adult configuration is attained by birth. Since the human corneal endothelium is unable to reproduce after birth (Kaufman et al. 1966), any changes in this layer are caused by the effects of age, disease, and environment.

2.5.4. Specular microscopy

The natural ageing process of the corneal endothelium means that from birth to the age of 5 years, the cell population is fairly uniform with the cell density as high as 5,000-6,000 cells/mm^2 (Sherrard et al. 1987). With increasing age, the population becomes increasingly heterogeneous and the spread of normal cell densities by age 50, ranges from 1000 to 3500 cells/mm^2, and by 80 years from 900 to 2500 cells per mm^2 (Laing et al. 1976). However the individual variation is high, and the variables of age and cell density cannot be considered as predictors of each other with any degree of confidence.

2.5.5. Endothelial cell morphology as seen by specular microscopy

Reports on the endothelium for the age range of birth to 10 years, reveal interesting findings (Sherrard et al. 1987, Speedwell et al. 1988). In the infant eye up to one year old, the cells are small and similar and give a very regular mosaic. Cells are polygonal, mainly hexagonal but the junction angles are rounded to give an almost circular shape. Pairs of cells which have no interposed cell border are scattered throughout many examples.

There is an apparent mean cell loss of 26% during the first year of life. This has been suggested to be due to corneal growth with mitosis if any, failing to keep pace with the increased corneal area (Sherrard et al. 1987, Speedwell et al. 1988).

A large study by Nucci et al. (1990) on normal children showed that there was a rapid decrease in cell density of 13% between the ages of 5 and 7 years and an additional decrease of 12% by the age of 10. Again there are no known growth or endinocrinologic reasons to explain the decrease in cell density, the most likely explanation being the absence of mitosis accompanying the corneal growth or perhaps prepubertal hormonal change. Once corneal growth has stabilised, any cell loss must then be real.

2.5.6. Age changes as seen by specular microscopy

Hoffer and Kraff (1980) reported their analysis of routine preoperative cell counts on 2000 eyes with cataract in the 40-90 age range. Their study revealed that the average mean endothelial cell count was 2400 cells/mm^2 with a range of 1500 to 3500 cells/mm^2. They noted a statistically significant decrease in cell count with age. This study again showed that age alone is not the single predictor of cell density for any eye.

The normal physiological cell loss with increasing age results in the lateral spread of the remaining cells which increase in angularity and clarity as seen by specular microscopy. Although in some subjects, the remaining cells enlarge to similar proportions to maintain a regular homomegethous pattern, many others show an uneven enlargement resulting in an irregular polymegethous mosaic. It has been suggested that the polymegethous endothelium fares less well than the homomegethous endothelium on insult (Shaw et al. 1978, Rao et al. 1984).

In the adult, it is generally agreed that cell enlargement and migration, but not mitosis, follow endothelial injury or disease to form a repair mechanism. If endothelial cells are damaged for example during anterior ocular surgery they are not replaced. Adjacent healthy cells slide to produce a normal endothelial barrier resulting in the normal regular mosaic of hexagonal cells being disturbed in the repair process.

Studies have shown that following cataract surgery there is a differential cell loss which is greatest close to the incision and least furthest from the incision (Leisgang 1991). This creates a cell movement towards the area of damage resulting overall in a reduced cell density, polymegethism and pleomorphism. Corneal grafts have also been studied by specular microscopy and Bourne and O'Fallon (1978) reported a 15-20% cell loss in a group of patients three months after keratoplasty.

2.5.7. Regeneration potential of corneal endothelium

As opposed to the corneal endothelium of other species, such as rabbits, mitotic activity is a rare finding in human endothelium (Tuft and Coster 1990). Olsen and Davanger (1984) reported an increased cell renewal process in uveal melanoma and discovered that if the damaged corneal endothelium of an eye enucleated for choroidal melanoma is put in culture medium, the endothelium starts proliferating with ample mitotic figures. This suggests that cells will renew only under abnormal circumstances, triggered by a specific mechanism activating the process.

Endothelial cell coalescence has been demonstrated in controlled animal studies and in clinical observations (Laing et al. 1983) and appears to represent another possible repair mechanism. Radioisotope studies have revealed H3- thymidine uptake in corneal endothelial cells of man, monkey (Matsubara and Tanishima 1983) and rabbit. The mitotic activity was much lower in the human cornea and not enough to explain the healing mechanism.

Some reports have appeared in the literature suggesting the possibility of cell division. Laing et al. (1984) reported mitotic figures seen by specular microscopy following keratoplasty in a patient followed up for 18 months. However, as yet no consistent significant evidence has been presented for endothelial mitosis in the human cornea.

A great deal of work has been done on investigating potential endothelial growth factors (Yoshida et al. 1989, Woost et al. 1992a, 1992b, Schultz et al. 1992). In a recent study by Happenreijs et al. (1992) their results indicated that, in human corneas, human epidermal growth factor (EGF) promoted endothelial wound healing, predominantly by cell migration. These growth factors may have wide ranging future clinical implications when considering disease of the endothelium.

Although Treffers (1992) reported the occurrence of DNA synthesis and mitosis in human eyes, corneal endothelial cells have been assumed to proliferate rarely. When the cells are damaged and debrided during surgery, the adjacent cells spread, migrate and re-surface the defects. Extra-cellular matricies such as fibronectin, laminin, and types 1 and 1V collagen aid the spreading of endothelial cells (Nishida and Otori 1991).

2.6. Effects of contact lenses on endothelial replication

Although the effects on corneal physiology of acute trauma to the endothelium from ocular surgery are well documented, and have been studied at the histological level, it was not until 1981 that Schoessler and Woloschak first described the long term effects of PMMA contact lens wear on the corneal endothelium as observed by specular microscopy. Since that time numerous researchers have documented the effects of contact lens wear on the morphology of the endothelium. Comparisons have been made to the acute effects of surgery and the normal physiological age changes in the endothelium, but differing opinions exist in the severity, reversibility and significance of the changes observed (Doughty 1989b).

Due to the difficulty in obtaining corneal specimens of previous contact lens wearers there are very few reports of histological studies on the cornea in the literature. Consequently, little or no histological data or evidence is available, on the effects of contact lens wear on the corneal endothelium. Therefore, a recent histological study of the endothelium, conducted on a small sample of human corneas of contact lens wearers, is of real interest (Bergmanson 1992). Six corneas were obtained, 3 normals and 3 with a history of contact lens wear. The 3 with lens wear had 3, 13 and 25 years of lens wearing history.

Bergmanson (1992) found that a young cornea (control age 4 years) consisted of cells that were relatively uniform in their cytoplasmic texture and contained a large number of mitochondria and rough endoplasmic reticulum. The lateral sides of the cells, although showing moderate to complex invaginations, were consistently positioned in an overall vertical orientation. He found the posterior surface showed mostly six sided cells with distinct bumps corresponding to the location of the nucleus. Interestingly the older corneas (two samples ages 23,40) showed great similarity in their ultra structure except for a slight reduction in the complexity of the invaginations of the lateral sides which had become somewhat slanted.

Bergmanson's work (1992a) also provides some histological evidence to support the well documented age related changes in cell size and shape that have been observed on specular microscopic observation of normal eyes such as a reduction in cell density and an increase in polymegethism (cell size) and pleomorphism (cell shape) (Bourne and Kaufman 1976).

Bergmanson's study will also be discussed in Chapter 6 with respect to comments on the technique of specular microscopy. It is hoped that further histological studies will be done when corneal specimens are available, to fully describe the normal endothelium at birth, with increasing age, and the effects due to long term contact lens wear. Correlation with clinical observation using slit lamp and specular microscopy can then be made.

2.7. Effects of contact lenses on the endothelial morphology

2.7.1. Bleb formation

Contact lens wear has been shown to produce both short and long term changes in the corneal endothelium. The classic 'bleb' response first described by Zantos and Holden in 1977 demonstrated that endothelial changes occur within minutes of placing a lens on the cornea. The blebs can be seen bio-microscopically as sudden onset circumscribed defects in the endothelial specular reflection. This response peaks at around 20 minutes and then diminishes over several hours, suggesting that it has little long term significance.

Blebs are usually smaller than cells, and may appear to be within or between cells. They are sometimes clumped and irregular in shape and distribution, and their presence is usually accompanied by loss of clear focusing of the endothelial mosaic (Hodson and Wigham 1988).

According to some observers, the blebbing response diminishes with repeated lens insertion over a long period of time, and in contact lens wearers it is assumed that this indicates that the cornea becomes accustomed to lens wear. The nature of the bleb has not been determined, although interpretation based upon specular microscopy and the negative relief images, suggests that they are localised enlargements of the intercellular spaces (Hodson and Wigham 1988). Their frequent apparent location within cells could be related to the tortuosity of the lateral cell membranes which, as seen by transmission electron microscopy, often underlap the centre of the cell.

Zantos and Holden (1977) believed that lens induced hypoxia was the primary cause of the blebs but further experiments by Holden et al. (1985a) demonstrated that blebs can be produced independently of pre-corneal hypoxia and they most likely represent localised oedema of groups of endothelial cells.

Holden et al. (1985a) suggest that a reduction in pH at the endothelium due to lactate or carbonic acid accumulation may induce these transient changes. They also found that blebs could be induced in the absence of increased corneal swelling, suggesting that this response does not compromise endothelial pump or barrier function at least in the short term (as discussed in section 2.4.). Stromal oedema is not a pre-requisite for the induction of endothelial oedema since hypercapnia causes oedema of the endothelium in the absence of stromal oedema (Holden et al. 1985b).

2.7.2. Polymegethism and Pleomorphism

In the longer term contact lens wearer, the endothelial changes that have been reported relate to variation in cell area (polymegethism) and in cell shape (pleomorphism). These changes along with a reduction in cell density (ECD) also take place normally with age so it then becomes important to differentiate those cellular changes which are age related from those caused by long term contact lens wear.

Certain types of contact lens wear may change the area of endothelial cells in that the population of endothelial cells in the centre of the cornea can show a larger range of areas than non lens wearing eyes. This variance in cell area has been called polymegethism or presented as the coefficient of variation (COV). The COV (of cell area) is usually presented as a mathematical ratio of the standard deviation of the areas of the cells analysed, divided by the arithmetic mean of all the cell areas. This index of polymegethism will identify endothelia that have a larger than normal variance in cell areas, usually on a decimal scale of 0.1 to 0.9.

A low grade endothelial cell loss could give rise to polymegethism and in a study by Caldwell et al. (1982), the authors reported a reduced cell density associated with hard contact lens wear, exceeding the usual decrease with age. They did not have however an adequate control group to support their findings. In fact, other workers have not found a significantly reduced cell density to confirm this finding in wearers of hard PMMA or extended wear soft lenses (Schoessler 1983, Hirst 1984, Holden et al. 1985a, MacRae et al. 1986). It is clear however that the uniformity of size and shape of the normal corneal endothelium may be lost during contact lens wear.

Polymegethism has been reported to occur with long term wear of hard lenses, long term extended wear of soft lenses, but to a lesser extent with very highly oxygen permeable silicone elastomer lenses (Carlson et al. 1990). Schoessler and Woloschak (1981) were the first to describe morphological changes and especially polymegethism in the endothelial cell layer of long term hard lens wearers.

These findings have been confirmed by several other studies (MacRae et al. 1985, MacRae et al. 1986, Stocker and Schoessler 1985). The more severe cases of endothelial polymegethism have been observed in patients who have worn PMMA lenses for 10 years or more. MacRae et al. (1988) found that patients who had worn PMMA lenses for over 20 years had a very large COV of 0.45 compared to 0.36 for less than 20 years.

Extended wear produces a more rapid increase in the COV than daily wear. Osborn and Schoessler (1988) compared 2 months of daily wear with 3 months of extended wear of gas permeable hard lenses. A slight increase in COV was found with extended wear, while daily wear caused little endothelial change. If corneal hypoxia is responsible for these changes, then a contact lens that provides the greatest amount of oxygen to the cornea should produce the least amount of endothelial change. This is in fact demonstrated in the study referred to earlier, where silicone elastomer lenses of high oxygen transmissibility produced little effect on the endothelial morphology (Carlson et al. 1990).

No significant reversal of polymegethism has been demonstrated when stopping lens wear or by changing to gas permeable lens wear. MacRae et al. (1986) found a significant persistence in polymegethism in PMMA lens wearers who had discontinued lens wear for over 4 years. Sibug et al. (1991) followed a few subjects up to 6 months after ceasing PMMA lens wear. Although no significant difference in COV was found, a slight trend indicated an improvement in endothelial polymegethism (reduction in COV), suggesting a very slow reversal process.

A recent study (Bergmanson 1992a) suggested an interesting variation on the significance of polymegethism by proposing that it was merely an artefact of specular microscopy. Although it is the first report in the literature describing histological information from the corneal endothelium of subjects who had worn contact lenses, the conclusions were drawn from what was inevitably a small sample of three eyes and three controls. An important question arises; that is, can a two dimensional picture (the specular photomicrograph), accurately predict endothelial cell size?

The marked re-orientation of the lateral wall of the endothelial cell noted in the contact lens wearers, allows for the possibility that cells with a large anterior surface may have a small posterior surface. Therefore, in a polymegethous endothelium, cells may have altered shape but neither lost nor gained volume. The specular microscope may therefore be unsuitable for three dimensional cell size estimates.

The concept, put forward by Bergmanson (1992a) is interesting, in that the suggestion of cell border overlap which he demonstrated histologically might explain polymegethism as photographed by the specular microscope. Schematically, it seems that it might be possible to explain a large cell bordered by a small cell using Bergmanson's hypothesis, but it would be impossible to explain some of the more unusual appearances found in long term contact lens wear, where large and small cells do not alternate, but can be found in groups or clumps (Doughty and Fonn 1993). This would require unrealistic degrees of cell border overlap.

Further work by Bergmanson (1992b) on endothelial adhesion provides conflicting evidence to his studies on cell border overlap. He suggested that based on his experiments on monkey and pig endothelium, that endothelial adhesion is not controlled by physiological forces, but that fine basement membrane material located in the space between the endothelium and its basement membrane acts as a glue to provide strong adhesion. Further reference will be made to this later in the thesis (Chapter 6), when highlighting specific endothelial changes found in long term contact lens wear. The technique of specular microscopy is described in Chapter 6.

Some of Bergmanson's claims (1992a) could be investigated by assessing Descemet's side of the endothelium, but attempts to view and describe the anterior side of the endothelium have proved to be difficult. Sherrard and Yew (1990) in attempting to investigate endothelial cell changes, conducted experiments correlating light microscopy with scanning electron microscopy on ox and human corneas.

It was found that the basal cell membranes extend processes to neighbouring cells and that some of these unite. If the basal membranes of the endothelial cells are extended into finger like processes and the lateral cell borders are similar then it seems probable that many of the presumed intracellular vacuoles and blebs seen by specular microscopy are actually extra-cellular.

It has been hypothesised that chronic contact lens wear induces a state of relative corneal hypoxia and a subsequent decrease in stromal pH (Bonnano and Polse 1987b). The physiological stress then produces changes in the endothelial cell morphology that appear to develop quickly and regress slowly. However, it has also been reported that in spite of the morphological changes in the corneal endothelium, corneal clarity, corneal thickness and endothelial permeability remain unaffected (Carlson et al. 1988).

Although corneal function assessed by corneal thickness, aqueous flow and endothelial permeability to fluorescein, does not seem to be affected by the change in size and shape of the cell, it is believed that these apparent endothelial abnormalities may be associated with some loss of functional reserve which would make them more vulnerable to a variety of insults. To my knowledge, loss of corneal endothelial cells associated with hard contact lens wear on normal eyes has only been reported in one paper (Caldwell 1982), although other studies report reduced cell densities with PMMA lens wear on eyes which have had previous ocular surgery (Matsuda et al. 1989a).

An interesting study by Matsuda et al. (1989b) looked at the effect of PMMA lens wear on the endothelium of corneal transplants which had been carried out to treat keratoconus. In a matched group study they found that the lens wearers showed a significantly higher coefficient of variation in cell size (polymegethism), a marked decrease in the number of six sided cells (hexagonality), and a significant decrease in the cell density, all relative to the non lens wearers.

The actual cell densities were low in both groups which meant that careful clinical checks on the endothelium were required on longer term follow up, particularly in the lens wearing group. The authors suggest the cause of these changes to be due to lens induced hypoxic stress of the corneal endothelium which could be an important factor in the management of post keratoplasty eyes corrected optically with contact lenses.

Polymegethism has been defined as a disorder of the corneal endothelium characterised by an increased variation in cell size. The most commonly used index of polymegethism in the literature, is the coefficient of variation (COV) in cell area (sd/mean). The endothelium of a healthy individual in the age range of 20-30 years typically has a COV in cell area of approximately 0.22 to 0.25 and this index of variation increases with age (Doughty 1990).

Since 1981 when Schoessler and Woloschak first reported on the polymegethous changes in the corneal endothelium of long term PMMA wearers, several other studies have established that contact lens wear causes an increase in endothelial polymegethism (Hirst et al. 1984, Stocker and Schoessler 1988, MacRae et al. 1985, MacRae et al. 1986, Carlson et al. 1988, Schoessler 1982, Holden et al. 1985).

Endothelial polymegethism has been related to the total duration of lens wear. The more severe cases of endothelial polymegethism have been observed in patients who have worn PMMA lenses for over 20 years and have an average COV of endothelial cell area of 0.45 compared to a mean value of 0.36 for patients who had worn PMMA lenses for less than 20 years (MacRae et al. 1986). Extended wear of contact lenses appears to produce a more rapid increase in the C.O.V. of endothelial cell size than daily wear (Holden et al. 1985).

Several studies suggest that recovery of endothelial polymegethism is insignificant or at best, very slow, although no long term studies on a reasonable sample have been done. MacRae et al. (1989) demonstrated a significant persistence of polymegethism in PMMA lens wearers who had discontinued wear for an average of 4.3 years. Opposed to this Yamauchi et al. (1987) found a slight recovery from polymegethism in a group of PMMA lens wearers who had stopped lens wear for variable periods.

Specular microscopy of the corneal endothelium has been done on previous hard contact lens wearers who had ceased wear for up to 60 months. (Sibug et al. 1991). However their sample was very small (3 patients) and although a slight but statistically insignificant recovery of polymegethism was noted, further studies on the reversibility of morphological changes need to be done.

Highly oxygen permeable materials such as silicone elastomer have been shown to produce virtually no significant endothelial changes, confirming the link with hypoxia attributed to PMMA. Carlson et al. (1990) prospectively evaluated the effect of silicone elastomer lenses on the endothelial mosaic of aphakic subjects.

They found no statistically significant change in mean cell size or COV. Central corneal thickness was decreased after one year but morphological changes usually associated with contact lens wear did not occur with the silicone lenses. Another feature of silicone elastomer lenses is that corneal thickness is not increased on overnight wear studies assessing the effect of the closed eye to contact lens wear (La Hood et al. 1988).

Most contact lenses have been shown to produce 8 to 12% increase in corneal thickness in the overnight closed eye situation. The degree of the increase is proportional to the oxygen transmissibility of the lens. The absence of an increase in corneal thickness associated with silicone elastomer lenses has been explained by the high oxygen permeability of the material creating an oxygen 'reservoir' at the corneal surface (La Hood et al. 1988).

If corneal hypoxia is responsible for these morphologic changes, then a contact lens that provides the greatest amount of oxygen to the cornea should produce the least amount of endothelial change, and conversely, long term PMMA wear on a regular basis would create the worst possible hypoxic situation. One of the difficulties in interpreting these studies is knowing the extent to which reported data on lens wearers and controls can be relied upon. Contact lens wearing histories are often extremely complicated relying on the patient to provide the necessary details of periods of either lens wear or no lens wear.

Holden (1989) has questioned the continued use of PMMA and other low Dk contact lenses, with patients who appear to have tolerated PMMA wear and do not have any obvious symptoms. This is based on the number of PMMA lenses fitted in Spain, the UK and Japan, the basis of his argument being that all the studies which suggest that if the cornea's requirements for oxygen are not met, the product will eventually fail. Currently it is felt that 12% oxygen concentration (a partial pressure of 90mmHg) under a contact lens is required to maintain normal corneal metabolism. Consequently Holden (1989) recommends that practitioners should be using ultrathin soft or moderate to high Dk (50-100) RGP materials.

In summary, the important conclusions reached by Holden (1989) were (a) banning the use of PMMA (b) increasing the use of high Dk RGP lens materials (c) using thin mid water or high water content soft lenses and (d) all plus soft lenses to be in a high water content material.

2.8. Corneal exhaustion syndrome

The possibility of contact lenses inducing stresses on the cornea leading to some form of rejection was discussed by Korb (1961) where he described the term 'corneal exhaustion'. He referred to intolerance to hard lenses after 2-6 months of wear, which seems very short term, but more recently corneal exhaustion or corneal fatigue are terms that have been used to describe cases of contact lens failure after long term wear of PMMA or HEMA lenses (Holden 1988).

These patients had symptoms of lens discomfort, spectacle blur and reduced wearing time associated with oedematous corneas which typically exhibited keratometer mire distortion and fluctuating changes in corneal curvature and spectacle refraction after cessation of lens wear.

Sweeney (1992) in a recent paper describes her investigations on 4 subjects who had sudden intolerance to lens wear and episodes of acute oedema after short periods of PMMA lens wear. Symptoms included ocular discomfort, reduced vision and photophobia. Posterior corneal changes included distortion of the endothelial mosaic and moderate to severe endothelial polymegethism. The 4 subjects were all successfully refitted with lenses of high oxygen transmissibility which eliminated the previous symptoms.

Sweeney (1992) described the condition as 'corneal exhaustion syndrome' and proposed that long term hypoxia and acidosis accompanying PMMA lens wear may be responsible for this syndrome, which is characterised by endothelial dysfunction resulting in inadequate regulation of corneal hydration and subsequent intolerance to contact lens wear. Clinical features of this syndrome are reported to be lens discomfort, periods of corneal oedema, and a general loss of tolerance to the contact lenses.

Since these symptoms are fairly common in contact lens wear, other possible causes can not be excluded. It seems important, at this time, to view 'corneal exhaustion' as anecdotal rather than scientifically proven, until more convincing evidence is presented to support the case for its existence, and/or sufficient cases are presented in the literature. Endothelial morphometry data would need to be correlated with corneal function, in such cases.

2.8.1. Surgical damage to the endothelium

There are correlates with 'corneal exhaustion', when looking at the adaptive reserves of the cornea, following intra ocular surgery. Patients who exhibit higher degrees of polymegethism before surgery face greater risks of serious corneal oedema and reduced vision postoperatively (Rao et al. 1984). There are numerous sources of endothelial damage during intraocular surgery including physical trauma, chemical toxicity, and complications (e.g. bullous keratopathy).

Bourne and Kaufman (1976b) conducted specular microscopy after intraocular lens surgery and highlighted the risk of damaging the endothelium and the consequences of a reduced cell population. Since then, several specular microscopy studies have helped differentiate the effect on the endothelium of intra capsular cataract surgery, extra capsular cataract extraction, phacoemulsification and the use of implants (Tuft and Coster 1990).

After cataract surgery there is an immediate decrease in the percentage of hexagonal cells with increased cellular elongation and increased coefficient of variation most marked in the superior cornea (Schultz et al. 1986). This vertical disparity is absent in non surgical corneas. Earlier studies suggested that the morphological changes resolved within 3 months, although recent studies suggest that endothelial cell loss continues for years even after uncomplicated cases (Liesegang 1991).

However, in the case of the combination of contact lens wear and intra ocular surgery, there is insufficient evidence relating to corneal function postoperatively. Until a significant number of long term hard lens wearers require cataract surgery, the question will remain unanswered. Also the numbers of PMMA wearers are reducing all the time since currently few are being fitted, many are and have been refitted with gas permeable lenses, and others have dropped out of contact lens wear altogether. Nevertheless, there are still some PMMA wearers who, by their own criteria, are successful in achieving and maintaining comfortable, problem free contact lens wear. This group are discussed in detail in Chapter 6.

2.9. Examination of the Corneal Endothelium

Instrumentation that allows a view of the endothelial cells, gives the opportunity to carry out morphometry of the cells, common to many other aspects of medicine. Qualitative judgements are also possible concerning the health and appearance of the cell structure. As outlined in the general introduction to this thesis, one of the major aims of the project was to photograph the endothelium of long term contact lens wearers and conduct measurements on individual cells. In Chapters 6.1. and 6.2. the techniques of specular microscopy and image analysis of photomicrographs are reviewed in detail.

There are currently three clinical instruments for imaging and/or recording the appearance of the corneal endothelium. These are the photo slit lamp bio-microscope, the non-contact specular microscope, and the contact specular microscope.

Although routine slit lamp microscopy of the endothelium (using specular reflection at approximately 25x magnification) can give an overall qualitative assessment of the cells, contact specular microscopy offers significant advantages mainly as a result of increased magnification and resolution. It therefore provides the best means of obtaining photomicrographs from which reliable and valid data on endothelial morphology can be determined from image analysis techniques.

Recent advances in instrumentation relating to non-contact specular microscopy show improved results which may well be suitable for routine clinical purposes where detailed morphometry does not need to be carried out (see Figs.3a, 3b). These new instruments utilise automated focusing and alignment methods to avoid corneal contact and they may be suitable in pre and post contact lens fitting. The manufacturer of one of these instruments has linked such an instrument to an automated image analysis system (Topcon Europe, personal communication 1993).

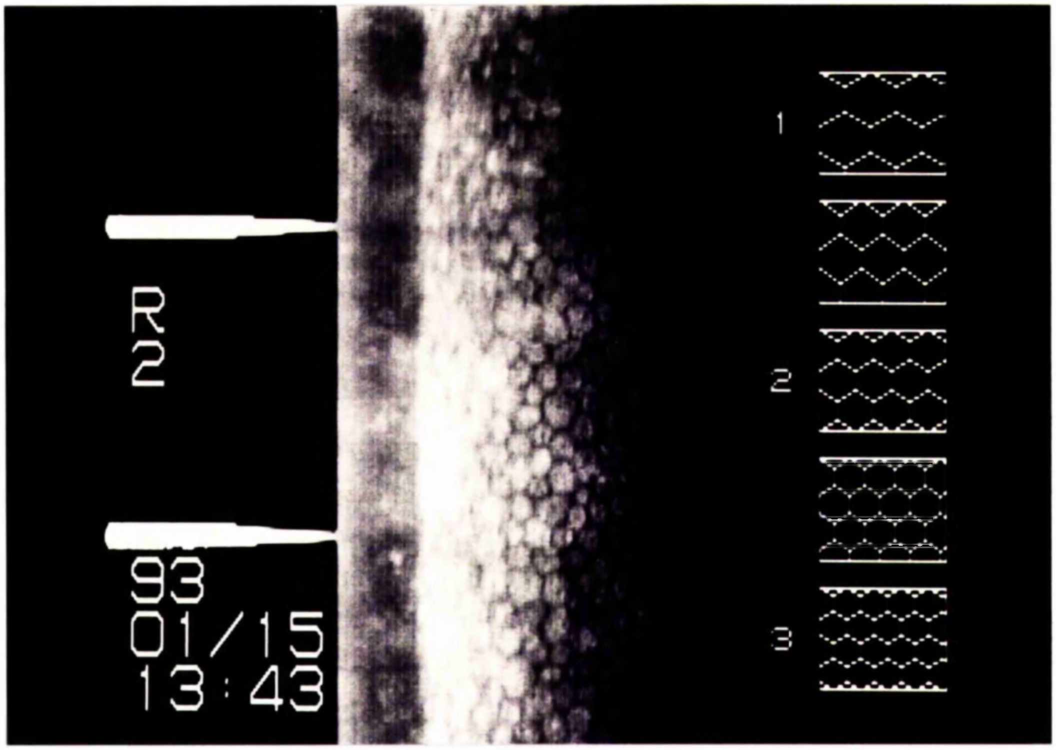


Fig.3(a)

Specular photomicrograph of the author's RE taken with the Topcon non contact specular microscope.

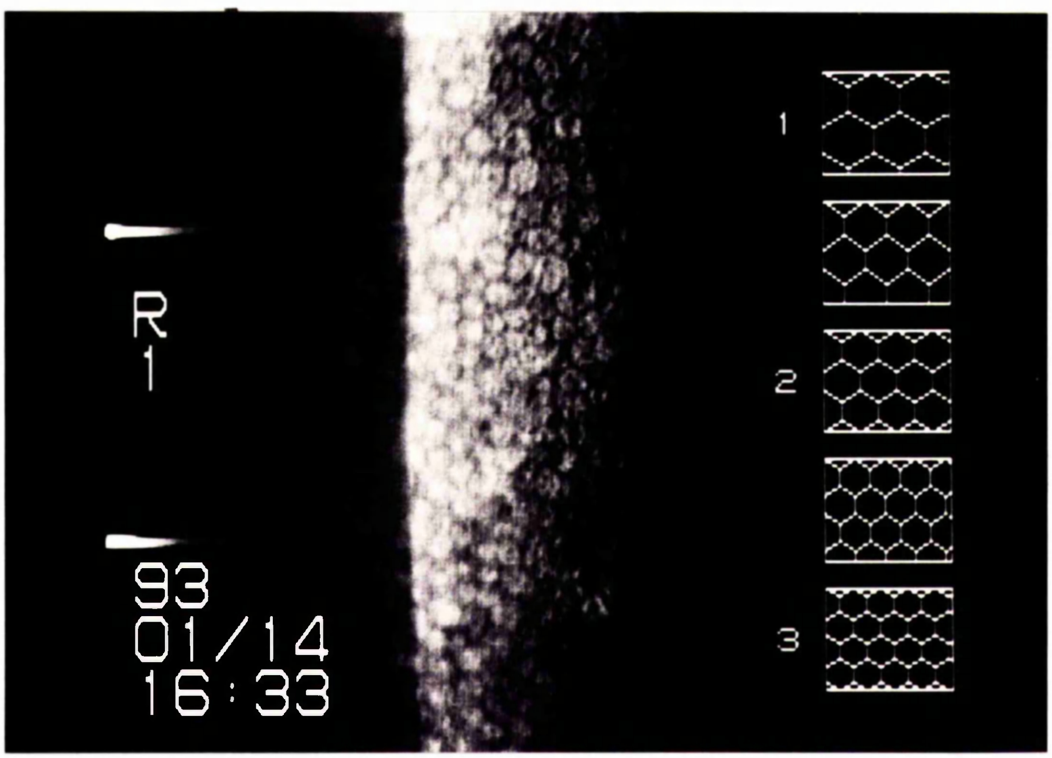


Fig.3(b)

Same eye as in 3(a) 30mins after inserting a soft contact lens. Note the dark spots or 'blebs'.

2.9.1. Developments in Specular Microscopy

Vogt (1920) published the first report in the literature of a method of examining the corneal endothelial mosaic by specular reflection using the slit lamp microscope. He described the specular appearance of normal and diseased corneal endothelium and the excellent illustrations in his series of monographs demonstrate his skill in observing subtle corneal changes. The effects of various inflammatory conditions on the endothelium are clearly demonstrated in his drawings in a way that has only since been improved by the benefit of modern day photographic documentation to allow changes in the endothelium to be studied.

However nearly 50 years elapsed before Maurice (1968) first reported the use of a specifically designed laboratory corneal microscope, to observe the endothelium of the rabbit cornea at 400x magnification. Maurice coined the term 'specular microscope'. He realised the difficulty in viewing individual cells because of the interference with light scattering from the stroma and the epithelial surface and managed to create an image sufficiently defined to see individual rabbit endothelial cells.

His microscope viewed objects illuminated from above. The objective lens acted as the condensing lens with light passing from inside the microscope out through the objective lens to arrive at a focus near the focal plane of the lens. If this position coincided with a reflecting surface the focused light was reflected back through the objective lens and viewed through the eye piece of the microscope.

Since then various modifications have been made to increase the clinical usefulness of the technique, such as the wider field of view or the variable magnifications obtainable with current instruments. Wide field contact specular microscopes capture a much larger area of the endothelium and allow for both quantitative and qualitative assessment of a greater surface area of the endothelium (Bourne and Kaufman 1976a, Waring et al. 1982).

Video cameras have more recently been incorporated into the microscope to provide dynamic views where required, and 'in line' analysis of individual frames (Nishi 1988; Nishi and Hanasaki 1989). As a result of these developments, the specular microscope has made a significant impact on laboratory and clinical studies of the cornea (Hodson and Sherrard 1988).

Specular microscopy can be achieved by either contact or non contact methods. However significantly better pictures have been obtained with the contact method due to less interference from fixation changes and eye movements. The increased magnification also allows individual cells to be viewed.

All instruments work on basically the same principles. That is, light from the slit beam is reflected and refracted at the corneal surface. Some of the transmitted light is reflected at the back surface (endothelium) and if the bright epithelial reflection can be ignored then endothelial detail may be seen in the microscope. The specular reflection is a bright mirror like reflex observed when the angle of incidence of the light source and the angle of reflection to the observer are equal.

The primary feature of these instruments is the microscope housing or optical head. The internal optics of these microscopes project a beam of light on a near normal axis for observation and recording. The field of view produced is determined by the magnification available from the specific optical system in use. The early contact microscopes produced a narrow slit like field of view at 200x magnification. Wide field microscopes were developed to capture more of the endothelium in a single frame (Hodson and Sherrard 1988). In common with many optical instruments, reduced magnification yields a larger field of view and typically, the area being viewed is approximately $1mm^2$, with the current generation of instruments. Optical ray diagrams have been shown by Mayer (1984).

Due to the similarity in refractive indices of the cornea and aqueous, much of the incident light is transmitted through the aqueous. The small difference in refractive index at the posterior corneal surface means that less than 0.02% is reflected back into the cornea from the aqueous interface in a mirror like fashion in which the angle of incidence is equal to the angle of reflection (Sherrard and Buckley 1981). It can be calculated that for near normal angle of incidence used in the specular mode the proportion of incident light reflected is given by

$R=[(n_1-n_2)/(n_1+n_2)]^2$ where n_1 and n_2 are the indices of refraction of the two regions considered.

Light is also reflected at the anterior surface of the cornea and the stroma. A portion of this reflected light is also collected by the objective lens and an image on the film plane is produced. The illuminating beam encounters a series of different refractive indices as it traverses the cornea. At each of the interfaces of the optically distinct regions some light is reflected back into the microscope. The larger the difference in refractive index the more intense will be the reflected light beam.

If the slit beam is narrowed sufficiently, four zones of reflection can be distinguished. If the slit beam is widened a large field will result, but because a large field illuminates more of the corneal stroma and epithelium, interference in the form of light scatter from the stroma increases, and the greater amount of scattered light obscures endothelial cell detail.

This is the main limitation of specular microscopy, in that it is more difficult to view and photograph the endothelium in cases where it would often be desirable, such as in various corneal pathological conditions. However, with contact lens wearers and normal controls, image degradation due to stromal oedema and hence light scatter, should be relatively low. Laing et al. (1976) has described the various components of the image formed by the reflected and scattered light from the different surfaces of the cornea. Thus the final specular micrograph is a composite of all individual zones, some of which cancel or interfere and obscure the individual cell detail.

Various optical modifications to the applanating objective cone can improve the final image and, in at least one of the major instruments (Keeler Konan), the tip of the applanating cone is replaced by fluorite cemented by canada balsam. The face of the fluorite is coated with magnesium fluoride, and the effect is to further reduce the refractive index of the cone tip to minimise the difference created at the cone/cornea interface. Alternatively, soft contact lenses have been tried successfully as a means of reducing the refractive index difference between the cone and the corneal epithelium, and they have the extra benefit of providing corneal protection during applanation (Mayer 1984).

The topography of the posterior corneal surface is dependent on the properties of the endothelial-aqueous interface. Clinical specular microscopes image the endothelial cells if the cornea is reasonably clear, but they are more limited when endothelial blemishes exist as a result of diseased or disturbed endothelia.

These disturbances or events are caused by disruption of the specular reflection from the endothelium-aqueous interface due to the presence of protuberances from the posterior cornea e.g. corneal guttata, swollen endothelial cells or keratic precipitates. These abnormal entities may reflect light away from the specular path and thus produce dark, shadow like events, or may diffusely and/or specularly reflect more light than usual to result in bright events (Bourne and Kaufman 1976).

Sherrard and Buckley (1982) described a fascinating non-specular mode of use of the clinical specular microscope in which the topographic features of the retrocorneal surface and foreign material associated with it can be observed and photographed in apparent relief. The method has been called the 'relief mode' to distinguish it from the specular reflection mode and it has proved to be useful in understanding the endothelial changes in pathological conditions such as Fuchs' Dystrophy (Mayer 1984) and in a number of secondary endotheliopathies (Hodson and Sherrard 1988).

Therefore during specular microscopy, a variety of cell patterns can be seen. It is difficult to interpret them until some form of photographic record is obtained which can be studied. Cell pattern differences can then be categorised under the headings of cell configuration, cell boundaries, posterior corneal surface, and structures within the cells themselves. Clinically, in reviewing the technique of specular microscopy for the Committee on Ophthalmic Procedures Assessment, Hoffner (1991) concluded that although not essential prior to routine cataract surgery, the procedure is indicated in situations which include;

1. prior to cataract extraction and other anterior ocular surgery.
2. eyes where a history of ocular complications exist.
3. eyes that contain intra-ocular lenses suspected of causing intra-ocular inflammation.
4. eyes where the fellow eye has a history of corneal oedema.

2.10. Oxygen Tension at the Corneal Surface

The term oxygen tension is synonymous with the term partial pressure and indicates the number of oxygen molecules present. Oxygen tension is the term normally used in the medical literature. When a gas mixture is dissolved in a liquid and the gas mixture is allowed to stand over the liquid, an equilibrium will be reached. At this time it is said that the tension of each component of the gas mixture is the same in the gas and liquid phases (Fatt 1978). When the eyelids are open, the partial pressure of oxygen dissolved in the tears is equal to that of oxygen in the atmosphere. The term oxygen tension is a way of expressing the concentration of oxygen and is used in this context throughout this report.

It is now well established that one of the most likely causes of physiological disturbance to the cornea from contact lens wear is hypoxia produced by any type of contact lens. In order to maintain aerobic metabolism, the cornea, and especially the epithelium must have an adequate supply of oxygen. As a result of aerobic metabolism adequate energy is created to maintain the cornea in a normal deturgescent state.

When a contact lens is in place, oxygen is supplied to the cornea by either of two routes. One is by 'pumping' oxygenated tears under the lens and the other is the passage of oxygen through the lens. Atmospheric oxygen dissolved in the pre-corneal tear film is pumped under the lens surface by blinking and by lens movement. Oxygen will only pass through the lens if the material is gas permeable. With hard lenses, it has been estimated that the pump effect accounts for about 20% of the tear volume on each blink but with soft lenses, this is reduced to 1% and can therefore effectively be ignored (Mandell 1988).

With PMMA hard lenses, the pump is more important than with soft lenses since hydrogels allow some diffusion of oxygen through the lens. If a contact lens material does allow the passage of gas through the lens it would appear to be more effective than the tear pump since corneal oedema has been more commonly observed with PMMA lenses than pHEMA soft lenses. The greater the gas exchange through the contact lens the more adequately corneal metabolism can be maintained and so the less likely the occurrence of oedema (La Hood et al. 1988). This also applies to carbon dioxide which builds up behind a contact lens.

2.11. Pachometry

Ang and Efron (1989) have shown that there is a direct relationship between the oxygen transmissibility and the carbon dioxide transmissibility of a lens. It was not a one to one relationship but carbon dioxide values were found to be eight times those of oxygen. Prevention of hypoxic changes to the cornea with lens wear requires an understanding of gas transport through the lens and the oxygen requirements of the cornea.

All contact lenses produce some degree of corneal thickness increase following a period of lens wear. The degree of corneal thickness increase as a result of overnight lens wear has been widely used as an index of lens performance in allowing optimal aerobic metabolism. That is, a measurement of corneal thickness is a direct indication of the degree of corneal oedema assuming that base line values are known. The principles of optical pachometry are interesting, and the basis of the measurement of apparent corneal thickness is shown in Fig.4.

Incident light from a slit lamp biomicroscope on the cornea is both reflected and refracted at the epithelial and endothelial interfaces. This forms a corneal 'section', and when viewed by the biomicroscope, is a measure of the apparent corneal thickness.

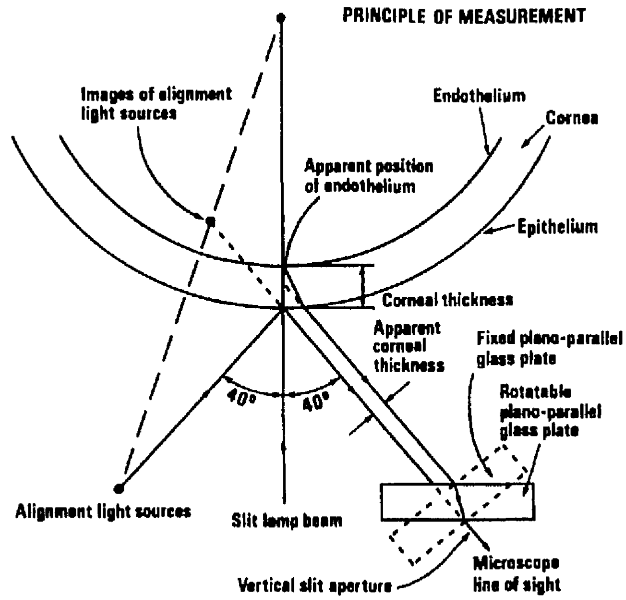


FIG.4

The optical principles of pachometry which measures the width of a corneal section and therefore, apparent corneal thickness.

The amount of corneal swelling produced by a contact lens can be compared to that produced purely by lid closure during sleep. Overnight, lid closure without lens wear typically produces around 4% corneal swelling. Again it has been shown that there is a direct relationship between corneal swelling as determined by pachometry and the oxygen transmissibility of the contact lens. With extended wear as much as 10-12% increase in corneal thickness can occur with soft lenses.

An increase in the oxygen transmissibility (Dk/t) of a lens decreases the amount of corneal swelling. Holden and Mertz (1984) established that a Dk/t of 24 x 10^{-9} units or an EOP of 10% under open eye conditions was required to achieve zero corneal swelling during daily wear. To avoid overnight corneal swelling an EOP of 18% and Dk/t of 87 x 10^{-9} was required.

2.11.1. Oxygen Thirst

Pachometry is therefore an important method both clinically and experimentally of determining the oedema response of the cornea to contact lens wear. An alternative method of determining the corneal response to different types of contact lenses is by measuring the relative oxygen thirst following periods of lens wear (Wilson and Roscoe 1984).

This is measured by polarography where a sensor is placed in contact with the cornea after removal of the particular contact lens. The polarographic sensor is connected to an ammeter which records an electrical current. The tip of this electronic sensor is covered by a polyethylene membrane that has been saturated with air just prior to the test. The contact lens is quickly removed from the eye, and the membrane covered tip of the sensor is touched to the anterior surface of the cornea.

Depending upon how much oxygen had reached the cornea, it is in a state of greater or lesser hypoxia. When the air saturated membrane is touched to the cornea, oxygen flows from the membrane into the cornea. As oxygen leaves the membrane the polarographic current decreases. The data can be plotted as the oxygen tension record of the cornea.

The oxygen requirements of the cornea have only recently been defined. Holden et al. (1984) determined the minimum pre-corneal oxygen tension to avoid corneal oedema. By exposing the eyes of subjects to gases of varying oxygen concentrations, they found considerable individual variations both in the degree of corneal swelling and in the concentration of oxygen required to avoid swelling. The pre-corneal oxygen concentration required to avoid corneal oedema in all subjects was 10.1% (oxygen tension of 74mmHg). This is significantly higher than the previous levels of oxygen which researchers had suggested were necessary for normal corneal function (Mandell et al. 1970, Polse and Mandell 1970, Mandell and Farrell 1980).

Using soft contact lenses worn under extended wear conditions Holden et al. (1983) found that again individuals who were previously adapted to contact lens wear showed large variations in their degree of overnight corneal swelling (range 9.7%-15.1%) and that the mean corneal deswelling for the group of subjects for the 12 hours following sleep was found to be limited (mean =8.2%). They suggested that the desirable maximum overnight swelling with extended wear contact lenses is 8% as this level of oedema allows the cornea to regain normal thickness during the day.

These results were confirmed by Mitzutani et al. (1987) and Hamano et al. (1985). Using very high Dk/t rigid and silicone lenses, they have shown that it is possible to reduce corneal swelling to approximately 4% overnight. According to La Hood et al.

(1988), silicone elastomer lenses can produce even less overnight corneal oedema than no lens at all. This has been explained by suggesting that there is a substantial reservoir of oxygen on the surface of these lenses due to their very high oxygen transmissibility.

The physiological process by which corneal oedema develops as a result of hypoxic conditions at the corneal surface due to contact lens wear has never been fully understood, but recent work by Huff (1991) provides valuable information obtained from experiments on excised rabbit corneas.

By placing PMMA hard contact lenses on isolated superfused rabbit corneas and measuring the change in corneal thickness under a number of different pharmacological conditions (bathing solutions), Huff found two important results. One was that although there was a decrease in pH within the stroma and therefore acidosis, the most significant change was an increase in lactate concentration also in the stroma. When this was countered by pharmacological means, less swelling occurred.

The second important finding was that none of the pharmacological agents used as calcium blocking bathing solutions (diltiazem, dexamethasone, fructose and adenosine), completely controlled the oedema response, whereas the pyruvate dehydrogenase stimulant sodium dichloroacetate used on the tears side, ameliorated the oedema. These agents are known to relieve lactic acidosis systemically, and Huff's results indicate they had no effect on corneas without lenses. This could have future clinical significance in contact lens wear.

The main conclusion of Huff's experiments was that the contact lens induced oedema did not involve the acute cytotoxic mechanisms seen in severe tissue ischemia or hypoxia. The oedema appeared to result from stromal lactate accumulation. This work provides a better understanding of the oedema response, and further experiments are required to determine the pathophysiology in the human eye, where control of corneal oedema could be considered in a range of clinical conditions.

The findings also provide experimental laboratory confirmation for the results of the experiments carried out by Bonnano and Polse (1987a, 1987b, 1987c) on 'in vivo' corneal hydration control referred to earlier. Their experiments consisted of using a thick soft lens to swell an individual's cornea by up to 10% and then by means of optical pachometry, monitor the rate of deswelling.

This rate allowed an index of corneal hydration control (CHC) to be determined for an individual, which may well be a useful indicator of corneal function. That is, once swelling or corneal thickness has been produced, these authors determined how effective the cornea was, in returning its thickness to base line levels.

The primary aetiological mechanism underlying the changes in corneal physiology that have been discussed, are believed to be a hypoxic or hypercapnic effect on the corneal epithelium and endothelium. Other changes which have been reported in the literature, and attributed to hypoxia or hypercapnia, are alterations to the afferent corneal nerve supply and vasodilation of the limbal vasculature (Millodot and O'Leary 1981, Efron 1987).

A brief mention of the limbal vascular response to contact lens wear will be made at this point, since it was originally intended to be part of this project. It is a significant area for further research, and is a topic on which a great deal of misunderstanding exists. Difficulties with instrumentation in conducting video fluorescein angiography of the anterior eye curtailed the experimental work which was planned for this project. However, the pilot study on the assessment of limbal vascular changes associated with extended wear of soft lenses, revealed some interesting observations which require to be followed up with further study.

Corneal vascularisation is one of the most common biomicroscopic signs of the inflammatory response induced by soft contact lens wear (Cunha et al. 1987). The ingrowth of vessels is typically at the subepithelial level, although deeper stromal vascularisation also occurs (Nirankari et al. 1983). Superficial vessels are continuous with the limbal vasculature, unlike deep stromal vessels which disappear from view at the limbus (Efron 1987). The link between hypoxia due to contact lens wear and limbal vascular changes, needs to be further tested.

There have been isolated clinical cases where corneal neo-vascularisation as a result of cosmetic contact lens wear has been so extensive that keratoplasty has been necessary (Ghafour and McEwan 1987, WR Lee 1991, personal communication). However, in any of the reported studies it is evident that the aetiology, grading, assessment, and clinical management of the vascular response to contact lens wear is not well understood. Further studies should be done to understand, and if possible, avoid inflammation in contact lens wear.

SECTION 2

EXPERIMENTAL WORK

Chapter 3. Physical Characteristics of Contact Lenses

The following two chapters cover the measurements carried out to determine two important basic properties of contact lens materials, that is, oxygen permeability and material flexibility and the relationship between them. These are two important contact lens properties since by definition a rigid lens, although being gas permeable, is not flexible but a soft lens being flexible, is very comfortable to wear. Hence the relationship between the two needs to be determined particularly in respect of gas permeable materials which have increased in number and type over the last few years.

3.1. Oxygen Diffusion Through Contact Lenses

Since oxygen diffusion through a contact lens is the major source of corneal surface oxygenation, it is important to understand how oxygen passage can be measured and whether contact lens materials can be produced with significant oxygen permeability. The measurement of oxygen transmitted through a contact lens has been approached in two distinct ways. These have created a very confusing situation and arguments exist to support either concept. One has to distinguish between a material property which is intrinsic, and an ocular response which is variable, and only relevant to the eye from which the measurement has been made.

3.2. In vivo Eqivalent Oxygen Percentage (EOP) concept

This method determines the amount of oxygen depletion of the cornea that occurs after a period of contact lens wear. Using polarographic sensors to measure the rate of oxygen uptake, it can be shown that the cornea has a 'thirst' for oxygen immediately after wearing a contact lens. This thirst is greatest following PMMA lens wear (zero oxygen transmissibility) and least after a gas permeable lens of high oxygen transmissibility. Based on the original work of Hill and Fatt (1960), this technique compares living eye responses with lens wear to the eye's response to oxygen environments up to 21% (the EOP).

It is really a measure of the corneal response, and therefore not a direct indication of a lens property. One criticism levelled at this approach has been that since the inter subject response is so great, then no generalisation can be applied (Fatt 1984).

However with respect to a defined measurement scale, then it is well understood that all measurements are relative to a no lens situation which is equivalent to 21% oxygen.

3.3. In vitro Dk Concept

A more fundamental means of measuring the ability of a lens material to transmit oxygen, is to measure its oxygen permeability (Dk) by electro-chemical methods. When attempting to classify material properties, this would seem to be a more applicable approach assuming that a reliable and valid method could be demonstrated.

Polarography has been extensively used for the purpose of oxygen permeability measurement but has never been fully accepted in the contact lens literature as a reliable method of measurement, and results have certainly been misused by the contact lens industry in the past. As an analytical method it has many attractions and its application to the measurement of contact lenses has been well demonstrated.

The oxygen transmissibility of a contact lens (Dk/t), usually stated as $cm^2/sec\ mlO_2\ mmHg$, and the oxygen permeability of materials (Dk), have become important contact lens and material parameters. It was considered at the outset of this project that if a reliable method of measurement could be developed, it would have both clinical and experimental significance.

3.4. The Measurement of Oxygen Transmissibility of Contact Lenses

3.4.1. Introduction

In recent years there has been an increasing demand for the determination of oxygen levels in the important fields of medicine, physiology, biochemistry, food and drug production, environmental control and corrosion assessment. Consequently polarographic oxygen probes have been developed and constructed to ensure that as sensors they allow rapid precise and cheap measurement of oxygen concentration under a wide range of experimental conditions.

Oxygen determination by polarography is also one of the three most commonly used measurement techniques in analytical chemistry alongside fluoride ion and hydrogen ion concentration determination. It is not surprising therefore to note the various attempts that have been made to apply polarography to the measurement of oxygen transport through contact lens polymers (Fatt 1984, Brennan et al. 1986, Hammano et al. 1985).

However in doing so, much confusion has been created due to differing opinions on the appropriate measurement conditions and in the subsequent analysis of the results. Consequently, there have been lists of oxygen permeability (Dk) values published for gas permeable materials (Hamano et al. 1985), later modified (Brennan et al. 1986) and again further modified (Fatt et al. 1987), as measurement problems became apparent.

The 'edge effect' phenomena first described in detail by Hitchman (1978) and by Linek et al. (1979), has received considerable attention in recent literature when considering measurement errors and the overestimation of Dk values (Brennan et al. 1987a, Brennan et al. 1987b). Correction for the edge effect using flux ratios and involving a simple calculation has also been proposed by Fatt et al. (1987) but correction for potential boundary effects involves multiple single chamber polarographic measurements of the oxygen transmissibility of the same material at different thicknesses.

Dk and Dk/t have been measured by the single chamber polarographic method (D is the diffusion coefficient, k is the solubility coefficient, t is the lens thickness). Using this single chamber technique, it has been possible to accurately and reliably measure the oxygen parameters of many hydrogel and RGP lenses. Dk has been shown to be directly related to the water content of hydrogels and to the silicone content in siloxane RGP's. In the single chamber technique the chamber above the sample is filled with water saturated air, as opposed to the double chamber method where the chamber above the sample is filled with air saturated water.

Instead of offering appropriate guide-lines, researchers have created a situation whereby the real significance of oxygen permeability cannot be evaluated. Consequently, polarography has been questioned as an appropriate analytical method likely to be accepted as a standard system for the measurement of oxygen permeability of contact lenses (Ang and Efron 1989).

A large number of hard gas permeable polymers have been developed in recent years for the manufacture of contact lenses. The main reason for the proliferation of materials has been with respect to their fundamental property of oxygen permeability. Research has shown that changes occur in each layer of the cornea when oxygen levels are reduced by the wearing of contact lenses (Holden et al. 1985).

Hard gas permeable contact lenses have become a significant proportion of the total contact lens correction world-wide and various polymers have been developed which their manufacturers claim may transmit sufficient oxygen for extended wear. It follows that the measurement of oxygen permeability needs to be carefully determined, understood, and the results properly applied by researchers, clinicians and manufacturers.

One of the main criteria for selecting a gas permeable material from an abundant range is still on the basis of oxygen permeability in relation to a given clinical situation. Such varying situations would include a minus lens, a plus lens, a toric, a bifocal, refitting a long term wearer of PMMA. or in cases of extended wear.

Since there is no laboratory standard for this measurement either in the U.K. or world-wide, the main purpose of this series of experiments was to determine the repeatability of the polarographic technique as applied to contact lens measurement. A series of gas permeable materials of varying oxygen permeability, were compared to PMMA. The results of this study would therefore determine the potential use of polarographic oxygen sensors in measuring oxygen transport through contact lenses.

3.4.2. Materials And Methods

Since lenses rather than flats were to be measured, an oxygen sensor designed to measure curved samples was obtained. A custom designed cell was manufactured by Titron (Victoria, Australia) and based on a design originally described by Fatt (1981). The sensor cathode was made of platinum, had a diameter of 4mm and was machined to a radius of 8.00mm. The cathode surface area was therefore 12.77mm^2. The anode was a silver wire within the electrolyte 0.1M KCl. The cell body was remade so that the unit could be filled with electrolyte and operated in the inverted position (Fig.5). The cell was clamped above a water bath to ensure constant humidity (95%) and temperature (35^{o}C).

A potentiostat was used to apply a polarising voltage of -0.74V, and the current flow was displayed on the potentiostat and recorded on a pen recorder for subsequent analysis. The apparatus is shown schematically in Fig. 6.

It was found in a large number of experimental trial runs that if the lens sample was carefully applied to the sensor tip and the method carefully repeated on every occasion, consistent readings could be obtained without the use of cigarette paper acting as a conductor between the sample lens and cathode. A thin layer of electrolyte was always trapped between the cathode and lens and ensured a satisfactory flow of current throughout the measurement sequence. Measurements without the use of cigarette paper thus overcome the difficult problems of quantifying it's contribution to the total resistance to oxygen transport (Fatt 1988).

The lens sample was securely held in place by a rubber 'o' ring and thin stockinette using the sensor clamp. The rubber 'o' ring ensured a clearance between the stockinette and the sample lens thus preventing droplets of condensation forming on the outside surface of the lens samples and eliminating a potential source of error. Although it is impossible to simulate the 'in eye' situation, the measurements were taken with the back surface of the lens in contact with the electrolyte and the front surface exposed to humidified air. This is therefore representative of the actual normal lens environment.

The method of measurement for each lens involved cleaning the cathode surface with lint free tissue, placing 3-4 drops of electrolyte on the cathode surface, placing the lens sample over the cathode and holding it in place by means of the rubber ring and stockinette. The whole electrode was then inverted and placed above a water bath. When applying the polarising voltage, the cell was allowed to run for 5-6 minutes to allow the current to stabilise. The typical chart recorder response is shown in Fig.7. The initial high current level is due to oxygen in the electrolyte trapped between the lens and the cathode. Within a few minutes this drops to a steady state level as oxygen is depleted and the differential gradient between the two lens surfaces is reduced.

For calibration purposes a PMMA lens was used before and after each measurement session. The average 'residual' or 'dark' current was found to be 0.24 micro amps. This would indicate very slight permeability of the material or side diffusion of oxygen from the sample edge. These possibilities were checked by placing the sensor inside an oxygen free environment (argon) and demonstrating zero current. This finding supports the idea of very slight side diffusion of oxygen at the sample edge. Thus this 'residual current' value was subtracted from all subsequent measurements on gas permeable materials.

To determine the repeatability of the measurements, five representative, commercially available gas permeable materials were chosen and coded. These consisted of two low to medium Dk materials (one silicone acrylate, standard gas permeable (SGP) one fluoro-silicone acrylate Boston Equalens, two medium Dk materials (fluoro-silicone acrylates Fluoroperm 60, Fluorex 700) and one higher Dk material (fluoro-silicone acrylate, Fluoroperm 90), as described by their respective manufacturers. Two further experimental higher Dk materials (fluoro-silicone acrylates, Quantum 2, Boston Equalens 2) were tested when they became available from the manufacturers.

Samples in the form of lenses were prepared especially for the polarographic measurement. They had a base curve of 8.00mm, an overall diameter of 9.4mm and consisted of four samples of different thickness in each material (0.1mm, 0.2mm, 0.3mm, 0.4mm). The sample 'lenses' were made on a manual contact lens lathe, from material buttons supplied by each manufacturer.

All samples of each material were supplied from the same batch number. The determination of oxygen permeability of any one material, involved 5 measurements on each of 4 samples, giving a total of 20 measurements per material. These special lenses had parallel polished surfaces giving uniform thickness, avoiding the need to determine lens average thickness for Dk/t computations.

The errors involved in these computations have been previously documented (Fatt et al. 1987). Ideally very thin samples (<0.10mm) should be used for measurement but these are very difficult to produce in lens form and are less stable when placed on the polarographic electrode. Samples of the two experimental materials were supplied by the manufacturer (Bausch and Lomb/Polymer Technology) to the specifications requested.

Each sample of each material was measured in a random order until 5 readings of each lens had been obtained. At the completion of all measurements, the chart records were measured and the polarographic current values for each sample determined. The mean and standard deviation of the 5 readings for each sample of each material was then calculated. Polarographic current values were transformed to measures of Dk/t using the equation described by Mancy et al. (1962), adapted for polymeric films by Aiba et al. (1968) and also by Brennan et al. (1987b) when using a similar sensor to that used in this study.

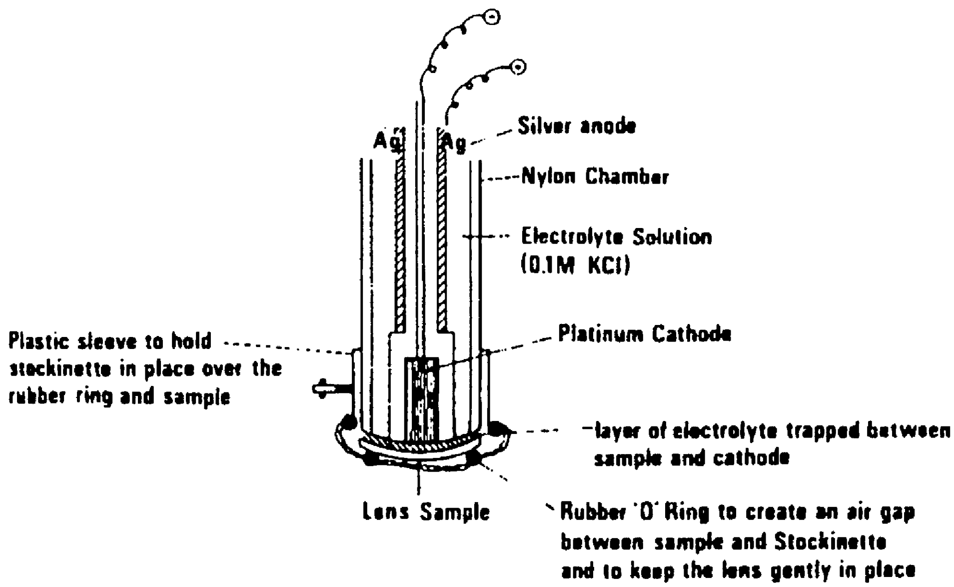


FIG. 5

The polarographic electrode designed for the measurement of oxygen permeability.

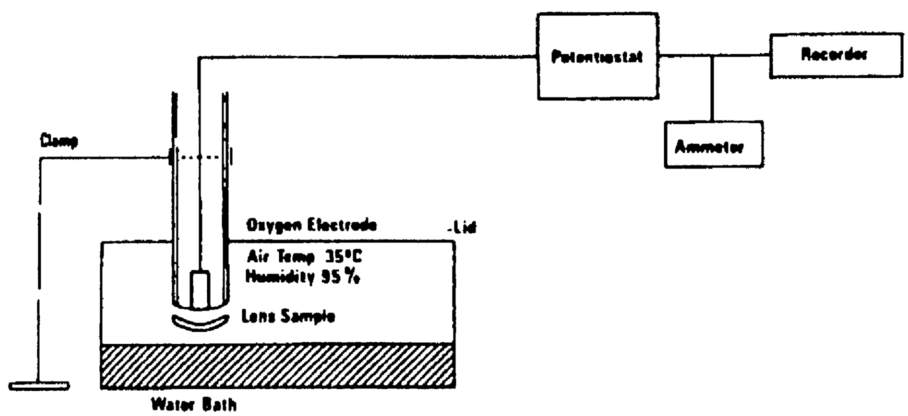


FIG. 6

The experimental apparatus used to record polarographic currents when oxygen diffused through the lens samples.

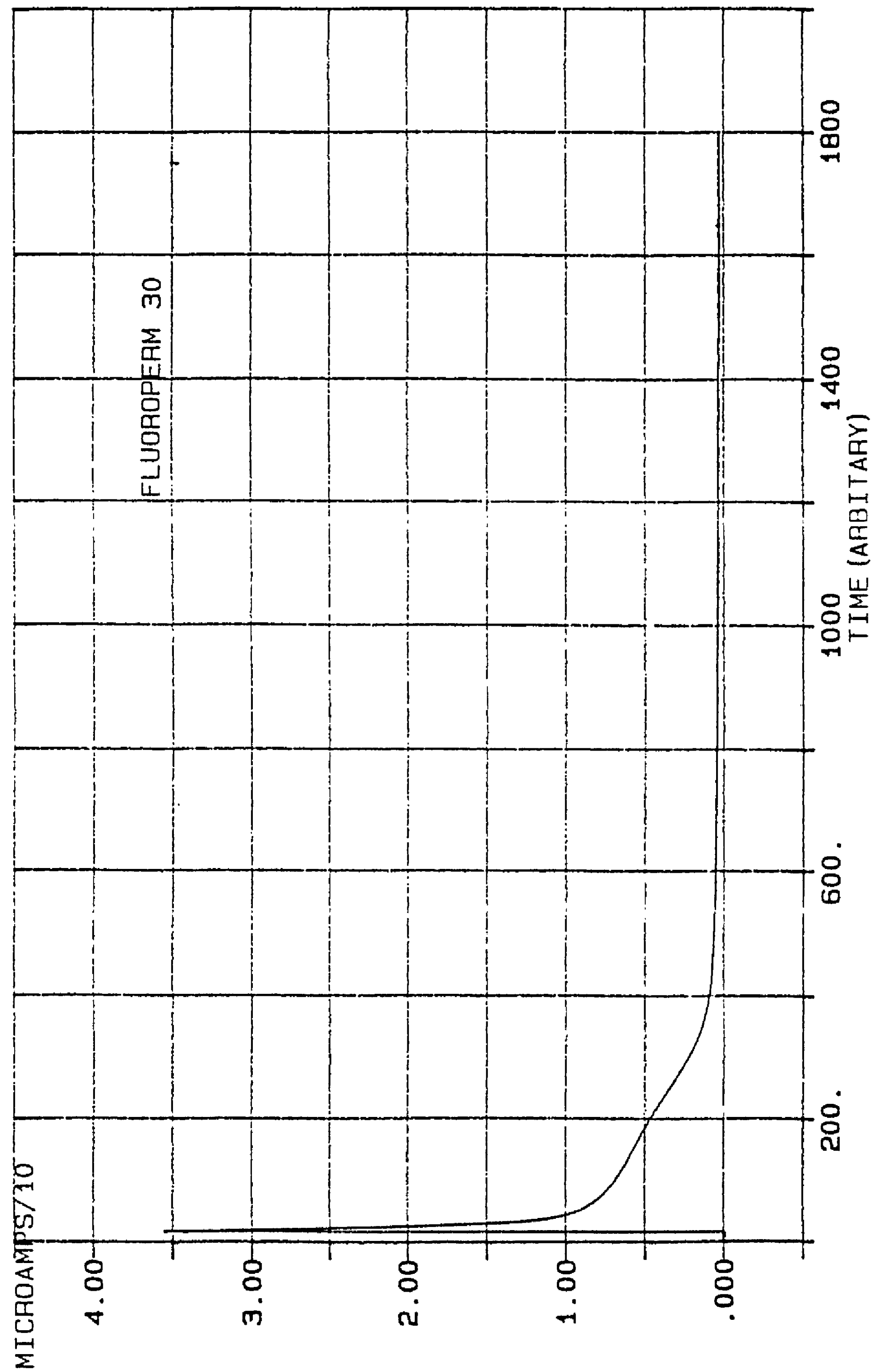


FIG.7

The typical chart recorder response obtained from the polarographic cell. In this case the response (electrical signal) has been input to a computer and the units are arbitrary. The initial high current level falls rapidly to a steady state level as oxygen is depleted at the cathode.

3.4.3 Results

Fig.8 shows the relationship between sample thickness and the measured polarographic current for each of the five standard materials. The error bars show ±1 standard deviation. Predictably it can be seen that as sample thickness decreases polarographic current increases in the curvilinear manner shown. In all five cases a third order quadratic was the best fit.

It is clear from the standard deviations, that the spread of results is minimal for each sample, the average standard deviation for a series of 5 readings on one sample being ±0.15 micro amps. The average standard error of the mean of a typical series of 5 readings was ±0.07 micro amps. Expressing the standard deviation as a percentage of the mean gave an average error in the measurement of 3.2% across all six materials. This contrasts with much higher error values quoted previously for a polarographic method (Brennan et al. 1986).

The greatest variability was found in the highest oxygen permeability material in the standard group. However, even in this case, the variability was well within the experimental error and the likelihood is that it was more difficult with that material to achieve a constant thickness water layer between the lens and the cathode with the thinner samples, due to their flexibility.

Therefore contrary to previous reports it would seem that the repeatability of polarographic currents as demonstrated in this study is extremely good if the procedures referred to in the measurement section are rigidly adhered to. It was also noted that using these procedures, very similar results were obtained between two observers independently measuring the same samples.

To provide both the contact lens profession and the industry with meaningful information the values of current need to be converted to oxygen transmissibility values (Dk/t) as previously mentioned.

The equation allowing the calculations of the oxygen transmissibility (Dk/t) of polymer films from the current flowing in a polarographic cell is

$$P = Dk/t = i/nFAP$$

where i is the current (micro amps),
n is the number of electrons involved in the reduction of one molecule of oxygen,
F is Faraday's constant, (9.65×10^{-4} coul/mol^{-1}),
A is the surface area of the cathode ($12.77mm^2$) and
PO_2 is the oxygen tension at the lens surface (155mmHg).

For the electrode system described in this paper the above equation can be reduced to $Dk/t = i \times 2.932 \times 10^{-9}$ (cms/sec)(mlO_2/ml/mmHg) by appropriate substitution of values.

The average current (i) for each sample of each material was therefore converted to a value of oxygen transmissibility, the reciprocal of which gave the total resistance of that sample. A graph was then plotted of sample thickness against resistance. This showed a curvilinear function for four of the materials, and a linear function for the fifth (Fluoroperm 90), demonstrating the likely presence of the edge effect. Non linearity has been explained by Brennan et al. (1987b) who have proposed a method of correcting the 'edge effect' using curvilinear functions rather than linear regressions.

Fig.9 shows the curves obtained for the five standard materials measured. In each case except for Fluoroperm 90 (fluoro-silicone acrylate), a second order quadratic equation gave the best curvilinear regression. A simple linear regression fitted the data for Fluoroperm 90 and therefore had no correction for the 'edge effect'. In Fig.9, the reciprocal of the slopes of the lines at zero thickness gave the 'true' permeabilities corrected for the 'edge effect'.

It is clear that the 'edge effect' is thickness dependent and ideally very thin samples should be measured to increase the range over which measurements were made. However it is technically very difficult to manufacture lens samples thinner than 0.1mm in high oxygen permeable materials. When this was attempted, the samples were easily broken or chipped. Therefore, this remains a limitation of the polarographic method described.

Using the above regression analysis and the graphical method of correcting the edge effect using the procedure described by Brennan et al. (1987b), the oxygen permeability values of the five materials measured were found to be 18, 36, 38, 43, and 76×10^{-11} (cms^2/sec.)(mlO_2/ml/mmHg.) respectively. The value for Fluoroperm 90 may be artificially high due to the lack of any edge correction factor.

These figures are shown for comparison with manufacturers' figures in Fig.10 and with other published data in Table 3.

Fig. 10. shows that in each case the measured value of Dk is less than the quoted value, due probably to the lack of 'edge effect' correction in manufacturers' figures. This again highlights the need to review currently used Dk values quoted in manufacturers technical specifications of material properties. The difference between quoted and measured values is consistent however, which suggests that a correction factor could be applied to manufacturers' permeability data to give actual values (approximately 2:3).

The two experimental (high Dk) materials were dealt with separately for three main reasons. Firstly they became available later in the project, secondly the manufacturers quoted the Dk values to be greater than 100, and thirdly, following experiments conducted by Fatt et al. (1987), an 'edge effect' correction using flux ratios for high Dk materials was proposed. All tests done on these materials were identical to the procedures described earlier.

Since the flux ratios proposed by Fatt et al. (1987) for the measurement of RGP polymers, were based on a system which used a cigarette paper between the sample and the cathode, his proposed alternative hydrogel ratio was used. In the case of both of the experimental materials measured, the values for current were converted to Dk/t and then t/Dk, before being corrected by the flux ratio term from Fatt's equation (1987).

This was taken as
Flux Ratio = 1+ 5.88L(cms), where L= sample thickness.

Fig.12 shows the corrected data plotted as the sample thickness against the resistance (t/Dk). A linear regression for each sample gave the best fitting line and again the reciprocal of the slope gave a measure of the Dk of the material. The Dk of Equalens 2 was 66.4 and for Quantum 2 the Dk was 70.6, both figures corrected for edge effect. The uncorrected figures were 89.2 and 95.5 respectively (Table 3).

Interestingly, the data for these two high Dk materials was found to be linear before correction but was corrected because;

1. the configuration of the sample lens on the probe was such that theoretically, an edge correction was required;

2. for materials of Dk greater than 70×10^{-11} it has been shown that the edge corrected data is in closer agreement with the coulometric data which does not suffer from the edge effect problem (Newton-Howes 1990),

3. other centres conducting similar measurements on contact lens materials quote values based on this correction.

Fig. 11 shows the increase in current with the decrease in sample thickness for the two experimental materials. Fig.12 shows the linear regressions for the corrected data, the reciprocal of each slope giving a measure of the Dk of each material.

Brennan et al. (1987b) have pointed out that as the sample thickness approaches zero, the flux lines approach the condition for flow in a vertical cylinder. These authors suggested that a quadratic equation be empirically fitted to the thickness versus resistance data points, and that the slope of this fitted curve at zero sample thickness be taken as the reciprocal of the true permeability. This is the method that was applied to the data reported in this study.

The main disadvantages of this method have been pointed out by Fatt (1988) who chose to solve the problem of funnel shaped oxygen movement in the sample by decomposing the sample into small elements and solving the diffusion equation in each element. Hence, he proposed the use of 'flux ratios' referred to earlier. However, whether the polarographic data needs correcting for the edge effect, requires some careful analysis, since it was observed that in this series of experiments, the edge effect was less apparent with the higher Dk materials than it had been when measuring the lower Dk materials.

Also Fatt (1988) has pointed out that for oxygen permeabilities greater than 200, the flux ratio theory is less accurate. As pointed out by Newton-Howes et al. (1990), the edge effect correction does not seem appropriate, possibly because the efficiency of the oxygen probe falls off at higher current densities. A thorough scientific justification of ignoring edge effect correction in the higher Dk materials is necessary, by detailed and careful calibration of the polarographic electrodes.

As outlined by Holden et al. (1989), it is important to quantify the error in the measurement of Dk. The Dk for each material was determined by the reciprocal of the slope of the best fit straight line as originally described by Fatt and Chaston (1981). The standard error of Dk (SE Dk) = SEm/m^2 where m is the slope of the straight line, and SEm is the standard error of the slope (Holden et al. 1989).

To illustrate this, and to indicate the typical measurement error in the calculation of Dk described in this Chapter, Fig. 13 shows the error for the Quantum 2 experimental high Dk material indicating the slope, the SE of the slope, and the 95% confidence limits of the slope. The data in Fig. 13 is corrected for edge effect.

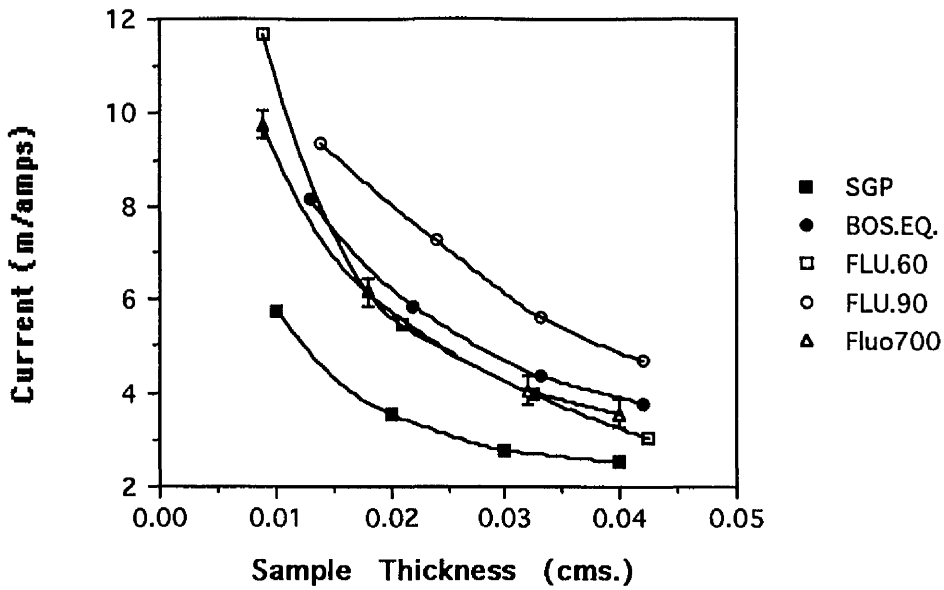


FIG.8

Polarographic current plotted against sample thickness for each of the material samples tested. The error bars show ±1SD for the Fluorex material.

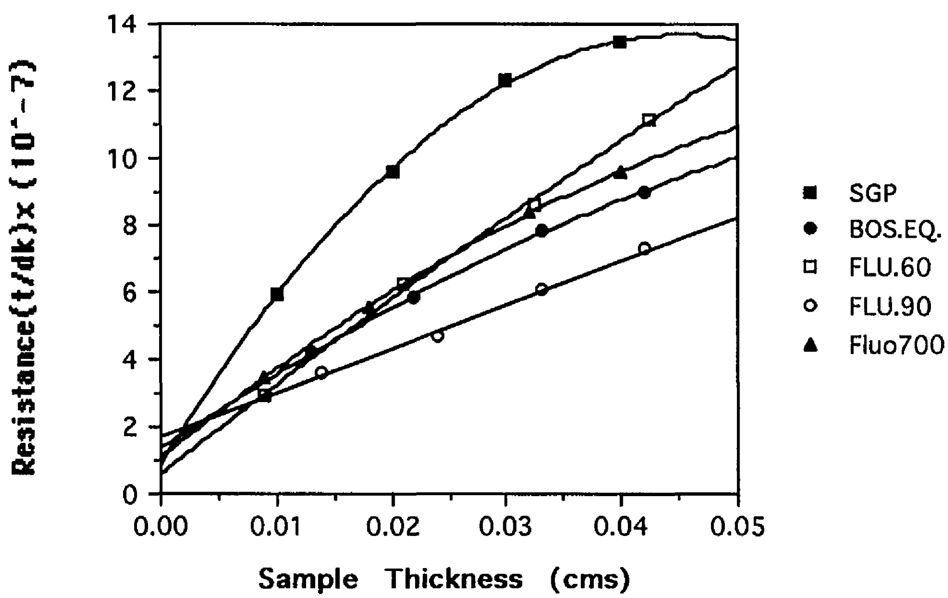


FIG.9

Sample resistance plotted against thickness for each of the materials tested. The reciprocal of the slope of each of the lines at zero thickness gives a measure of oxygen permeability (see Table 3 for Dk values on these materials).

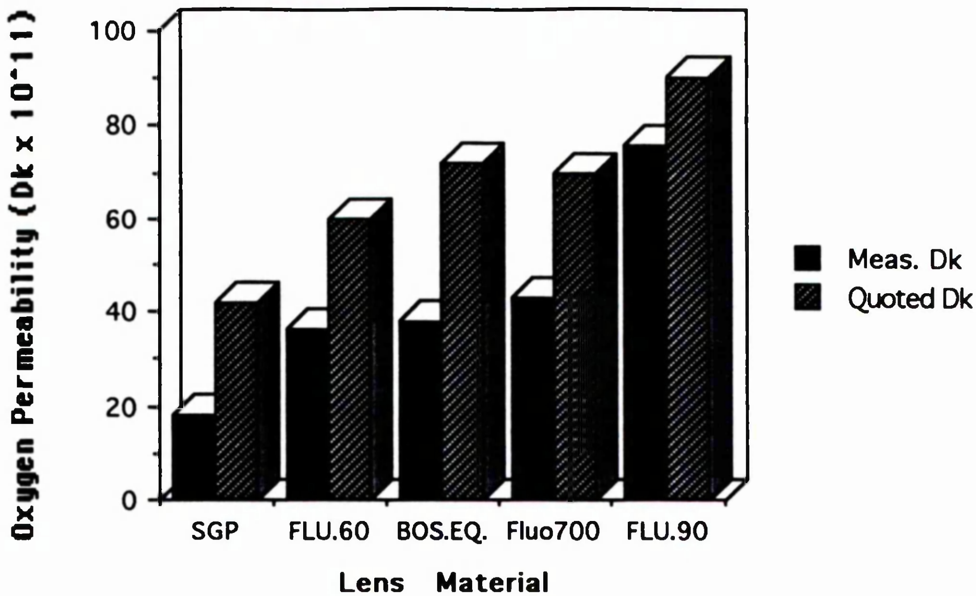


FIG. 10

A comparison of the measured and the manufacturers' quoted oxygen permeability values showing the consistent trend of the measured values always being less than those normally quoted in the literature. The likely explanation for the consistent difference is that no account has been taken for 'edge effect' in the data quoted by individual manufacturers.

MATERIAL	Fatt*	CCLRU*	Stevenson*	Coulometric
SGP	11		18	7.5
Fluoroperm 30	28			
Fluoroperm 60			36	41
Fluorex 700			43	
Fluoroperm 90		57	76	
EQUALENS	48	49	38	43.4
Quantum 2	99		95.5/70.6**	130
Equalens 2			89.2/66.4**	

TABLE 3

List of Dk values obtained by different research centres for the more commonly listed materials. Due to the complexity of materials and differences between countries, very few materials have been common to all researchers to allow a proper comparison of the Dk data.

*Fatt (1988); Fatt and Ruben (1993).

*CCLRU- Cornea and Contact Lens Research Centre, Sydney, Australia.
Holden et al. (1989); Newton-Howes (1990).

* Coulometric method, Ciba Vision, Atlanta, USA.
Winterton et al. (1987,1988).

*Stevenson, see Chapter 3.2.

** Figures presented are (1) uncorrected (2) corrected for edge effect as determined in this study.

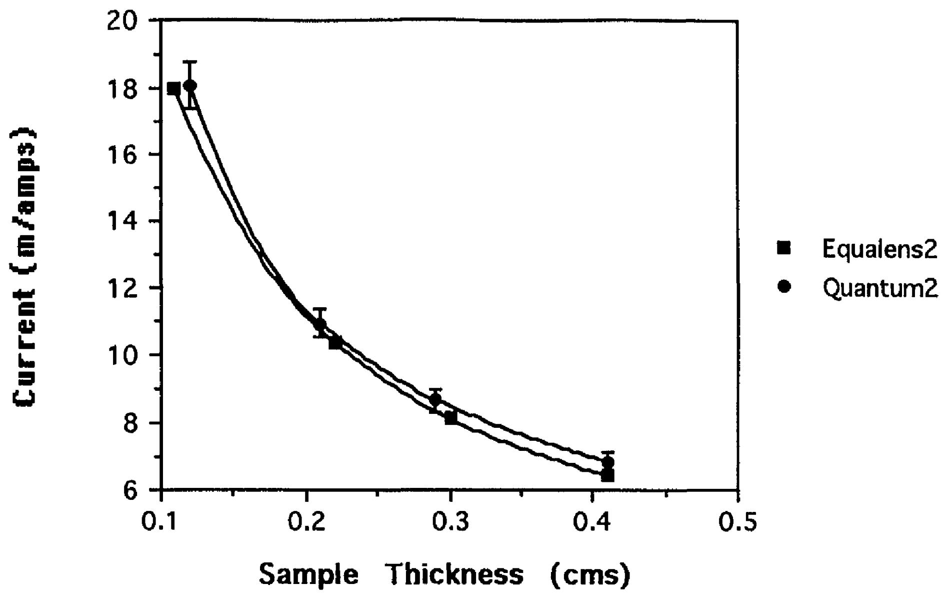


FIG.11

The polarographic current plotted against sample thickness for the two experimental high Dk materials measured. Error bars show the typical sd of each measurement.

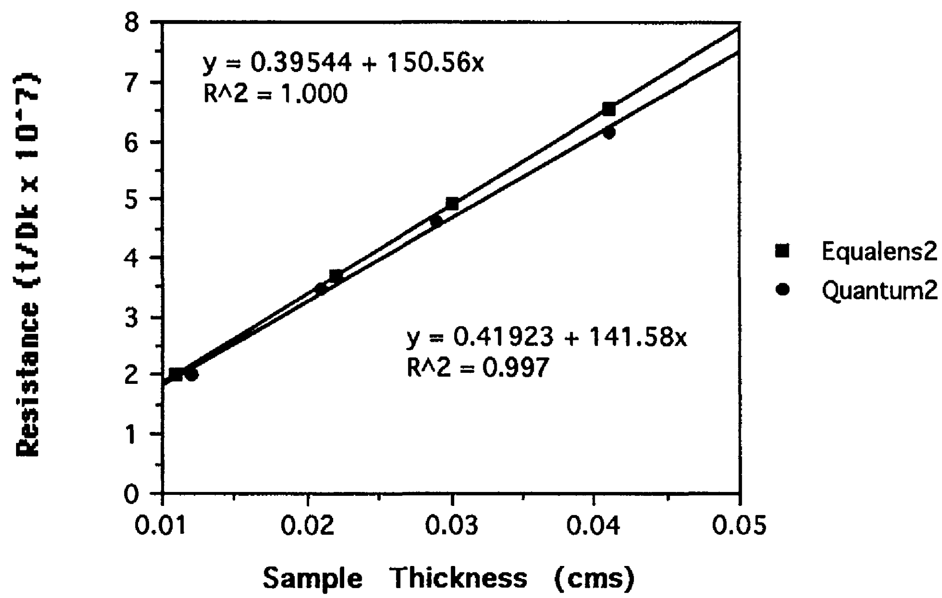


FIG.12

The sample resistance plotted against the sample thickness for the two experimental high oxygen permeability materials. A linear regression has been fitted to the data corrected for edge effect. The reciprocals of the slopes of each line gives the Dk of the materials. These values are **66.4** for Equalens 2 and **70.6** for Quantum 2.

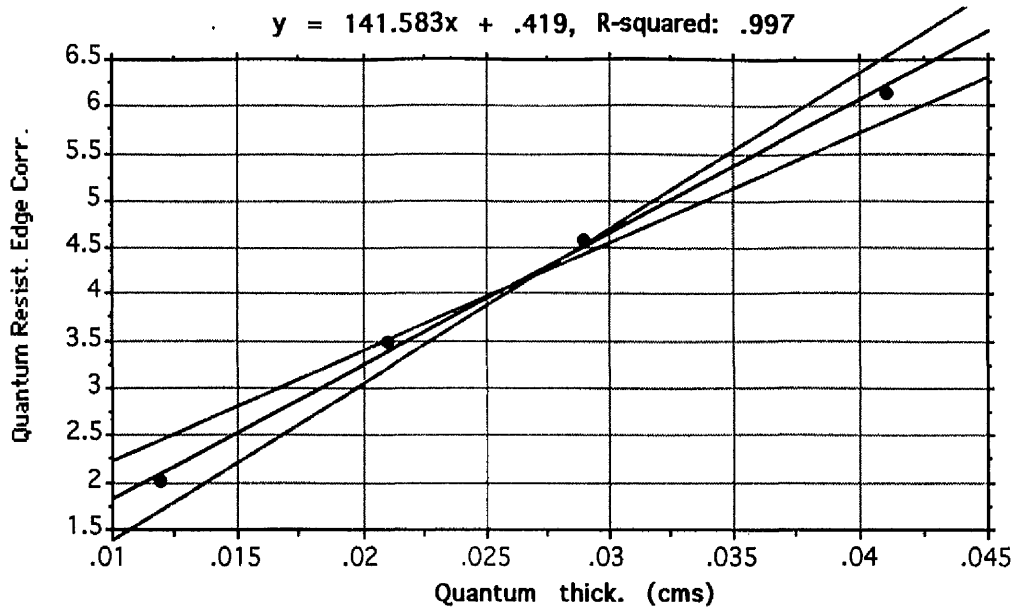


FIG. 13

Taking Quantum 2 as an example, this shows a linear regression fitted to a plot of the data, relating the thickness of the sample to the reciprocal of the transmissibility. The 95% confidence intervals have been fitted to the slope of the regression line. The slope of the regression is 1.4×10^9 and the 95% confidence interval is 1.16 to 1.66×10^9. This indicates that the Dk of Quantum 2 corrected for edge effect, is between 60.2 and 85.6×10^{-11}. Again all units of Dk are $\times 10^{-11}$ $(cms^2/sec.)(mlO_2/ml/mmHg.)$

3.4.4 Discussion

Good repeatability of the polarographic current has been shown in a range of currently available gas permeable materials. It should therefore be possible with careful choice of sensor and minor modification to take contact lens samples, and use strictly controlled measurement procedures to obtain reliable results. The most important points were found to be cleaning the platinum cathode surface before every measurement and eliminating a paper membrane between the lens and cathode.

The method used in the first part of this experiment to correct for the 'edge effect' (Brennan et al. 1987a) is relatively easy to use assuming the availability of the appropriate regression analysis programme on a micro-computer (Statview/Apple Macintosh). The curve fitting is done automatically, with the goodness of fit (r^2) indicating the best fitting curve to the data. The reciprocal of the slope of the line at zero thickness gave the permeability of the material.

Fatt et al. (1987) suggested the use of flux ratios to correct for the 'edge effect', a technique based upon finite analysis. However Fatt's equations were designed for an electrode system incorporating a paper membrane between the lens and cathode, and thus separate equations would be required for the system described here. However his flux ratios for hydrogel materials were felt to be appropriate and were used to correct for the edge effect. A comparison of the two methods for correcting the 'edge effect' is required as evidenced in the measurements within the thickness range used in these experiments.

Following recent work by Newton-Howes (1990), and on the basis of the results found in this experiment, it is clear that there is some non-linearity with the polarographic system across the permeability range of gas permeable materials. Comparison of polarographic results with the coulometric method has shown good agreement with low to medium Dk materials, when the data is corrected for the edge effect, whereas in the higher Dk materials, the comparison is better with uncorrected data (Newton-Howes 1990).

This is consistent with the results in this project which show the presence of the edge effect in the low Dk materials (e.g. SGP) but much less in the high Dk materials (e.g. Fluoroperm 90). As previously mentioned, the data on the higher Dk materials appeared linear before edge effect correction, so this point requires further investigation if polarography is to be developed as an acceptable working method in industry and research centres.

The errors in the measurement of Dk in this work have been consistent with the previously published reports. Taking Quantum 2 as the example in Fig. 13, the reciprocal of the slope of the straight line gives a Dk of 70.62. The SE of this measurement was 2.88. The uncorrected data for the same material gave a Dk of 95.52 and a SE of 8.6. Holden et al. (1989) quote SE's of their Dk measurements corrected for edge effect, to be between 0.1 and 4, and for the uncorrected measures, to range from 0.3 to 7.

Fatt and Ruben (1993) state that the standard deviations of their Dk values uncorrected for edge effect, range from 2% to 7% of the Dk value. When corrected for edge effect the standard deviations were somewhat smaller. Again this is similar to the standard deviations (%) shown in Figs. 8 and 11 for the measurements obtained in this study.

The method described in this work could be considered as a possible standard for the measurement of oxygen permeability. It is hoped that a number of centres world-wide (UK, Australia, USA, Japan) could agree on the Dk values of a series of standard reference materials measured independently (Newton-Howes and Holden 1990).

Alternatively, calibration of a polarographic oxygen sensor system would be possible against a known standard, the oxygen permeability of which could be obtained by coulometric methods, as has been suggested previously (Winterton et al. 1987, Winterton et al. 1988). Collating data on oxygen permeability from the published research studies, shows some degree of agreement between the major centres and with the results obtained in this study (Table 3).

However, the limitations of the coulometric method in measuring both hydrogel and hard lens materials, and the expense of the apparatus and manpower involved in large numbers of lens measurements, mean that the polarographic method should continue to be used, particularly as the measurement difficulties are now better understood and controlled.

Despite the doubts cast by some workers, a reliable method of measurement of oxygen permeability can be obtained if satisfactory calibration can be achieved. Accuracy and repeatability of polarographic measurement has been demonstrated recently in experiments by Weissman et al. (1991) who found that by 'stacking' hydrogel lenses one on top of the other, reproducible results were obtained of the oxygen permeability of that material.

If contact lens materials of significantly higher oxygen permeability than those presently used are going to be continually developed in response to the demand from clinicians, then their measurement remains a problem. Weissman et al. (1989) measured silicone elastomer material using a single chamber polarographic technique with edge and boundary corrections. They arrived at a value of 190 but with a standard deviation of ±79. The excessive standard deviation suggests that an improved method to evaluate contact lens materials with Dk values of 100 and more should be determined. Alternative methods such as those described by Mizutani (1989) and Fatt (1991) based upon gas to gas methodology may be worth further investigation.

If the results of this experiment can be interpreted properly, accurately, and corrected for the 'edge effect' to give values of material oxygen permeability then clinicians, researchers and the contact lens industry can look forward to more useful guidance on this very important clinical aspect.

At the present time, although imprecise, it may be sufficient to know from a clinical viewpoint that a high oxygen permeable material has a Dk value of greater than 70 x 10^{-11} $cms^2/sec\ mlO_2/mlmmHg$. More specifically, it is suggested that Dk values could be considered to be

(a) low : Dk up to 30
(b) medium : Dk from 30-70
(c) high : Dk 70 and above.

Holden and Mertz (1984) determined that a Dk/t value of 24 x 10^{-9} was necessary for successful daily wear and a Dk/t value of 87 x 10^{-9} was necessary for extended wear. If these values are accepted as clinical guidelines, then materials from the medium category suggested above would need to be used for daily wear applications and that current lens materials are unsuitable for extended wear even when materials from the high Dk group are used.

3.4.5. Summary

A polarographic method of oxygen determination across contact lenses was developed. A custom designed polarographic cell with a platinum cathode and a silver anode was used to measure a series of specially prepared contact lens samples made from five standard commercially available and two experimental gas permeable materials* in a range of oxygen permeabilities (Dk). The polarographic cell was inexpensive (approx. £200) and the apparatus took about 15 minutes to equilibriate and calibtate prior to each experimental run.

On a series of measurements the polarographic current was shown to be highly reproducible without the need for a wet membrane between the lens and the cathode as suggested by other researchers. A layer of solution was adequate. Values of current were converted to oxygen transmissibility (Dk/t) and the reciprocal taken as the resistance (t/Dk). The results were plotted comparing the sample resistance (t/Dk) to the sample thickness.

Four of the five standard materials showed non-linear functions thus demonstrating the 'edge effect' and were fitted using second order quadratics. The reciprocal of the slopes of the curves at zero thickness gave the Dk's of each of the five materials. The remaining material (Fluoroperm 90) showed a linear function and the reciprocal of the slope of the regression line gave a measure of the Dk.

The data from the two experimental high Dk materials although linear was corrected using the flux ratios method. The resistance values (t/Dk) were plotted against sample thickness and a linear regression of the data gave the best fitting line. The reciprocal of the slope of this line gave the corrected Dk of the materials.

The significance of this system towards setting up a standard for the measurement of oxygen permeability of contact lens materials is discussed and a comparison made with other proposed methods and published data.

* these materials have subsequently become commercially available.

Chapter 4. THE FLEXIBILITY OF CONTACT LENS PLASTICS

The Measurement of Young's Modulus of Elasticity

4.1. Introduction

Various anecdotal reports suggest that contact lens materials which are more oxygen permeable tend to be more flexible. Some data is available to support this theory (Fatt 1988) although the materials measured were not typical of those available at the present time. The clinical significance of this possible link between permeability and flexibility, is that lenses made from such materials may bend or flex in situ, giving rise to induced astigmatic effects. The intention of this part of the experimental work was to try and specify the flexibility of some gas permeable materials across a wide range of oxygen permeability based on a measurement method designed specifically for contact lens samples.

The determination of some of the fundamental mechanical properties of contact lens materials is important when considering the relative properties and merits of various materials. In the engineering industry, test methods are well developed using prepared samples of the materials to be tested. This also applies to the plastics industry but however, in the contact lens industry, it may be more appropriate to test lenses rather than material samples which can be difficult to obtain in the necessary forms.

One of the difficulties associated with the measurement of the mechanical properties of lens materials is that no single property measurement reflects accurately, the 'in eye' situation. Tensile strength indicates the resistance of the material to deformation under tension; tear strength the resistance of the material to tear propagation from a notch or imperfection (more relevant to soft lens materials), and rigidity modulus the resistance to deformation under compression.

The first two parameters relate to the behaviour of a lens material in handling and the third (modulus) indicates the extent to which various forces will deform it, most likely when in lens form. A hard or rigid material will have a high rigidity modulus whereas a soft material will have a low modulus. Following on from this, and as previously suggested, a low rigidity modulus is likely to be associated with greater lens comfort.

However, poorer visual results may be obtained when fitting astigmatic eyes, due to residual astigmatism resulting from lens flexure. Again this has been traditionally a soft lens problem, but as indicated later, can also occur with RGP's, and in particular some of the recent high oxygen permeable (Dk) polymers which have been described as flexible fluoro-polymers (Isaacson 1988, 1989).

Mechanical property testing of materials in the engineering industry typically involves the application of a force or load to a sample and a measurement of the way in which the sample responds. Results are usually expressed in units of strength or modulus.

The strength of a material is defined as the force per unit area required to cause failure when the material is subjected to a test procedure e.g. tensile, shear, impact, tear. The modulus is defined as the true stress (force per unit area) required to produce a true unit strain, i.e. deformation in the direction of the force e.g. tensile modulus, rigidity modulus (Smith,1990).

Resistance to breakage is important for RGP materials to ensure durability in RGP lens usage. However if this means increased flexibility and associated difficulty in masking corneal astigmatism and greater difficulty in lens manufacture, then the balance of properties may be inappropriate. In designing new polymers for RGP lens use, then this balance needs to be carefully considered.

In engineering laboratories, materials can be tested under tension or compression and the results of these tests can give detailed information on the strength, deformation characteristics, stiffness and toughness of a material.

(a) tension

The tensile test provides a stress/strain curve as the load is applied and the associated measures are (a) tensile strength (load at break/cross sectional area) (b) tensile modulus (stress/strain) and (c) percentage elongation at break (extension at break/original length x 100%).

(b) compression

As previously indicated, unlike true elastic materials, most polymers are viscoelastic, that is they deform time dependently when a load is applied and recover time dependently when the load is removed, and this may result in a progressive deformation of the materials. In this way, stress/relaxation curves can be generated and these may be applicable to the contact lens industry.

Various instruments allow the indentation of any test sample by a small sphere under a small load. The recovery of the specimen can also be measured as a function of time. The value of the rigidity modulus indicates the force necessary to compress the material by a given amount.

Although it gives a good understanding of the flexibility of the material, the rigidity modulus bears no relationship to properties that are measured in tension. It is important to remember that on the eye, RGP lenses are acted upon by relatively small forces associated with lid blinking, lid tension, eye movements, and the surface tension of tears. Some of the effects of these forces have been measured directly on the eye and they show interesting interactions between lid tension and corneal astigmatism (Wilson et al. 1982). Clearly, these forces may also have an effect on a contact lens 'in situ'.

Units

One of the difficulties when comparing lenses is that units used in the measurement of 'stress' often differ. The SI unit of stress is now Newtons per square metre (N/m^2) although MN/m^2 is more useful to contact lens materials (mega Newtons per square metre = $10^6 N/m^2$). However other units such as $Kgms/mm^2$ and $dynes/cm^2$ are also used and conversions may be necessary if comparison of materials is to be made.

$1 dyn/cm^2 = 0.1 N/m^2 = 10^{-7} Mn/m^2 = 1.02 \times 10^{-8}\ kg/mm^2$

Values used may depend on the method used to test materials.

The data derived from stress/strain measurements on thermoplastics are important from a practical viewpoint, providing as they do, information on the modulus, the brittleness and the ultimate and yield strengths of the polymer. By subjecting the specimen to a tensile force applied at a uniform rate and measuring the resulting deformation, a curve of stress against strain can be constructed (Smith 1990).

The initial portion of such a curve is linear and the tensile modulus E is obtained from its slope. Eventually a point is reached beyond which a brittle material will fracture and the area under the curve to this point is proportional to the energy required for brittle fracture.

If the material is tough, no fracture occurs, and the curve then passes through a maximum or inflection point, known as the yield point. Beyond this, the ultimate elongation is eventually reached and the polymer breaks.

Rigidity modulus and related measurements have been made on a number of contact lens materials using a micro indentation apparatus (Tighe 1989), a loading applied to prepared material samples (Fatt 1988), and to finished lenses by Fatt (1986,1988). These measurements are discussed later in this chapter.

The widespread use of PMMA in industry made manufacturers of this material carry out comprehensive engineering studies on its elastic behaviour. However there have been very few published reports of RGP elastic moduli, mainly as a result of the materials being custom designed for the contact lens industry.

Methods of quantifying material flexibility should be developed so that manufacturers can then quote a measure of flexure in the technical specification of their product. The most common measure used in the engineering field to describe the elasticity of a material is the Young's Modulus of Elasticity, relating stress to strain when loading a sample of a given material, under specific experimental conditions.

The first part of this experiment was designed to determine the total bending of a sample of material to a relatively high load which meant that (a) some samples broke under load and (b) bending of the sample beyond the elastic limit of the material was produced. No attempt was made to quantify the modulus of elasticity using this method. Such an experimental procedure is likely to evaluate the fracture resistance of a polymer, and this may well be a mechanical property worthy of further investigation.

4.2.1. Fracture Characteristics of Polymers

If mechanical loading of polymers goes beyond their elastic limit then their fracture characteristics can be evaluated. This can be useful for manufacturing purposes and may be relevant to patient handling of RGP lenses. That is, depending upon the shape of the stress/strain curve, materials can be classified as soft and week, soft and tough, hard and brittle, hard and strong or hard and tough.

Questions have been raised in the literature regarding the difficulty of relating material measurements to subsequent lens performance where design factors may be as significant as material differences (Tighe and Kishi 1988).

Fatt (1986) measured flexure in vitro by mechanically compressing the chord diameter of lenses and determining the force needed to produce the change in diameter.

Since the experimental work described in this chapter, was concerned with a material rather than a lens property, it was felt that the modulus of elasticity would be the most relevant measure to determine, and that the samples used would be flats rather than lenses. As a standard engineering measurement, the modulus of elasticity, relating stress to strain is well defined. Using flat samples instead of lenses, eliminated the important variable of lens design.

The purpose of the second part of the experiment was therefore to measure Young's Modulus in a range of materials of different chemical classification and oxygen permeabilities, in an attempt to determine whether the modulus differed significantly between groups of materials, and was related in some way to oxygen permeability. This may provide a basis for measures of elasticity to be considered as 'standards'.

4.2. Materials and Methods

The measurement method used a Mercer Engineering gauge (122L) adapted and mounted on a specially designed platform as shown in Fig.14. This gauge is normally used in the engineering workshop to allow machine tools to be calibrated. The custom designed platform was made in mild steel and was used to support the Mercer gauge. It also provided a base, into which a sample of polymer material could be placed for testing. Fitted to this platform was a vertical mild steel rod having either a round tip providing point contact with the sample under test, or in the later experiments, a V shaped tip giving contact across the width of the sample. All dimensions of this apparatus are shown in Fig.15.

Brass weights were then added progressively to the support on the central rod which acted directly on the sample under load. An exploded view of the modified unit incorporating the Mercer gauge is shown in Fig.15.

The sample material was placed in the support and weights were added in 20gm. steps from 20-300gms for flexibility testing in the first experiment, or in 10gm steps from 10-60gms for Young's Modulus measurement in the second experiment. As the sample was deflected by the addition of weights, the Mercer gauge measured the bending or deflection of the sample and converted the movement via a transducer to an amplified electrical signal (Fig.15). This reading was registered on a scale and the sensitivity of the instrument was such that deflections could be measured to the nearest 10 microns. The apparatus therefore measured the bending of the material sample under the specific test conditions.described, and did not necessarily replicate test methods used in the engineering and plastics industries.

Two different approaches were used in evaluating the flexure characteristics of the different materials. The first was to load the samples beyond their elastic limit, a procedure which assessed both the flexibility of the materials and also their fractural resistance properties.

The second procedure was designed specifically to obtain stress/strain curves within the elastic limit of the material. This allowed the Young's Modulus of the material to be determined. For the first series of experiments, sample flats in the form of discs were prepared from contact lens blanks supplied by the materials manufacturer. Each sample was cut to the required thickness using an engineering workshop lathe fitted with a tungsten carbide cutting tool which was air cooled.

All samples were cut parallel sided having a thickness of 0.2mm (±.005mm). It was possible to produce even thinner samples down to 0.1mm by this method, but they were more prone to break or chip in handling.

To check the linearity of the complete measurement system, mild steel discs of 0.2mm uniform thickness were prepared in a similar manner to the plastic samples, and loaded with weights from 20gms to 200gms.

In the first experiment, a series of material samples including PMMA and 5 gas permeables, were tested by the method described above, noting the amount of bending for each weight added. Four samples of each material were measured and the mean value of flexing or bending for each weight was calculated. In addition, 4 samples at each of three different thicknesses of PMMA were measured (0.1, 0.2, 0.3mm) to consider the effect of thickness on flexibility using a standard material. All measurements were made at 20^{0}C.

The range of gas permeable materials (n=5) was chosen for flexibility testing with all samples having the same uniform thickness, but of different oxygen permeability. These were the Optacryl family of silicone acrylates (18, 32, 84 Dk, Pilkington USA), Paraperm EW (Paragon Optical USA) also a silicone acrylate, and Boston Equalens (Polymer Technology USA) which is a fluoro-silicone acrylate. These trade names all relate to materials made in the USA, but also available in the UK at the time of the experimental work.

In a number of experimental runs, the sample broke (see Fig.18) before the maximum weight (300gms) had been reached. As a result of the measurements obtained and problems encountered in the first part of the study due to going beyond the elastic limit of the polymers, two main differences were incorporated into a second series of experiments.

Firstly, making the assumption that the polymers to be measured were perfect elastic bodies, any assessment of the stress/strain relationship should be kept within their elastic limit (Fatt 1988). Hence the range of loads applied in this experiment were much less than those used in the first experiment when the break point or fracture point of the material was reached in some cases (Fig.18). The actual range used was 10gms. to 60gms. in 10gm. intervals. In this experiment, all loads applied were well within the fracture resistance range of the materials.

The second difference concerned the preparation of samples. The first experiment used 0.2mm thick circular discs of material prepared from lens blanks. As has been pointed out (Fatt 1988), a significant increase in the sensitivity of the measuring technique can be obtained by using small rectangular plates of material, such that more bending for a given load is obtained. Therefore although they were more difficult to produce accurately, rectangular flats of different thicknesses, all 4mm wide, were prepared from standard contact lens buttons. The loading device was applied as a knife edge across the width of a sample as shown schematically in Fig.16.

Ideally as in the engineering industry rods of material would be the preferred form to test but rarely is it possible to obtain gas permeable materials in this form. For any one material, six samples of different thickness were produced. Each was measured twice and an average slope of the amount of bending relative to the weight added was determined.

A value for Young's Modulus(E) was calculated using the standard formula (Roark and Young 1975) $E = L3 / 4ab^3(Y/W)$, where L is the separation of the supports, a is the width of the bar, b is the thickness of the bar and Y/W is the slope of the line relating bending to weight added. The mean and standard deviation of all six values of E were then calculated to give the measured Young's Modulus of Elasticity for that particular material.

For elasticity testing (Young's Modulus), the materials measured (n=8) were Optacryl 18, SGP 2, Paraperm EW as silicone acrylates and Fluoroperm 30, Fluoroperm 90, Boston Equalens and Fluorex 700 as fluorosilicone acrylates. These were all commercially available materials. PMMA (Dk 0) was measured as a reference and the Allergan Advent lens (3M 100Dk) value given by 3M (R Franz, Research Director, personal communication) was added to the data for further comparison.

The Advent 3M material (Fluorofocon A) was not available in button form to allow samples to be produced for flexibility testing of the material. The lenses were however, very flexible to handle, which appeared to correlate with the low value for Young's Modulus supplied by the 3M company (R Franz, Research Director; 0.4 $\times 10^{-4}$ Kgms/cm^2).

The 3M lenses were a recent addition to the range of gas permeable lenses commercially available in certain states of the USA before being made available to the world market..They were described as 'flexible fluoropolymers' (Isaacson 1988, 1989) and for this reason it was interesting to use the reported data for comparison with other materials. The clinical performance of these lenses with respect to their degree of flexure on astigmatic eyes is discussed in Chapter 5 (page 119).

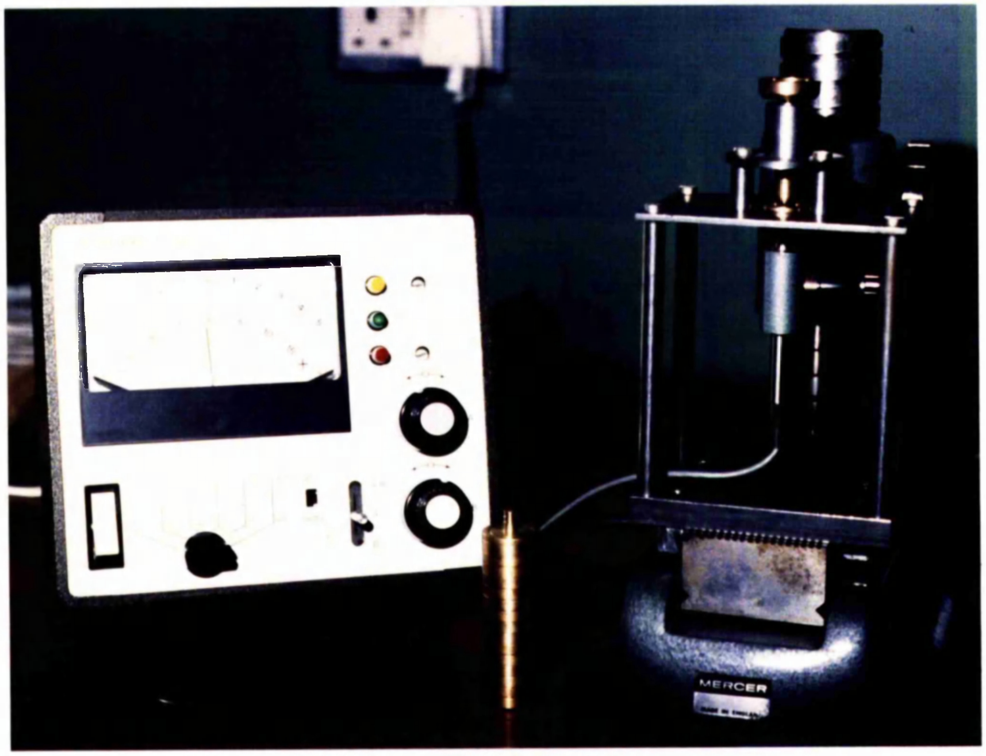


Fig.14

A photograph of the apparatus used for the measurement of Young's Modulus of RGP materials.

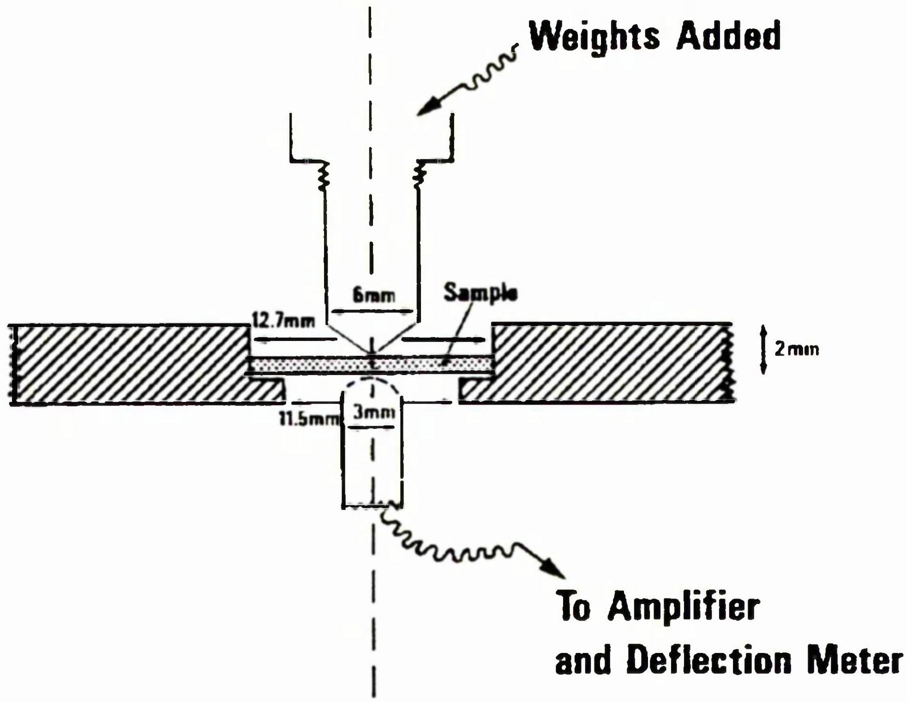


FIG.15

Exploded view of the sample holder and gauge for the measurement of lens material flexibility. The weights were added to the central support and the bending or flexing of the sample was detected by the probe underneath and converted to an electrical signal.

4.3. Results

To determine the linearity of the measurement system, the response obtained relating bending of the sample metal disc to the weight added, was found to be linear in the range of weights used ($r^2=0.997$, $p< 0.05$) (Fig.17).

Fig. 20 shows the stress/strain response for each of the 6 materials measured indicating the relative flexibility of each of the materials. It appeared that the trend was for the higher oxygen permeable materials to flex more than the lower oxygen permeable materials.

The fracture characteristics of Boston Equalens were observed when loading 10 equal thickness samples of the material with weights up to 300gms. Fig.18 shows the response obtained which gives some idea of the variability between samples. Four samples out of those measured, broke at the levels indicated. These breakages all occurred beyond the initial linear part of the response but showed a lack of reproducibility in the fracture point which would have allowed a meaningful value to be specified.

The results obtained with the three PMMA samples of different thickness show a significant difference in the slope of the response with the 0.1mm sample relative to the 0.2 and 0.3mm samples (t test, $p<0.05$). This comparison was made at the 200gm. level, prior to the fracture point of the thinnest sample.

Fig.21 shows two typical stress/strain slopes obtained with comparable thickness samples of PMMA and Boston Equalens, the difference reflecting the relative flexibility of those two materials. The linearity of the responses obtained shows that the weights used (10-60gms) were within the elastic limits of the materials. This was unlike the first part of the experiment where the limits were exceeded to in some cases the fracture point of the material.

The value of each slope is the Y/W term substituted in the equation given above to calculate Young's Modulus(E). Fig.22 shows a bar chart of all the materials measured the error bars being one standard deviation from the mean. The 3M material has no standard deviation shown since this was not an experimentally determined value. The test retest correlation on the slope of each material was always equal to or greater than $r=0.94$ ($p<0.05$) showing the measurement technique to be repeatable.

Table 4 shows the values obtained for Young's Modulus for each of the materials tested. The drop in the value for Young's Modulus from PMMA to the RGP materials becomes significant at the Fluoroperm 30 material (Mann-Whitney, $p<0.05$) suggesting that 3 main groupings of materials can be considered as far as flexibility is concerned. These would correspond to low Dk materials (PMMA, OC18, SGP), medium Dk materials (P/P EW, Bos.Eq., F700, Fluoroperm 30,90), and high Dk materials (3M Advent).

The evidence on the high Dk materials is only based on one lens type and is therefore limited. Samples in the form of rectangular plates of the two high Dk experimental materials used in the oxygen permeability experiments were not forthcoming from the manufacturers to allow an increased sample in this group of polymers to be tested.

Clinically, the significance of these groupings is that standard thickness lenses in the first group would not flex but have low oxygen transmissibility, those in the middle group would flex in thin design negative powers, but have moderate oxygen transmissibility, and those in the third group would flex in all powers but have high oxygen transmissibility.

In terms of material classification it would appear from the results obtained in this experiment that most silicone acrylates would be in the first group, most fluoro-silicone acrylates would in the middle group, and the fluoropolymers in the last group. It was interesting to note that the fluoro-polymer lens (3M Advent) was made by a moulding method and could not be manufactured by normal lathing methods from 'buttons', due to the mechanical properties of the plastic.

Correlating the Young's Modulus of the materials measured, to their oxygen permeability measured by polarography, showed a significant correlation (Fig.23, $r^2= 0.969$ $p<0.05$). The relationship with Young's Modulus is an inverse one as shown by the linear regression ($r=-0.98$) in Fig.23. The Dk of each material used in the regression analysis and the method used in the measurement of oxygen permeability have been described in the previous Chapter 2.1.

Material	Modulus	sd (±)	Fatt	ICI
PMMA	3.29	.9	3.00 ±.8	3.00 ±.1
O/C 18	2.86	.32		
SGP	2.6	.37		
F 30	2.40	.35		
P/P EW	2.31	.54		
BOS EQ	2.18	.37	2.2 ±.25	
FL 700	2.11	.37		
F 90	1.98	.36		
3M	0.4			

TABLE 4

Values of Young's Modulus for the range of materials tested. All units are x 10^{-4} Kgms/cm^2. Fatt's data (1988) are shown for comparison along with that available from ICI for PMMA. No published data was available for the other materials.

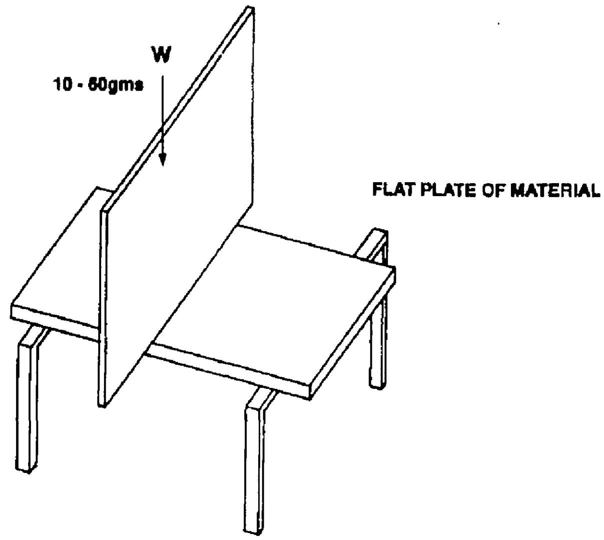


FIG.16

A schematic representation of the apparatus used in testing stress/strain relationships of RGP contact lens materials. A thin rectangular sample of material is supported at either end and is loaded in the middle across the full width of the sample.

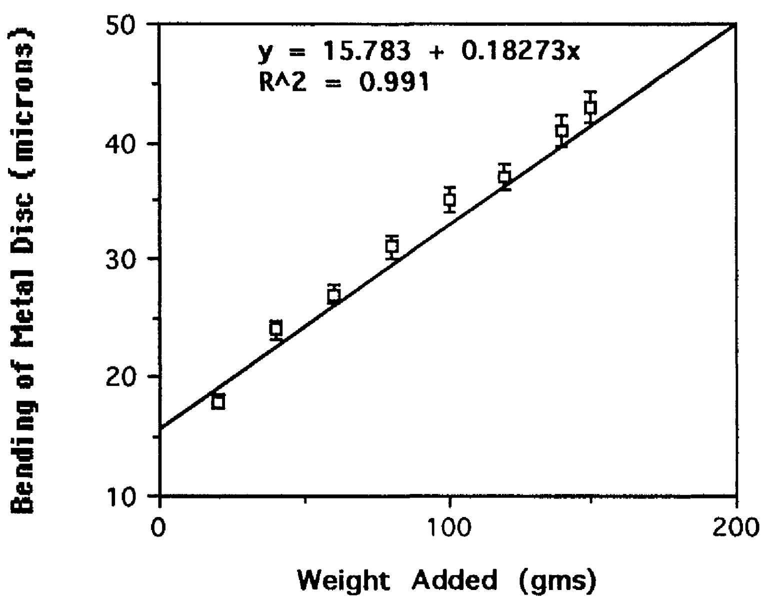


FIG.17

The bending of a metal disc to show the linearity of the measurement system. The error bars show ±1sd.

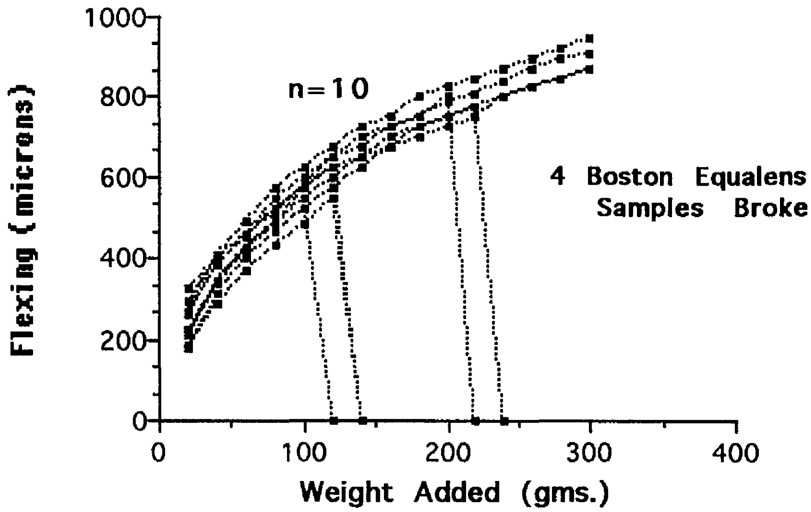


FIG.18

Testing Boston Equalens material by loading samples up to 300gms. Note that in this test, 4 of the samples broke under load.

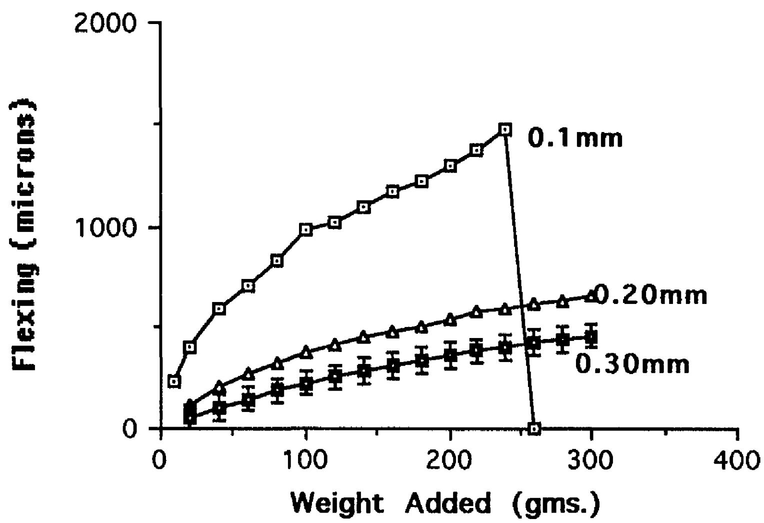
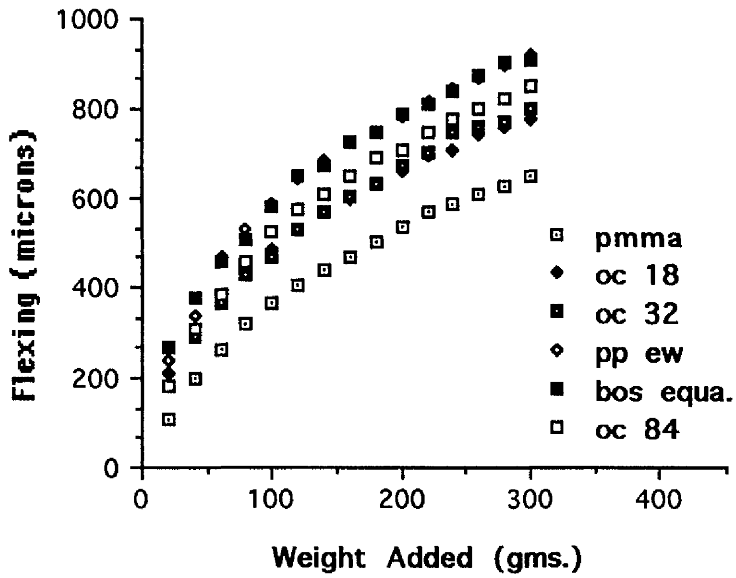


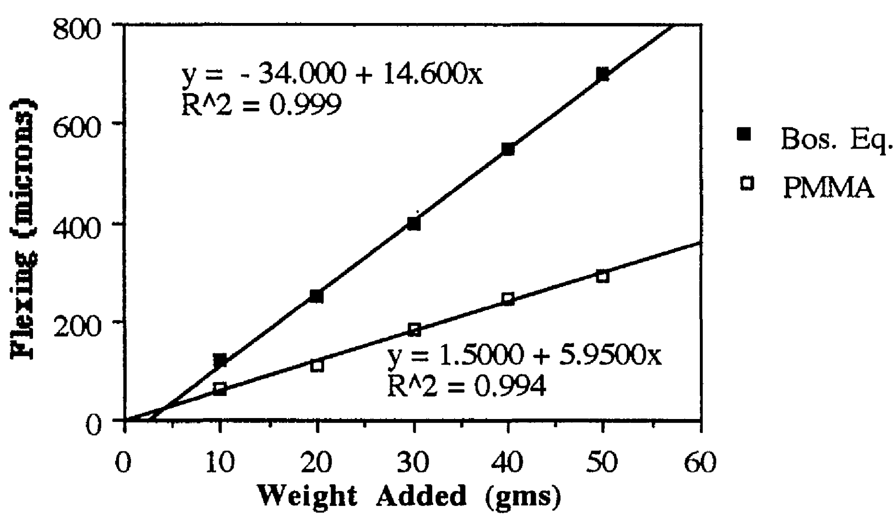
FIG.19

The relative flexibility of 3 sample thicknesses of PMMA. The thinnest sample broke when the load reached 240gms. Each point is the mean of 4 sample measurements and errors bars(±sd) are shown for one sample thickness.



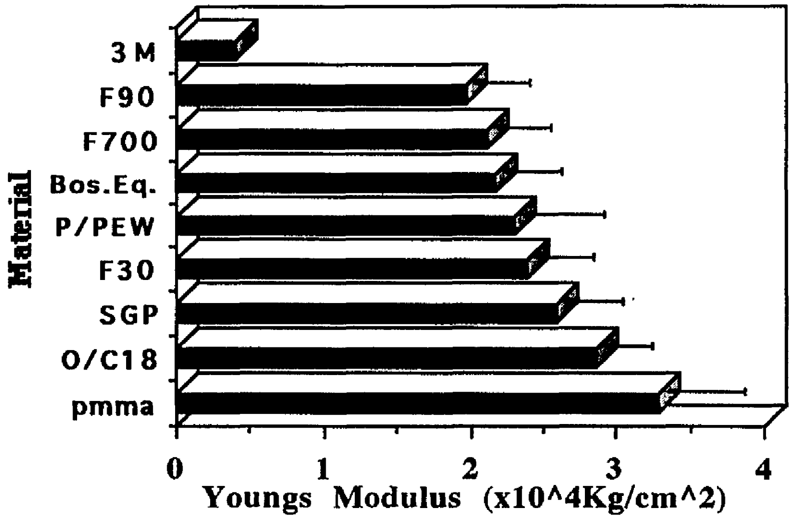
<u>**FIG.20**</u>

The relative flexibility of a range of gas permeable materials. Each point is the mean of one measurement on each of 4 samples.



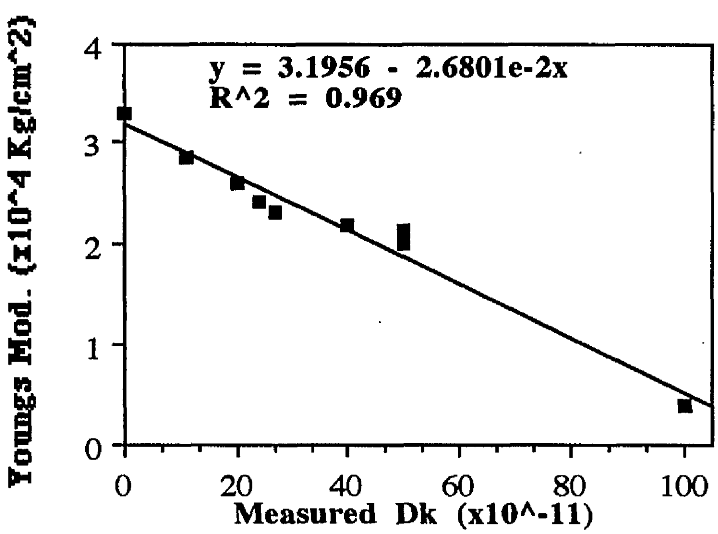
<u>**FIG.21**</u>

A comparison of the flexibility of PMMA and Boston Equalens when loading small samples of each material. The greater slope indicates increased flexibility. Each point is the mean of 1 measurement on each of 4 samples.



<u>**FIG.22**</u>

The bar chart shows the Young's Modulus of Elasticity in the range of materials tested. The error bars are ±1sd. No sd is shown for the 3M material since this value was provided by the manufacturer.



<u>**FIG.23**</u>

Correlation between Young's Modulus and oxygen permeability. If the point at Dk 100 is ignored the correlation is still significant (variance r^2=0.912). The Dk's of 6 of the materials were obtained from the experiments described in Chapter 3.4. The other 2 (Paraperm EW, Optacryl 18) were subsequently measured using the same method.

4.4. Discussion

The results of this experiment confirm clinical observations with lenses. That is, the higher the oxygen permeability of the material the more flexible are lenses made from that material. One notable exception to this rule has been cellulose acetate butyrate (CAB), which is a low Dk material producing flexible lenses, even when manufactured in standard thickness form. This indicates that material chemistry can be a major contributing factor to flexibility in addition to oxygen permeability.

Therefore polymer chemists in the development of new gas permeable materials should consider the property of flexibility and to what extent practitioners view it as an advantage or disadvantage. The general rule of an increase in oxygen permeability giving rise to increased flexibility was confirmed in the range of samples tested.

Individual lens design has an important influence on flexure, centre thickness or average thickness being the most crucial lens parameter to balance oxygen transmissibility (Dk/L) and flexure. Measuring lenses rather than flats as discussed by Fatt (1988) would be a logical procedure since they are more readily available, but several materials in identical design or the same material in several designs would be required for testing.

A personal clinical observation has been that a thin toric base curve lens does not flex as much as a spherical design on an astigmatic cornea. Previous publications have related the centre thickness of PMMA and low Dk materials to flexure and residual astigmatism.

Harris and Chu (1972) found that 0.13mm was the crucial centre thickness of PMMA below which significant flexure occurred. Harris et al. (1987) using two different low Dk silicone acrylates, found 0.15mm to be the critical centre thickness. It has also been shown that fitting lenses steeper than the flattest 'K' on a toric cornea will cause more flexure (Herman 1983) or conversely, fitting on 'K' or even flatter than 'K' would minimise any potential flexure.

The main point of this study was to determine material flexure characteristics and hence the measurement was conducted on prepared samples rather than on lenses in which lens design would be an uncontrolled variable. When looking at flexure of lenses in situ then of course fitting relationships are also crucial. At the present time it is recommended that manufacturers should quote values for the Young's Modulus of all materials allowing practitioners to predict those likely to flex when lenses are manufactured in a thin lens design to maximise oxygen transmissibility.

Very little published data is available on Young's Modulus values for contact lens materials. Most of the previous work has been done by Fatt (1988). He tested nine contact lens materials and found that seven had Young's Modulus values between 2×10^{-4} and 3×10^{-4} Kgms/cm^2. Of the nine materials tested, only two were repeated in the present study. The remainder were not available from the manufacturer. The two materials common to both studies were PMMA and Boston Equalens, and the values obtained by Fatt (1988) for each are shown in Table 4.

Also the value of 3.29×10^{-4} Kgms/cm^2 for PMMA is in close agreement with Fatt's data (1988) and also that given by Imperial Chemical Industries (ICI), makers of the perspex brand of PMMA. They give however an sd of ± 0.1, but ICI made their study using large rods where dimensions are more accurately known. The deflection of a bar loaded midway between two supports is shown by the equation for Young's Modulus (see page 105) to be a function of the third power of bar thickness and the distance between supports. If this is also true for RGP lenses then lens thickness, and the location of supporting points will have a more important influence than Young's Modulus on lens flexure.

Practitioners also need to be aware of the range of thicknesses used by lens manufacturers across the power range typically specified. This would be of particular importance when looking at new high Dk polymers being developed. PMMA and the 3M lens values for Young's Modulus could be used for reference purposes, the majority of lens materials falling within these two limits. Manufacturers' fitting manuals often recommend increasing the centre thickness of lenses when using the higher Dk materials to offset the flexure response.

It may be that flexure is desirable in some clinical situations where optically induced astigmatism can be used to advantage (Sorbara et al. 1992). How the elasticity modulus is determined is open to debate, with the method described in this experiment, essentially a laboratory test on prepared samples. Since it is normally easier to obtain lenses than specially prepared samples, a simpler test on lenses rather than flats may help practitioners understand the significance of lens design in relation to the concept of lens flexure. However the value of this study has been to demonstrate that Young's Modulus can be measured reliably and that materials do differ in their elastic properties.

The flexure of a contact lens has important consequences. When considering the general relationship between the rigidity modulus of a lens material, and the visual performance and comfort of a lens, then as the rigidity decreases the comfort increases but the visual performance may decrease (Stone and Phillips 1989). This is most likely to occur when fitting an astigmatic cornea, when a lens of low rigidity modulus is more likely to bend to the shape of the cornea.

For the analysis of RGP lens bending in the eye, it seems logical at this time to assume that the Young's Moduli at 35^0 C will maintain the same relationship to each other that they had at 20^0 C. A more precise procedure will need to be developed to detect temperature effects on the elastic modulus. Also, some knowledge of the glass transition point will be needed to estimate the effects of temperature on elastic properties.

The question of induced astigmatism due to lens flexure is not a simple relationship and is discussed in the next chapter (Chapter 5). Practitioners need to consider mechanical, optical, and physiological factors when choosing and fitting hard gas permeable lenses, particularly with new generations of high oxygen permeable plastics.

4.5. Summary

A measurement system was devised and built to allow contact lens polymer samples to have flexure characteristics and elasticity tested. On specially prepared contact lens material samples, the relative flexibility of a range of gas permeable materials was measured and in some cases the fracture resistance observed. The Young's Modulus of Elasticity of a range of commercially available gas permeable contact lens materials was also measured using specially prepared samples.

Significant differences were found in the Young's Modulus of the materials tested, such that they could be arranged in groups on the basis of their oxygen permeability. The highest value of Young's Modulus was found with the lowest Dk (PMMA) and the lowest measured value with the highest Dk (Fluoroperm 90). The value quoted by the manufacturer for the 3M(Advent) lens material correlated with its apparently high Dk(100).

The results confirmed that with the apparatus and methodology described, it was possible to obtain reliable measures of Young's Modulus and that the greater the oxygen permeability of a material the greater will be its flexibility. Lens design and particularly centre and edge thickness, are important factors in governing the flexure of gas permeable lenses.

When fitting and designing lenses for a toric cornea, practitioners must balance any increase in centre thickness to offset flexure with the corresponding decrease in oxygen transmissibility. Conversely it may be that lenses can be designed to flex in cases where optically this would be an advantage.

It is suggested that contact lens material producers provide data on Young's Modulus along with other properties, to help contact lens practitioners more fully understand the materials they prescribe. This will be particularly relevant when new high Dk materials are made available for clinical use.

Chapter 5. Flexure of High Oxygen Permeable Lenses

5.1 Introduction

Contact lens materials have a wide spectrum of rigidity, but are categorised into two groups of soft and hard. However the definition of a hard or a soft lens should describe the behaviour of the lens on the eye and is a combination of the material properties, the lens design, and other factors.

In contact lens practice spherical back surface contact lenses are often the first consideration when fitting moderately astigmatic corneas. The principle of replacing a toric cornea with a spherical tear layer is the basis behind successful correction of corneal astigmatism. Significant levels of corneal astigmatism can be corrected with spherical back surface lenses until a point is reached where either the lens edge clearance from the cornea is too great causing discomfort, or lens pressure on the cornea is unacceptable, resulting in a disruption of corneal integrity.

It is well recognised with soft lenses that flexure on toric corneas results in residual astigmatism (Stone and Phillips 1989). Toric soft lenses become necessary when residual astigmatism results in unacceptably reduced levels of visual acuity. As gas permeable materials have developed, much greater levels of oxygen permeability have been achieved, allowing lenses to be fitted with minimum physiological disturbance in daily wear. However, gas permeable lenses have been observed to flex on toric corneas, which in theory, should result in induced astigmatism at the lens back surface/tear film interface.

The relationship between oxygen permeability and flexibility, evaluated in Chapter 4, showed that the Young's Modulus in a range of gas permeable materials, decreased as the oxygen permeability increased. This has obvious fitting and design implications if high Dk lenses have to be made thicker to both enhance base curve stability and resist flexure.

The oxygen transmissibility of a lens is thickness dependent resulting in a potential conflict between on the one hand maximising oxygen permeability, and on the other, resisting flexure. Published data on gas permeable lens flexure is mainly on low to moderate Dk materials (Dk<50) (Fatt 1986) and therefore, with the development of new high Dk materials (Dk>100), it is important to consider the flexibility properties of all these materials.

One such lens has been described as a 'flexible' fluoro-polymer (Isaacson 1988, 1989), and therefore the optical as well as the physiological response, needs to be evaluated. Since it has also been suggested that the base curve to cornea fitting relationship will influence the amount of lens flexure (Herman 1983) it was decided to determine whether these flexible fluoro-polymer lenses would flex on toric corneas as a function of the fitting characteristics as specified by the lens base curve to cornea relationship.

The visual results of fitting spherical soft lenses in cases of corneal astigmatism are well documented and a general fitting rule in contact lens practice has been that a gas permeable hard lens would be the lens of choice in such a case. However if gas permeable materials are developed having high oxygen permeability and significant flexibility, then the optical effects of these lenses will need careful evaluation.

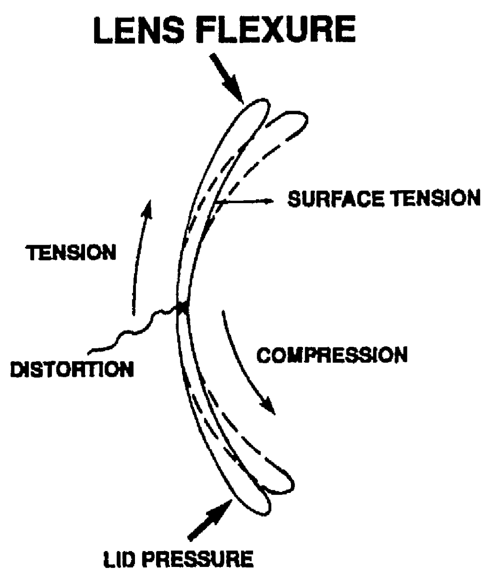


FIG.24

The forces acting on a lens 'in-vivo' which contribute to lens flexure and to stresses within the plastic.

5.2. Materials/Methods

Subjects

12 adapted contact lens wearers were recruited having varying degrees of corneal astigmatism (mean=2.21±.79, range 1.25-4.00D). The subjects were also chosen on the basis that corneal astigmatism equalled ocular astigmatism (within ±0.50D) to avoid the complication of lenticular astigmatism. Since within any one subject, astigmatism and lid configuration would be highly correlated, only one eye from each subject was used for measurement and data collection.

The lenses used in this experiment were Advent (3M) lenses supplied by Allergan (Irvine, California, USA). These lenses are manufactured by a moulding process and the material contains no silicone. They are classified as perfluoroethers and have a measured polarographic oxygen permeability of over 100 (Fatt 1989) and a Young's Modulus of 0.4 $\times 10^{-4}$ Kgms/cm^2 (R Franz, personal communication) which is significantly less than all silicone acrylates or fluoro-silicone acrylates (see Chapter 4). The lenses supplied were available in one diameter only and the centre thickness varied only slightly (range 0.160-0.195) across the available range of powers.

5 lenses were fitted in a random sequence to one eye of each subject. The lenses were fitted 0.1mm flat, in alignment and 0.1, 0.2, and 0.3mm steep relative to the flattest corneal meridian. All 'K' readings were recorded objectively using a Humphrey Auto-Keratometer (Humphrey Instruments, Irvine California). Each lens was left to settle for 5 minutes before measurements were taken. Front surface keratometer (FSK) readings were taken using the Humphrey Auto Keratometer. Five readings from the front surface of each lens were recorded to give the mean and standard deviation of the astigmatism on the front of the lens. This was assumed to be a measure of the degree to which the lens actually flexed on the eye.

The keratometer is automated and there is no subjective focusing or alignment on the part of the operator. The auto-keratometer uses three rays of near infra-red light projected onto the cornea or, as in this case, the front surface of a contact lens in a triangular pattern within an area approximately 3mm in diameter. Each measurement only takes about 3 secs. and therefore it was possible to get a rapid series of objective measures of lens flexure.

The auto-keratometer is calibrated for a refractive index of 1.3375 on the assumption that it is to be used for front surface corneal measurement and, since this study required the dioptric values measured from the anterior surface of the contact lens 'in situ', it was necessary to correct the actual measurements by multiplying the dioptric values by a factor of the ratio of the two refractive indices. In the case of the Allergan Advent (3M) lens this ratio was 1.39/1.3375=1.04. Therefore, when measuring from the surface of a lens, the indicated astigmatism (D) will in effect be less than the actual value. It is interesting to note that this lens material has a much lower refractive index than most other gas permeable lenses.

Halberg (1981) compared the auto-keratometer with a one-position manual type and found that on calibration standards, the auto-keratometer was accurate and needed no calibration shift. It was also found that the repeatability of both instruments was good for the power reading in the horizontal and vertical meridians. The advantage of the auto-keratometer for this type of experiment is that it is objective, fast, and allows repeated measures which can be stored in the instrument and later printed.

An over-refraction with an auto refractor (Humphrey 570, Humphrey Instruments, Irvine California) was carried out with each lens in place to determine the residual astigmatism. Five readings were taken in succession, and the cylindrical component of the result recorded. The mean and of the 5 readings was taken as the measure of residual astigmatism. The use of an auto refractor to determine residual astigmatism with contact lenses in place has been shown to be valid (Winn 1991, personal communication).

A conventional subjective over-refraction in addition to the objective auto-refractor was carried out with each lens in place using the crossed cylinder method to confirm the sphero-cylindrical correction. The cylinder component was assumed to be a subjective measure of the residual astigmatism but the repeatability of this measurement cannot be easily determined due to operator bias. The auto refractor reading was used for statistical purposes since it was objective, unbiased and the repeatability could be checked.

When measuring lens flexure, each FSK reading was taken following a blink when the lens had stabilised centrally on the cornea. Spurious results from lenses in a low or a high riding position were thus avoided. Any variation in lens flexure (FSK) was shown by the standard deviation of 5 readings taken with any one lens whereas the variation in residual astigmatism was determined by calculating the standard deviation of 5 readings of astigmatism from the auto refractor.

5.3. Results

Fig.25 shows the effect of lens base curve to cornea relationship on the amount of flexure on the front of the lens. The degree of flexure increases with the steepness of the lens fit, the difference being significant at the 0.2mm and the 0.3mm steep levels (t test, $p<0.05$). The error bars show the variability of the measurement between subjects with each fitting (± 1 sd). Fig.26 shows the relationship between the fit of the lens and the degree of residual astigmatism measured by the objective auto refractor. Again as with front surface flexure, the steeper the fit the greater the residual cylinder. However this was only statistically significant when the lenses were fitted 0.3mm steeper than flatter 'K' reading (t test, $p<0.05$).

An interesting finding was the variability of both the flexure and residual astigmatism response between subjects. This is shown by the large standard deviation of the mean astigmatic responses indicating a degree of inter-subject unpredictability. Since it might be expected that front surface lens flexure would result in a corresponding back surface flexure leading to induced astigmatism, and hence residual astigmatism, it was predicted that a correlation would exist between flexure (FSK) and residual astigmatism (RA).

Fig.27 shows a scattergram of all measurements from all subjects taken from each lens. Although a linear regression shows a significant correlation ($r = 0.75$, $p<0.05$) there is still sufficient scatter to show for example that some subjects show definite lens flexure but little residual astigmatism. Also it will not be a one to one relationship between the front surface flexure and the residual astigmatism since the corresponding back surface flexure will offset the effect of the front surface changes.

Therefore, as a clinical guide, taking front surface keratometer readings from a lens on the eye will indicate if flexure is present and give an approximate, although excessive indication of the likely astigmatism transmitted by the lens. A general rule based on the results here is that approximately half of the astigmatism measured by a keratometer from the front of a lens would be transmitted through the system and contribute to residual astigmatism.

The induced astigmatism created as a result of lens flexure was calculated using a computer programme which required an input of front surface 'K' readings from the lens, the refractive index of the lens material, the back optic radius of the lens and the 'K' readings of the eye (Morley Ford 1989, personal communication).

This programme was based on a similar programme published by Douthwaite (1987).

An assumption concerning the induced astigmatism, was that it would become residual, and have the effect of reducing visual acuity. Fig.28 shows a comparison of the theoretical calculated induced astigmatism and the final residual astigmatism for each subject when the lenses were fitted 0.1mm steeper than the flatter 'K' reading. The indications were that the final response was variable and unpredictable, suggesting that other effects were involved such as the lid tension, or the lens position. These factors need further investigation.

Fig.29 shows the scattergram of the plot of the measured FSK on the Advent lens fitted 0.1mm steeper than the flatter 'K' for each subject against the degree of corneal astigmatism. In other words, could the degree of lens flexure be predicted on the basis of the amount of corneal astigmatism? Since no significant linear relationship was determined for this particular lens base curve to cornea relationship, this generalisation cannot be made and again, individual evaluation would be required to determine the refractive result.

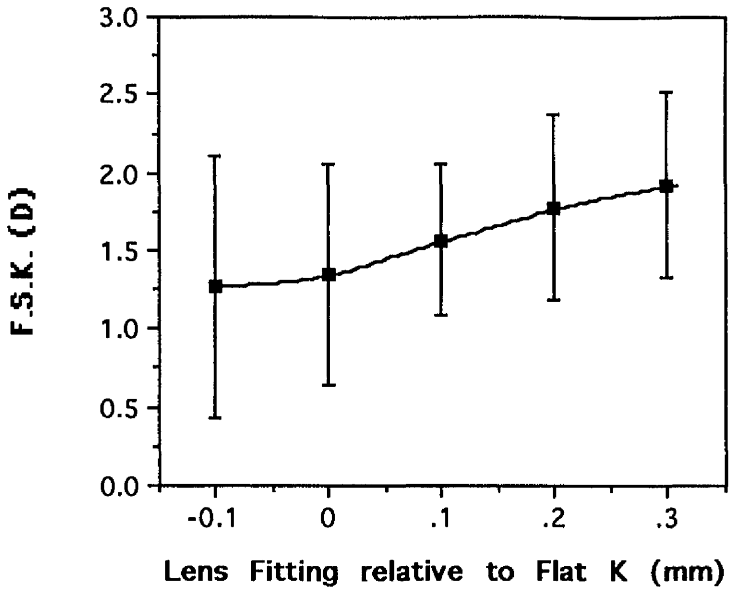


FIG. 25

The degree of flexure measured from the front surface of the 3M lenses increases as the fit of the lenses becomes steeper relative to the flatter 'K' reading.

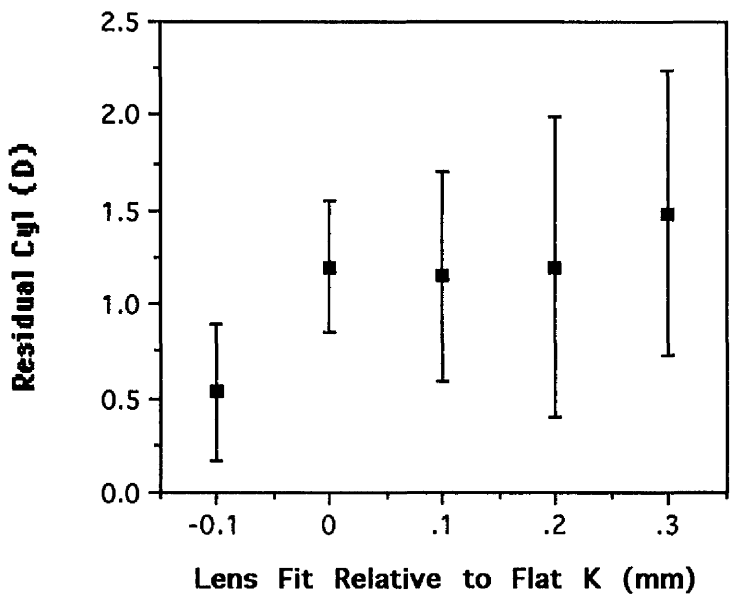


FIG. 26

The residual astigmatism increases as the fit of the 3M lenses steepens relative to the flatter 'K' reading.

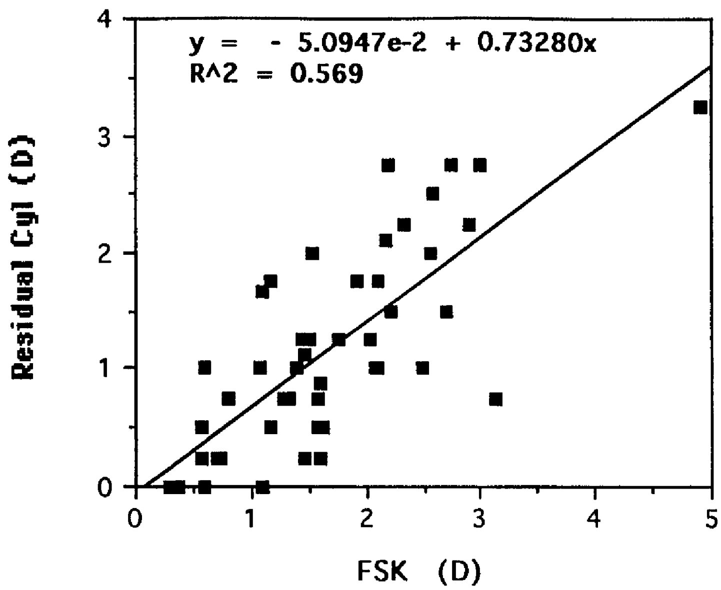


FIG 27

The amount of residual astigmatism is predictable from the degree of front surface flexure with the 3M lenses.

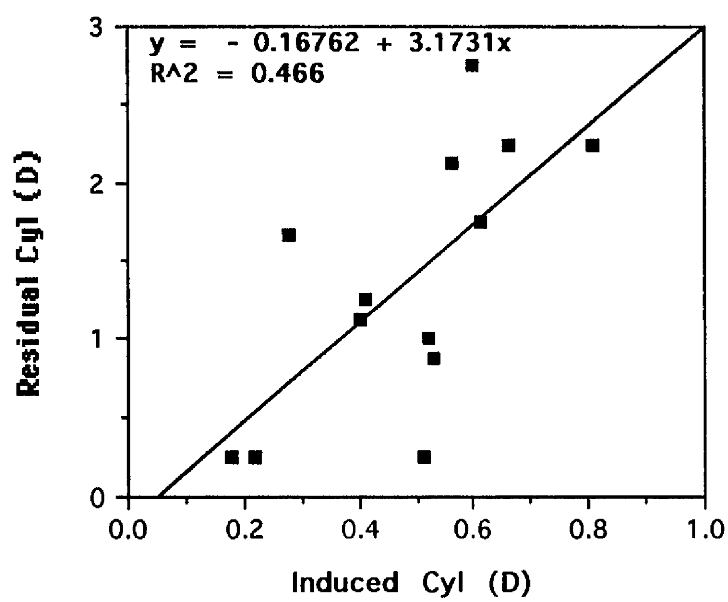


FIG. 28

A scattergram of all subjects for lenses fitted 0.1mm steeper than flatter 'K' relating the calculated induced astigmatism to the corresponding degree of residual astigmatism.

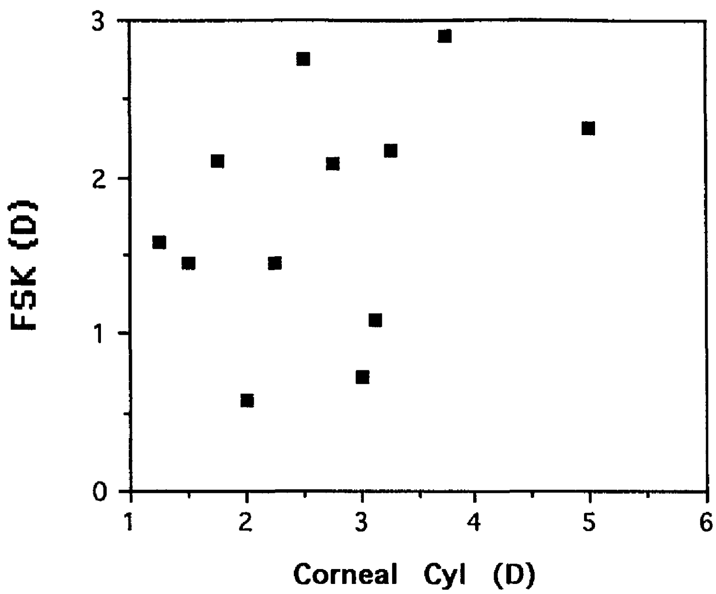


FIG. 29

The relationship between the degree of corneal astigmatism and the amount of flexure of the 3M lens when fitted 0.1mm steeper than 'K' to all subjects. No significant linear relationship was found.

5.4. Discussion/Conclusions

The implications of this experiment are twofold. That is, firstly it has been demonstrated that the fluoro-polymer lenses used in the study showed significant flexure, as determined by their response on a range of toric corneas. The base curve to cornea fitting relationship of these lenses is certainly one variable, but the response between subjects suggests that the effect is not merely lens dependent. It is also dependent upon patient characteristics and factors such as lid tension, corneal diameter, and lens position must also play a significant role in determining the degree of lens bending 'in vivo'.

Secondly, the optical result of lens flexure is not well defined in the literature and the assumption that induced and residual astigmatism will result from a lens bending on a toric cornea was not always found to be true in these experiments. That is, lenses could flex on some occasions with little apparent astigmatic effect being created, confirmed by an over refraction. This is not too surprising if one thinks of the response to bending lenses in air when measuring their back surface power in a focimeter. Little or no power change occurs, since the front surface bending is cancelled out by the equal and opposite effect of the back surface bending in air.

On the eye the effect is slightly different. That is, the refractive index difference at the lens/tears interface does not allow the front surface bending effect to be completely cancelled out, and as a result, some astigmatism is induced. Again this effect is reduced by the low refractive index of the 3M (Advent) lens material (1.39). A computer programme is available to calculate the induced astigmatic effect for a specific type of lens, given the specifications and the lens front surface 'K' readings (Douthwaite 1987).

It would appear therefore that the fluoro-polymer lens falls somewhere between a soft lens and a PMMA or silicone acrylate gas permeable lens as far as the flexure characteristics are concerned. Some flexure obviously occurs but is variable between individuals, and is partly dependent upon the base curve to cornea fitting relationship. Residual astigmatism is likely when fitting such lenses to toric corneas but not as much and less predictable than a soft lens. Since the results are variable, a fitting with a trial lens on an individual eye should be conducted to determine any residual astigmatic effect.

If lenses are fitted steeper to obtain better central fitting on toric corneas, then there is a greater likelihood that lens flexure will occur, giving rise to variable vision.

recommendation from this experiment is that when using spherical lenses on a toric cornea they should be fitted using a base curve close to the flatter corneal meridian to avoid negative pressure build up beneath the lens. The negative pressure would have the effect of pulling the lens towards the cornea creating the flexure. This effect would also be enhanced by the presence of a high lid tension forcing the lens to the shape of the cornea during a blink.

The value of the Young's Modulus quoted by the manufacturer for the 3M (Advent) lens and discussed in Chapter 4, is low (0.4×10^{-4} $kgms/cm^2$). It is less than PMMA and all the silicone acrylates but not as low as typical soft lens values (Fatt 1988). Therefore, this would be a guide to the expected response with lenses as determined in this experiment. The main difference as previously stated though is that PMMA and RGP lenses will normally successfully correct corneal astigmatism, soft lenses will not, and flexible fluoro-polymer lenses may in some instances, but not in others.

Practitioners may wish to use front surface keratometer readings to assess the flexing response but the results may be confusing and the only true means of evaluation should be to fit a lens, over refract, and assess the effect of any residual astigmatism on vision using both high and low contrast letter charts.

The levels of induced astigmatism as calculated using the computer programme were low presumably due to the relatively low refractive index of the contact lens material and the back surface of the lens cancelling out much of the front surface effect. It remains puzzling therefore to note those cases where a significant degree of residual astigmatism occurred. The mean residual astigmatism measured was usually higher than the induced values which were calculated. Questions raised or other factors which may have contributed to the residual values were;

1. Was the measure of residual astigmatism using an auto refractor an artefact? However the objective measures were usually confirmed by a subjective refraction indicating the measures to be reliable and valid.

2. Did the programme calculating the induced astigmatism have an error contributing to lower values? The programme was verified as correct and the optical principles used have been defined in standard contact lens texts (Douthwaite,1987).

More experiments need to be done looking at residual astigmatism with all types of contact lenses and the ability of auto refractors to measure the refractive error with the contact lens 'in-situ'. In the meantime, leaving oxygen permeability aside, back surface or bitoric gas permeable torics are probably preferable for eyes with substantial corneal toricity. In that situation, flexure is not likely to be a factor and moderate or high Dk materials can be utilised. Also in many cases of residual astigmatism it would appear that visual acuity is surprisingly good when measured with high contrast letter charts and that low contrast charts may be helpful in the clinical evaluation of such cases (Sorbara et al. 1993).

The fluoro-polymer lenses are more flexible than RGP lenses but do not bend to the shape of the cornea as do soft hydrogel lenses. Any future developments in high Dk lens materials should consider the mechanical effects of the material flexure and the optical effects of lens flexure on toric corneas.

5.5. Summary .

Thin gas permeable lenses have been used clinically to maximise the amount of oxygen reaching the cornea. A related clinical observation has been that thin lenses tend to bend or flex on a toric cornea which has theoretically an induced and residual astigmatic effect. Recent developments in highly oxygen permeable plastics specifically to enhance the physiological response of the cornea in contact lens wear have led to a new class of gas permeable lenses described as 'flexible fluoro-polymers'.

To evaluate the degree of flexure and the resultant optical effect, high Dk/t contact lenses (3M Advent) were fitted to one eye of 12 astigmatic subjects in a range of base curve to cornea relationships ranging from 0.1mm flatter to 0.3mm steeper than the flattest keratometer (K) reading.

Flexure of the lenses was measured by auto-keratometry (Humphrey Instruments, California, USA) from the front surface of the lenses and residual astigmatism determined by an objective auto-refractor and conventional subjective refraction. The theoretical induced astigmatism caused by the lens flexure was calculated using a computer programme and compared to the final residual astigmatism found on refraction.

Large individual variations were found in both the degree of flexure and in the amount of residual astigmatism. A general finding was that both lens flexure and residual astigmatism were greater as the fit of the lens was steepened relative to the flatter keratometer readings. Further experiments need to be done to explain the unusual results of residual astigmatism in the absence of lens flexure. The clinical implications are, that lenses used to maximise oxygen transmission to the cornea, may produce effects such as the optical changes reported in this experiment.

SECTION 3

CLINICAL EXPERIMENTAL WORK

Chapter 6. Specular Microscopy of the Corneal Endothelium of Long Term Contact Lens Wearers

6.1. Introduction

Observation of the posterior surface of the normal corneal endothelium with the specular microscope shows a regular, predominantly hexagonal, pattern of cells. The corneal endothelium is considered to be one of the most injury sensitive tissues in the eye. It has a barrier role and functions to maintain the corneal stroma in a state of relative dehydration, thereby preserving corneal transparency. Damage to corneal endothelial cells compromises both barrier and pump functions resulting in corneal oedema, opacification, and impaired vision.

Age, ocular surgery and disease have been shown to affect the morphology of corneal endothelial cells (Liesegang 1991) and in recent years several investigators have shown that prolonged use of hard PMMA contact lenses induces polymegethism (variation in cell size) and pleomorphism (variation in cell shape) of the corneal endothelium (Schoessler and Woloschak 1981, MacRae et al. 1985, 1986, 1987). Soft hydrogel lenses for daily wear and extended wear have also been shown to produce polymegethism and pleomorphism, although silicone elastomer lenses would appear to produce little or no morphological changes (Carlson et al. 1990). These effects were reviewed in chapter 2.7.2 (page 56).

6.1.1. Endothelial cell parameters

The factors discussed in chapter 2.7.2. highlighted the problem of quantifying polymegethism and pleomorphism. That is, how endothelial changes can be specified mathematically such that differences or changes in the mosaic, can be accurately determined and specified.

Due to the awareness of the potential for polymegethism, researchers are likely to continue to evaluate the areas of endothelial cells, although it should be recognised that the coefficient of variation in cell area can identify only the presence of a larger variance in cell area and not identify whether the cells have become smaller or larger, or whether the number of cell sides has changed (pleomorphism). A cell area increase has been reported to be associated with an increase in the number of cell sides (Schoessler 1987). The shape of endothelial cells as opposed to area changes has previously been analysed (Collin and Grabsch 1982).

(a) Cell Shape

The procedure in the experiments of Collin and Grabsch (1982), involved an assessment of the relationship between the area (A) of the cells and their perimeter (P) and was called the shape factor (P^2/A). Another index that relates area and perimeter is the figure coefficient ($4\pi A/P^2$) and this has also been used in relation to contact lens wear (MacRae et al. 1986). However these studies ignored the geometric principle that shape indices are different for different geometric figures (Doughty 1989).

For a symmetrical geometric figure such as a hexagonal cell in the corneal endothelium, in which all the sides are of equal length and the angle between all adjacent side intersections is 120°, the shape factor is equal to 13.867 and the figure coefficient is .907. The values for symmetrical geometrical figures (polygons) with numbers of sides ranging from 3 to 10 for both indexes are given in Table 3. These were calculated from standard equations that relate the area to perimeter for symmetrical geometrical figures (Doughty 1989).

No. of cell sides	Figure coefficient	Shape factor
3	.6050	20.791
4	.7851	16.00
5	.8640	14.53
6	.9076	13.867
7	.9347	13.445
8	.9475	13.264
9	.9596	13.096
10	.9627	13.052

TABLE 5

Figure coefficient and shape factor values for symmetrical polygons having different numbers of sides.

However following careful consideration of other studies where a form factor has been used, and in the light of more recent publications questioning such an approach, (Doughty 1988,1989) it was felt that the form factor would be a confusing term and mathematically incorrect.

In the real situation where, for example, in the human corneal endothelium there is a mixture of say 5, 6, and 7 sided cells, as the percentage of hexagons decreases the numerical average for the figure coefficient will also change, simply because the value is different for a pentagon versus a hexagon. The use of the figure coefficient does not therefore report on the actual shape of cells and is misleading, since two endothelia may have the same figure coefficient values, yet be vastly different in terms of the percentage of hexagonal cells. Alternatively, regular cells with different numbers of sides will have different form factors.

When looking at the endothelial cells of contact lens wearers, where the number of sides can range from 4 to 10, an analysis would require that all cells of equal sides be extracted and considered separately, so that several form factors could be calculated, an exercise which would be very time consuming and of questionable significance. The computation could however be programmed into the software of an image analysis system if felt to be worthwhile.

Other workers have used a measure of hexagonality, to describe the pleomorphism that can occur in the contact lens wearing cornea (MacRae et al. 1986, Carlson et al. 1988). With PMMA contact lens wear, the percentage of 6 sided cells has been reported to decrease from 71% to 61% and hence, the term 'hexagonality' has been used to describe pleomorphism (Carlson et al. 1988). This however has also recently been challenged as a valid index (Doughty 1989).

In a series of modelling studies, Doughty (1989) showed the marked influence that a change in the number of sides of an endothelial cell would have on the average value of the figure coefficient, as calculated for all cells in an endothelial mosaic. Using scanning electron micrographs of rabbit corneal endothelium, Doughty (1989) showed that 6 sided cells are not necessarily very symmetrical. He therefore suggested that such cells be reported as 6 sided rather than "hexagons", a term which carries the inherent risk of implying regularity, and thus stability of the mosaic of such cells.

Variation in cell shape (pleomorphism) would certainly seem to indicate a potentially important change as a result of corneal stress, but clearly the parameters used by various research groups as measures of variation in cell shape (pleomorphism) are unsatisfactory. The concept is interesting however, in that it has been suggested that the endothelium is thermodynamically stable by having a mosaic of predominantly six sided cells (Doughty 1989). Presumably any major deviation from this, or at least a change greater than that which occurs naturally with age, could be clinically significant.

(b) Cell Density

This parameter was calculated from the number of cells traced (100) divided by the total area of endothelium measured. e.g. if the total endothelial area measured in tracing 100 cells was equal to X sq.mm. then the endothelial cell density (ECD)=100/X cells/mm^2. There is very little published evidence suggesting that contact lens wear induces a loss of endothelial cells in the normal eye. However it is a fundamental issue which needs to be experimentally investigated, and since it is believed that corneal endothelial cells do not divide as part of a repair mechanism, the density is a parameter which is likely to continue to provide, important information.

Despite the criticisms of the relevance of other parameters used to quantify endothelial changes referred to earlier, there would appear to be general agreement that cell density provides probably the most clinically significant description of the status of the endothelium. There is ample evidence in the ophthalmic literature and in clinical practice to show that when cell density drops to a low level, (approx. 500-600 cells/sq.mm.) chronic oedema and corneal decompensation can develop (Mayer 1984, Tuft and Coster 1990).

Cell density has also been the most quoted statistic in endothelial studies because of the assumed non-regenerative properties of the endothelial cells, and the well established link between a low cell density and compromised corneal function in pathological cases such as Fuchs' Dystrophy (Mayer 1984). It is often difficult however to define what constitutes a significant reduction of the cell population for an individual due to the large variation with age and between subjects.

(c) Cell Area

The calculation of cell density was based on the cell area and in tracing cells for image analysis it was the area of each enclosure (cell) that was determined. Each individual cell area traced was measured and for a particular sample of 100 cells, the statistics were provided for total area, mean cell area, standard deviation of cell area and the minimum and maximum cell sizes within the sample.

A plot of the cell area distribution of the total sample measured, in the form of a histogram, provided the skewness and kurtosis of that distribution. During the process of tracing the cells, the Optomax image analysis VIDS programme gave an updated cumulative mean area and sample standard deviation which was helpful when determining the actual number of cells to be traced, counted, and measured, in the image analysis procedure.

(d) Coefficient of Variation in Cell Area (COV)

Polymegethism (variation in cell size) of the corneal endothelium has been documented as a result of normal ageing and intra-ocular surgery (Liesegang 1991). Since polymegethism has now also become a frequently used term to describe the endothelial appearance in contact lens wear, a measurement index of this phenomenon was considered to be important.

Although not differentiating between the presence of large and small cells, the COV in cell area (sd/mean) nevertheless is becoming a universally used index to describe the variation in cell area even although it has been recently reported that the appearance of polymegethism on specular micrographs may be an artefact of the angle of viewing in specular microscopy (Bergmanson 1992).

Also the ambiguity of the coefficient of variation in cell area has been highlighted by Doughty (1990). He has demonstrated by using a modelling analysis, that by substituting different percentages of smaller or larger cells into a group of six sided cells and calculating the COV, an ambiguous situation can arise. That is, the COV will always increase whenever abnormal cells are introduced into an otherwise normally distributed sample.

However the COV cannot distinguish between the introduction of abnormally small cells or abnormally big cells, which are those cells that contribute to a non gaussian distribution in the sample. The greater the abnormality in cell size the more substantial the changes in COV, even if small absolute numbers of cells are introduced to the model (Doughty 1990).

This will be referred to later in the discussion of the results section and evidence presented to show that polymegethism is more likely to be a real phenomenon and not an artefact of specular microscopy, as has been suggested by Bergmanson (1992). Several studies have shown that with increasing age there is a decrease in ECD and a concurrent increase in the variability of cell size (Laing et al. 1976, Sherrard et al. 1987).

(e) Central corneal thickness

The decline in endothelial cell density with age is not normally accompanied by an associated increase in corneal thickness. Reports of central corneal thickness as a function of age, seem to range from very slight increases, to no change, slight decreases and even to a marked decrease (Polse et al. 1989). As with endothelial cell density and age, these differences in central corneal thickness and age may be the result of gender or ethnic differences.

Polse et al. (1989) found that younger subjects had a thinner open eye steady state corneal thickness than older subjects but that the difference was not statistically significant. Interestingly, in conducting his 'stress' test of a thick soft lens on the cornea and assessing the rate of recovery (deswelling) in corneal thickness, he found no difference in the degree of swelling induced in the two age groups. The large and age dependent range of corneal thickness, even in the normal eye, means that endothelial cell density is a poor indicator of corneal thickness and vice versa.

Although only week correlations have been established between corneal thickness and any endothelial morphological parameter (Cheng et al. 1988), it is logical to establish what relationship might exist between polymegethism and central corneal thickness. Also, it is now well established that corneal thickness is directly related to corneal hydration (Polse et al. 1989).

It could be hypothesised that if long term contact lens wear is going to effect endothelial morphology, which in turn may influence corneal endothelial function (pump and barrier), then a measure of corneal thickness in a sufficient number of contact lens wearers should demonstrate an increase relative to non lens wearers. Alternatively, as found in the study carried out by Holden et al. (1985), it could also be hypothesised that long term contact lens wear with the resultant chronic hypoxia, could result in decreased central corneal thickness by a process of changing the structure of the stroma.

6.1.2. Morphometry using image analysis techniques

Image analysis has found wide application in research and development, quality assurance, and healthcare. The techniques have been utilised in the pharmaceutical, chemical, and food industries and in medical, material, environmental, and earth sciences. Essentially the principle is to create rapid, accurate, and comprehensive measurements from a variety of images created by a video camera and transmitted to a computer screen.

Two alternative approaches are possible, depending upon the quality and type of image generated on the VDU screen and the software available to handle the image in question. The first is a totally automated approach where the sample image can be stored in computer memory and then automatically manipulated and enhanced to allow counting and analysis. That is, if viewing for example, a number of cells which may have indistinct borders it can sometimes be difficult to tell whether two cells are separate and divided from one another.

Utilising grey scales and border enhancement techniques, greater definition can be obtained, and what started as a poorly defined grey image can be transformed into a sharply defined image of high contrast. These cells can be traced, counted and measured. Colour can also be used to provide increased definition and contrast of an image.

In order to define particular areas of interest in an image, frames of various shapes, usually squares or circles are used and where objects are only partially detected a fully automatic 'hole fill ' function ensures they are measured completely. Following the image enhancement, the measurement mode can be called up to perform the measurement and statistical analysis of the final image.

The second approach is to use a manual system of image configuration. This involves viewing the video image of a photographic print on a screen, and with the computer mouse and digitising tablet, manually tracing around the particular features of the image that are of interest as displayed in the video monitor.

In some applications of image analysis including this particular project, manual procedures were felt to be more accurate, since endothelial photomicrographs tend to be variable in contrast, clarity, and intensity across the image. An experienced observer in these situations can fill in holes in cells, or complete gaps in cell borders, more accurately than the computer (Nishi and Hanasaki 1989).

Once the desired image has been created on the screen, the counting and measuring process can be initiated to determine either the field specific or the feature specific parameters which have previously been chosen and defined for statistical analysis. Field specific measurements are those where the programme measures a number of parameters for each successive field whereas feature specific measures are those where individual objects in successive fields may be analysed to provide precise measurements.

Further features available on these systems are particle size analysis where particle size and shape can be determined, line densitometry, which provides a grey level profile of pre-selected single or multiple line scans in either the vertical or horizontal axis, and area densitometry where selected regions of the image can be scanned to provide the grey level value for each pixel.

The two approaches outlined utilise very sophisticated and powerful computer technology which has contributed significantly to an improvement in time and accuracy in the interpretation of corneal endothelial prints obtained from specular microscopy. Although previous methods such as fixed frame and random frame analysis have been used fairly successfully (Laing et al. 1976, Waring et al. 1980, Nishi 1988, Nishi and Hanasaki 1989), difficulties were encountered with manual tracing, bias in sampling and the time involved to obtain large samples of cells.

6.1.3. Descriptive statistics of the cell population

(a) The coefficient of skewness is a measure of the heterogeneity or asymmetry of the cell population in terms of individual cell area. It is a measure of the departure from horizontal symmetry of either a theoretical or empirical frequency distribution. If the cells are distributed symmetrically the coefficient of skewness equals 0.

When large cells appear, the curve that describes the histogram for an individual subject, skews to the right and has a positive coefficient of skewness. Since the lower limit of endothelial cell size is reported to be around 200 microns (Waring 1988) the curve can, but only rarely, skew to the left with a negative value. The young normal healthy endothelium may show a negatively skewed distribution (Waring et al. 1982).

However the significance of the coefficient of skewness is that it reports a very different type of change to that of the coefficient of variation in cell area (COV). Again Doughty (1990) has shown in an experimental model, that there is no ambiguity in the use of this co-efficient with the introduction of small or large cells.

In fact, Waring et al. (1982) listed the coefficient of skewness as one of the three important endothelial parameters that are meaningful and should be determined in any study. He showed that, for example, with increasing age in the normal population, the distribution of cell areas becomes more positively skewed as existing cells become larger to compensate for the natural reduction in cell density.

The equation used to calculate the coefficient of skewness on the VIDS programme is

$$\text{Skewness} = \frac{1}{n\sigma^3} \sum_{i=1}^{n} (x_i - \bar{x})^3$$

(b) the coefficient of kurtosis is a descriptive statistic of a sample distribution and indicates the degree to which a distribution is peaked relative to the standard normal bell shaped curve. It was included in the statistical analysis since in general terms a peaked distribution around the sample mean would indicate an endothelium which tended to have cells of all the same size and therefore a homogenous population (e.g. that typically found in the age range of birth to 10 years).

If however the sample distribution was relatively flat, then this would suggest a heterogeneous population and a greater variation in cell area. High values indicate a distribution which is leptokurtic, that is, highly peaked with little dispersion, whereas low values indicate much more dispersion (platokurtic).

There should be a general association between COV and kurtosis in that the greater the spread within a distribution the flatter the distribution will be, and thus measures of both parameters give a fuller description of a sample distribution which in the case of the endothelial cell areas, should provide a better understanding of the morphology. The equation used to calculate the co-efficient of kurtosis on the VIDS programme is

$$\text{Kurtosis} = \frac{1}{n\sigma^4} \sum_{i=1}^{n} (x_i - \bar{x})^4$$

Therefore for the purposes of quantifying endothelial changes within this study, it was decided to describe the three variables of endothelial cells suggested by Waring (1982) which most accurately define the corneal endothelial response to ageing, disease, and trauma, and could be determined by the image analysis system available. These parameters relate to cell size variation (polymegethism) rather than cell shape variation (pleomorphism).

That is,

1. cell size in terms of cell density or mean cell area.
2. the spread of cell size in terms of the SD or the COV of the sample measured.
3. the heterogeneity or asymmetry of the population expressed by the co-efficients of skewness and kurtosis.

Additionally, central corneal thickness, as measured by the pachometer on the specular microscope, was considered to be an important indicator of the overall effect of long term lens wear on the cornea relative to the non lens wearing controls. Specular microscopy is in fact the oldest method of measuring corneal thickness although it may be complicated by changes in refractive index of the cornea (Waring 1992).

Although not as precise as optical pachometry it was felt that the corneal thickness results from the specular microscope were sufficiently reliable and accurate to allow both statistical, and clinical evaluation. That is, measurements across a large range of subjects (experimental and control) were being evaluated rather than treatment differences within a small group, where the precision and sensitivity of optical or ultrasonic pachometry would have been necessary. Corneal thickness measurements can be expressed in millimetres or in microns.

Specular microscopy pachometry values have conventionally been expressed in millimetres because they are accurate to 0.01mm although optical and ultra-sonic pachometers have greater sensitivity and their values are usually quoted in microns (Waring 1992).

Due to the conflicting evidence in the literature, inconclusive results of studies, relatively small sample sizes where clinical details are sometimes unknown, inappropriate control groups and statistical methods, and non-standardised measures of endothelial change, it was decided to conduct an experiment which would both assess the corneal endothelium of true long term wearers on whom the clinical information was known to be accurate, and overcome the problems pertaining to other studies.

6.2. Materials and Methods

6.2.1. Patient Sample and Controls

Most studies in medicine eventually involve the comparison of two or more groups and the determination of differences between the groups as regards a single factor of interest. Statistical analysis of a study is performed on the results obtained, with the assumptions that the groups were randomly sampled from defined populations, and that measurements taken on the sample were true reflections on what one was trying to measure. In practice there are many biases in both the selection of study subjects and the measurements actually taken. Statistics cannot correct for these biases and careful study design must be used to avoid bias where possible.

As mentioned in the introduction to the project, the ideal situation would be to have each member of the experimental group act as their own control. This would require to find long term contact lens wearers who had only ever worn one lens. The non lens wearing eye would then act as the control. Although such patients do exist, reasonable numbers are difficult to find. Alternatively, a longitudinal study would allow subjects to act as their own control but this type of experiment would obviously take many years to obtain meaningful results and require much advanced planning.

A cross sectional study was therefore designed, incorporating two distinct groups. The experimental group of lens wearers were assumed to be a cross section of the hard lens wearers in the population and had to conform to certain criteria. These were (a) at least 10 years of continuous daily wear of hard (PMMA) lenses, (b) clinical details known to verify the lens type, (c) at least 6 days/week and 12 hours/day wearing time, (d) no history of ocular disease, (e) no prolonged break from wearing lenses.

Since over the last 10 years many hard contact lens wearers have been refitted with gas permeable lenses, as a result of practitioner education and awareness of the significance and associated problems with long term wear, many former hard lens wearers were not suitable for this project. At the beginning of the project a figure of 50 was felt to be a reasonable number to aim for given the constraints listed and the statistical requirements of the two group comparison.

In order to get these numbers, patients were recruited to the study from several sources. These included patients from my own patient base and some from the Contact Lens Clinic, Department of Vision Sciences, Glasgow Caledonian

University. In addition, three local contact lens practices were contacted, where practitioners were interested in having patients followed up with the investigation described in this experiment. Endothelial details of each referred patient were provided back to the referring clinician.

The practitioners involved were aware of the type of patient required, the procedures to be carried out, and the restrictions on each. They in fact screened all possible hard lens wearing candidates in their practices who presented for after care, and might have been suitable for the study. The main reason for rejection of referral to the study, was that in some cases the patient had not been fitted by that practitioner and consequently information on lens fitting details and the history of lens wear was missing. Following these guidelines, 57 suitable patients were enrolled into the study over a two year period and subsequently included in the experimental analysis.

A patient's contact lens history is often a complex sequence, that may involve periods of lens wear with different types of lenses or modes of wear and possibly interspersed periods of no lens wear. Also the impact of lens wear may differ depending on the number of hours of lens wear each day, the nature of the lens fit, and the solutions used. Consequently, a contact lens wearing history can be complex and multi-dimensional.

The fit of the lenses on each of the subjects was checked using sodium fluorescein prior to their removal for specular microscopy. All subjects were observed to have fitting patterns which were either, an alignment fit or an apical clearance fit. This was not too surprising given the hard lens fitting guidelines used over the period within which hard lenses were fitted. All lenses were found to be between 8.80 and 9.60mm in overall diameter and when worn, were positioned either centrally or held up by upper lid attachment.

Therefore it was felt reasonable to assume that all patients had acceptable contact lens fits in terms of the lens base curve to cornea relationship. It has been shown that long term wear of hard lenses which locate off the corneal apex and have minimal movement can cause significant corneal warpage (Wilson et al. 1990). Although many of the subjects commented upon their spectacle blur, none had lenses which located consistently 'off centre'.

It was felt that this sample constituted a unique group of patients to study. Extremely careful selection meant that the rigid criteria for inclusion would allow an ideal group for this type of experimental study design.

The control group consisted of 45 subjects (20 male and 25 female) within the same age band as the experimental group. To qualify for inclusion in the study each subject had to be in good general health, have had no experience of contact lens wear, and have no past or present history of ocular disease.

Also, all subjects were questioned about family history of eye conditions to rule out any possible effects on the cornea. Direct ophthalmoscopy of the media and fundi, and slit lamp bio-microscopy of the anterior segments of both eyes of each subject were carried out to check normality. Vision (corrected or uncorrected) was checked to ensure normal levels. Most of the control group were recruited from staff and employees of the Western Infirmary and the University of Glasgow.

The refractive error and sex of each subject were assumed to be unimportant for the purpose of the experiment. No evidence is available to suggest that refractive error has any influence on the corneal endothelium of the normal eye, or that there are differences between males and females (Bourne and Kaufman 1976a). Although not directly relevant to this project, an interesting finding in a study in Glasgow (Pitts and Jay 1991) suggested a link between Fuchs' Endothelial Dystrophy and hypermetropia. They concluded that a causal relationship was improbable, and that the association arises from a common factor giving rise to both conditions for genetic linkage.

A matched pairs approach has been used in a previous study of the corneal endothelium of hard lens wearers (Yamauchi et al. 1987). This experimental design requires random samples of equal size, and the individual members of one sample are paired with particular members of the other sample. The paired sample situation differs in design, and hence in analysis, from that in which there are two independent samples. However, inappropriate pairing of subjects can lead to chance differences (Bourke et al. 1985) since it is difficult to match each subject in the experimental group exactly. Therefore a comparison of the means and variances of two independent samples was considered to be a better approach.

6.2.2. Instrumentation

Contact specular microscopy was chosen as the best means of photographing the corneal endothelium. A Keeler-Konan model 580S (Fig.30) was used throughout the experiment. Fig.32 shows a diagram of a typical specular microscope and its main components. The instrument consists of an illumination source, an applanating cone, a flash light source, and a direct viewing system, to which can be added a 35mm camera back or a video camera.

In a pilot study, in which more than 50 normal eyes were photographed, the optimum conditions for obtaining good quality photomicrographs suitable for morphometric assessment of the cells, were determined. This concerned a number of factors, the two most important of which were the choice of film and the procedure used with the instrument to photograph the endothelium.

(a) **Film choice:**

Several black and white films of potential value in specular microscopy were tested to assess the final print for sharpness of cell definition. A number of the films tested gave very similar results, although as in many photographic situations, an increase in the speed of the film to enhance sensitivity meant an increase in grain. After trying 8 different black and white print films, Kodak T MAX 400 was chosen since it gave the required results combined with the advantages of 'in house' developing and printing. The grain was found to be adequate for individual cell detail, including intra-cellular highlights as well as cell borders and contours.

On obtaining the developed film from the photographic technician, an inspection of either the film negatives or a contact print of the film using a monocular magnifier determined the best frames for printing. A minimum of 4 suitable prints were required for each eye to meet the criteria of 25 cells to be traced from each of four separate areas (photographs). This gave a total sample of 100 cells.

(b) **photographic procedure in the clinical use of the specular microscope**:

Currently most wide field specular microscopes photograph an area of around 0.8 to 1sq.mm, assuming the full slit width of the illumination system of the instrument is used. This means that potentially up to 2000 cells may be imaged in a single photograph. However difficulties are created by light scatter and variations in lighting and image focus. Sharper pictures can be obtained by narrowing the incident slit width to reduce light scatter, thus cutting the overall field of view.

Patients were advised as to the nature of the ophthalmic investigation and about the data that was likely to be obtained. For the experimental group, contact lenses were removed and a drop of 0.4% benoxinate hydrochloride as a local anaesthetic was applied to the lower fornix of each eye. This was found to be easier than using a soft contact lens although the application of a high water content disposable soft lens was found to be equally successful for photography but more time consuming in its use.

The patient was seated at the instrument to allow their chin to be placed on a chinrest at the required height. The importance of maintaining fixation on the target was stressed, and normally the right eye was photographed first followed by a short break before completing photography on the left eye. Between patients, the applanating cone was disinfected using alcohol wipes (0.9%), then carefully dried and a drop of contact lens wetting solution or artificial tears (hypermellose) placed on the tip, to act both as a wetting agent and as a mechanical buffer.

Following contact of the tip of the cone on the tear film (Fig.31), the microscope was adjusted to focus on the epithelium and the digital pachometer was set to zero. Corneal epithelial cells were usually fairly easy to see and in fact the specular microscope has been used to study changes in epithelial cells in a number of clinical conditions (Tsubota and Yamuda 1992).

The focus was then automatically adjusted using the motorised mechanism on the instrument joystick to view corneal structures posterior to the epithelium, until the endothelial cells appeared clearly in view. It is very difficult to focus on any stromal detail although occasionally, a lamellar arrangement of collagen fibrils may be seen (Mayer 1984).

Prior to photographing the required areas of the endothelium a general scanning approach of the central and paracentral cornea was carried out to provide a qualitative assessment of the endothelium particularly noting any interesting variations. A number of pictures (approximately 9) were then taken in the locations and sequence shown in Fig.33 to ensure the coverage required.

Once the photographic procedure was completed the applanating cone was withdrawn from the cornea which was then checked with sodium fluorescein for any abrasion, before reinserting the patients own contact lenses. The digital reading of central corneal thickness was taken using the pachometer which had been previously calibrated against a plastic block of known thickness.

There are two lighting controls on the Keeler Konan specular microscope. These are the viewing illumination which was found to be best around the mid- point setting and incorporating a green filter for additional contrast and the flash tube setting which was mainly used at the highest level to obtain the greatest film exposure. Movement of the applanating cone around the central cornea was easily controlled by the X/Y controls of the instrument to obtain up, down, right and left positioning and excess applanating pressure was prevented by the warning buzzer operating within the instrument (Fig. 32).

The central area of the cornea was more easily imaged than the periphery since normal alignment of the cone was required to applanate the corneal surface to obtain the specular image. However in contact lens wearers, the central cornea is the main area of interest since it can be assumed that it will represent a zone of the corneal surface which is continuously covered by a lens during normal wear, allowing for eye movements and blinking. It will also be the area of the cornea exposed to the greatest level of relative hypoxia and coincide with the area through which the visual axis passes.

It was therefore convenient to assess a central zone of 5mm diameter by taking at least nine photographs in sequence and selecting the best prints for further analysis. Even with hard contact lens wear where lenses can move 1-2 mms on blinking the central area of the cornea will always be covered by the lens unlike peripheral areas which may be exposed on blinking and/or with eye movements depending on the fit and centration of a particular lens. This argument is confirmed by the well documented central corneal clouding associated with PMMA hard contact lens wear (Stone and Phillips 1989).

The central 5mm of the cornea is equivalent to a surface area of 20.6 sq.mm (assuming a spherical cap of radius 8.00mm, a sag of 0.4mm, and a surface area of $2\pi rh$) from which the sample of cells was taken for morphometric measurement. The total number of cells in this area will be approximately 50,000 assuming an average endothelial cell density of 2500 cells/mm^2. Since there are anatomical differences in the structure of the central and peripheral cornea and also differences in the endothelial morphology (Tuft and Coster 1990), results from this study were based on the central region around the visual axis.

To provide the photographs for image analysis, the central 5mm zone of the cornea was split into a 3x3 box matrix previously shown in Fig.30. This meant that areas above, on, and below the apex of the cornea were photographed and recorded for cell analysis.

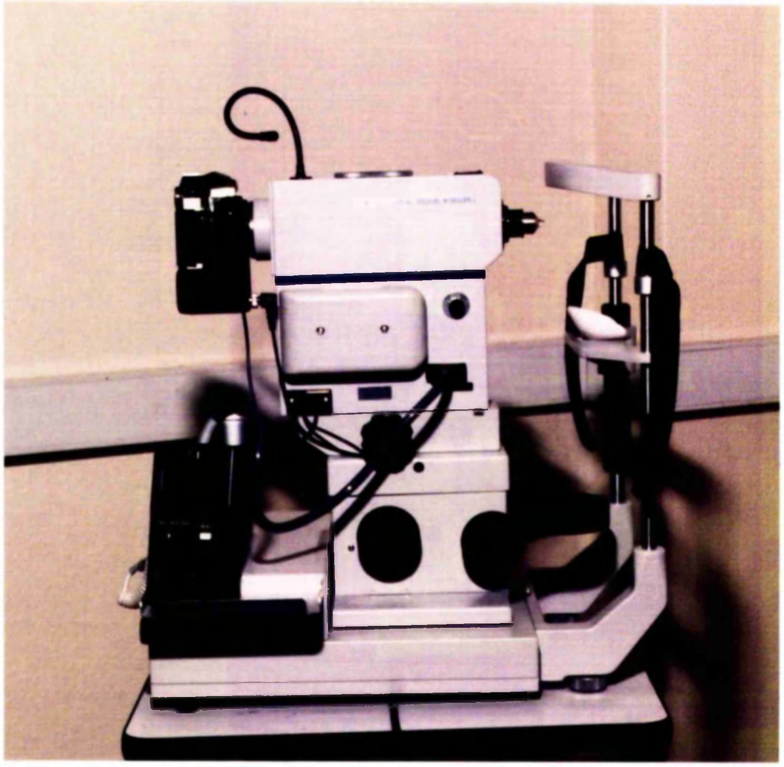


FIG. 30.

The Keeler-Konan Specular Microscope

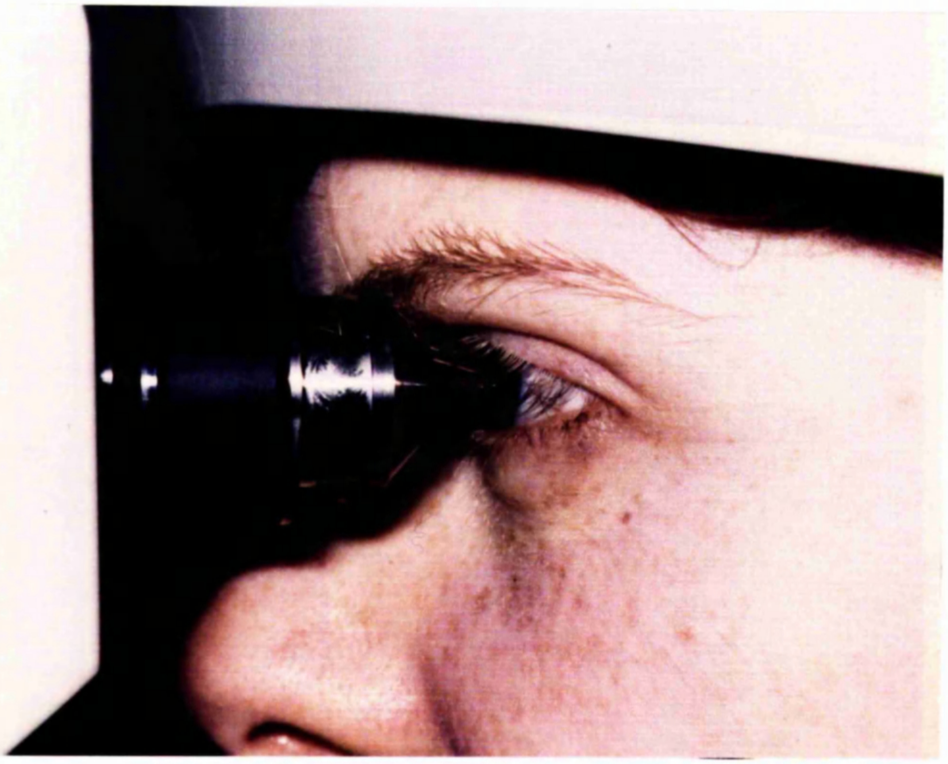


FIG.31

Contact of the applanating cone of the specular microscope with the anterior surface of the cornea.

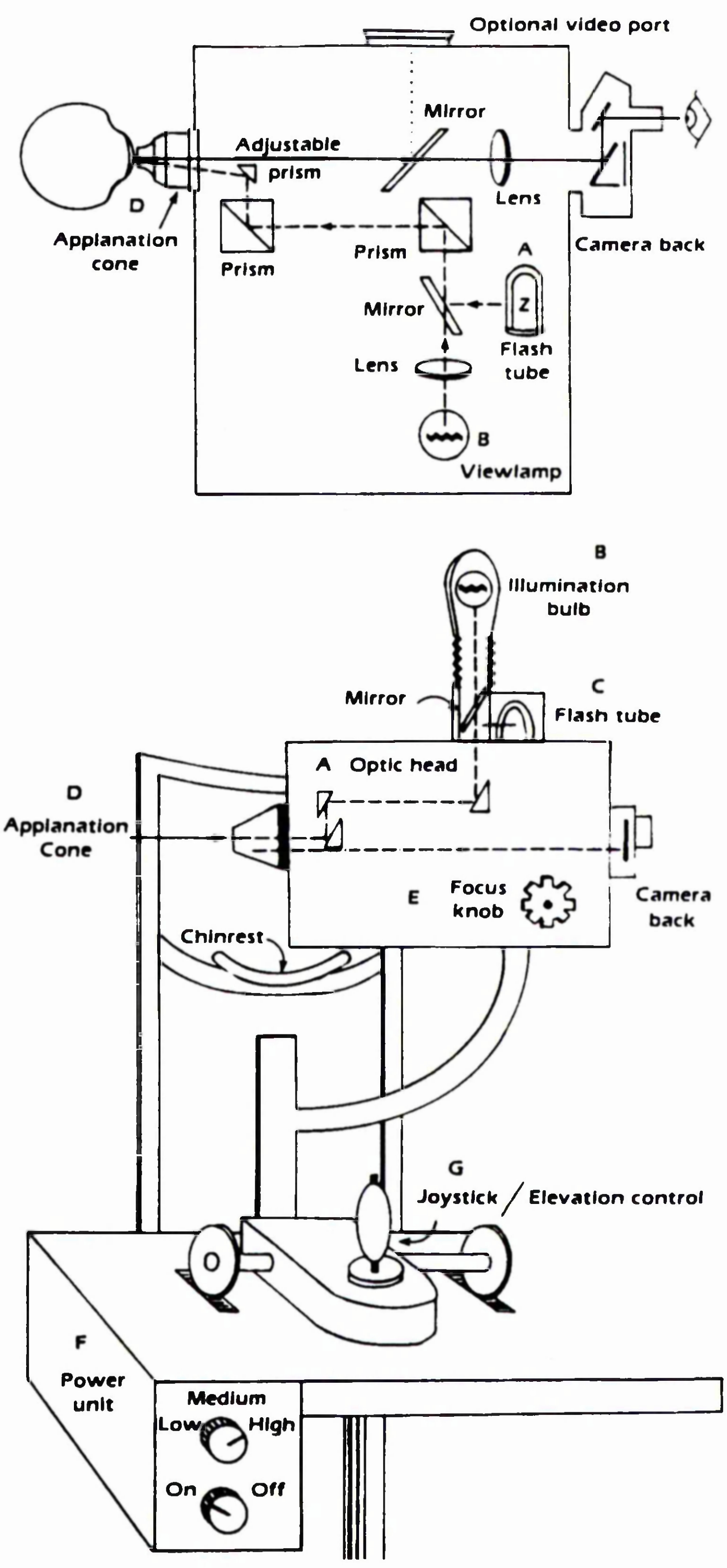


FIG. 32

The main features of a specular microscope showing (a)optical head (b)illumination system (c)flash unit (d)applanation cone (e)focus control (f)power unit (g)joystick control lever.

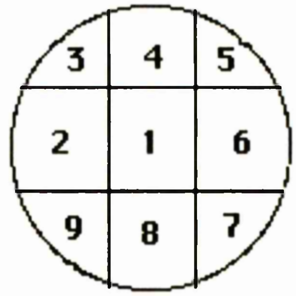


FIG.33

The arrangement and sequence for photographing the central corneal endothelium with the specular microscope.

FIG 34

The VIDS image analysis system used for the specular photomicrographs.

6.2.3. Image Analysis

The image analysis system used was the Optomax V Video Interactive Display System (VIDS) produced by AMS in Cambridge, England. The hardware consisted of a high performance tube video camera fixed to a purpose built macro stand on which the photomicrograph was positioned. The stand had a one metre high pillar and the camera was adjusted to give a magnification which allowed approximately 150 cells to be viewed on the monitor. A video camera captures a frame on the monitor allowing the image to be contrast enhanced or a digitiser to trace elements of the image to be analysed. The apparatus is shown in Fig.34.

The calibration of the system was then done utilising the graticule on the photomicrograph with the mouse on the digitising pad. Extremely good measurement accuracy was assured by high spatial resolution (704x560 pixels) coupled with 256 grey level detection. Measurements were then made by tracing the desired sample of cells, again using the mouse on the digitising board. Measurement data were displayed, stored on disc, and printed on a defined format. Statistical analysis was applied within the program and included the descriptive data and graphical displays.

The standard programme on the VIDS system lists 15 different feature specific measures of cells that can be obtained by image analysis. These were all considered for their relevance to this project and in relation to those measurements commonly referred to in the ophthalmic literature describing the corneal endothelium. They are listed below to indicate the range of measures that were possible with this system.

CELL MEASURES

Area	Horizontal intercept	vertical intercept
width	longest dimension	breadth
orientation	axial ratio	form factor
fibre length	fibre width	spherical diameter
perimeter	height	x centre of gravity
y centre of gravity	spherical volume	user defined parameters

TABLE 6

The range of cell parameters which could be computed in the image analysis system.

(d) **Cell count accuracy and precision:**

Since it is technically impossible to photograph every cell within the central corneal area for morphological measurement, a sampling method was necessary. This is typical of many similar procedures involving cell counts in medicine. When the cells were of fairly uniform size, without areas of guttata, and the image sharp, Irvine et al. (1978) found the accuracy of the cell count to be within 8%.

This could be regarded as being typical of the contact lens wearing endothelium. A change in thickness of the cornea can account for a variation in cell count of only 1-2% as noted by Bourne and Kaufman (1976a). Nevertheless, errors are present in any sampling technique and are often difficult to quantify. Careful controls over the technique as described, will help to minimise these errors.

Previous researchers have varied in the number of cells they have sampled when studying the corneal endothelium (Bourne and Kaufman 1976a, Yee et al. 1985, Hirst et al. 1989). Examples of sample size of cells to be measured and counted have ranged from 25 to 150 cells in total (Doughty 1989b). The compromise that has to be reached is that a minimum number of cells have to be counted to ensure a representative sample is obtained, allowing appropriate clinical and statistical conclusions to be made.

Equally, counting more than this minimum number would be counter productive. In the system used in this experiment, errors could have arisen from two sources. The first would be if the sample of 100 cells was not representative of the total population and the second if the tracing method was variable with poor reproducibility.

Since a reasonable period of time was required to actually trace a sample of cells, and a sufficient number of individual eyes needed to be evaluated in both experimental and control groups, it was felt that 100 cells would be an appropriate number for two main reasons.

(1) In a series of photomicrographs from 20 normal eyes, morphometric image analysis was conducted on 100 cells within a photomicrograph, recording the cumulative mean and standard deviation in cell area after 25, 50, 75, and 100 cell counts. In comparing the mean and standard deviation at each of these cell counts with the completed 100 cell sample estimate, it was found that the percentage difference between the 50 and 100 cell count values was always less than 5%.

In other words, it would seem that in normal corneas with homogenous endothelia, even an analysis of 50 cells could be valid to quantify endothelial morphology.

Therefore, in considering both controls and experimental subjects where cell variation can be greater, an increased sample of 100 cells was felt to be acceptable from the central corneal endothelium. The main aims in sampling were to ensure that cells were (a) not replicated and counted twice, and (b) representative of the overall appearance of the central endothelium. That is, photographic overlap was avoided and pictures did not concentrate on a particular feature which was specific to one area.

Researchers differ in the criteria used to sample cells from the corneal endothelium for morphometric measurement (Hirst et al. 1989, Doughty and Fonn 1993). The aim in this study was to obtain a reliable and valid measure of cell area from the fewest number of cells traced. Although it could have been argued from the pilot study that 75 cells may have been sufficient to achieve this, a sample of 100 cells was used to increase the validity of the sample from which measurements could be taken.

To avoid potential bias, and to ensure a cross section in sampling, 25 cells from each of four photomicrographs (prints) of different zones within the central cornea were counted to give a total of 100. Usually this meant starting at the left hand side of the photograph next to the calibration grid and tracing 25 cells. The second photograph was then chosen and a further 25 cells were traced. The third and fourth photographs followed to give the total number of cells traced equal to 100. Despite the argument that counting 100 cells from one photomicrograph would have ensured that no cell was counted twice, sampling cells from four different regions within the central zone provided a better cross section of cells, particularly in the lens wearers.

Prior to each set of 25 cells being traced, the system calibration was checked using the graticule which was superimposed on the print. The video camera magnification was set to give an area of corneal endothelium imaged on the monitor containing a minimum number of approximately 100 cells.

The typical screen picture of cells traced is shown in Fig.31 and in this print out, the sequence of the cells traced is numbered, and the long axis of each cell is drawn. These are optional features which can be deleted if required. For illustration purposes, 100 cells have been traced from one specular photomicrograph.

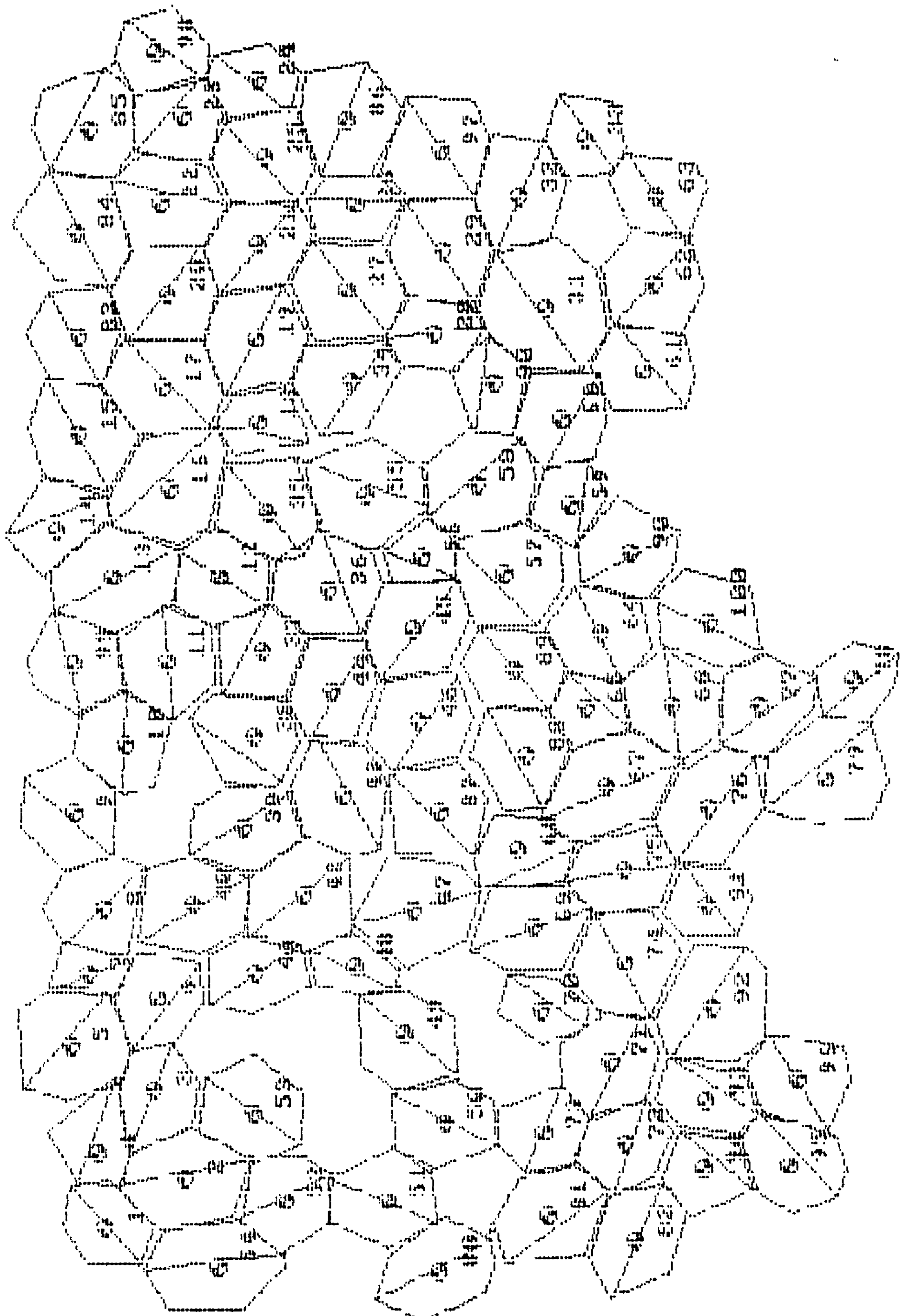


FIG.35

A 'screen dump' of an example of the cells traced on the Optomax image analysis system.

(2) The time necessary to carry out the complete procedure on a patient, including the image analysis, was approximately 30 minutes. This was felt to be reasonable given that the total number of patients and controls to be photographed and measured was around 100.

The cells were individually traced using the mouse and digitising pad, linked to the computerised image analysis system. Each apex of the cell borders was marked by the cursor, the position of which was controlled by the mouse on the digitising pad, and the line joining two adjacent apices formed a cell border. As previously stated, manual tracing was chosen instead of the automatic image enhancement.

Preliminary investigations in a pilot study using the VIDS image editor, showed that with many corneal endothelial photomicrographs, cell borders were obscured by other detail and were subsequently better defined by the human eye than the computer. That is, the computer software filled in gaps and left blank spaces within a sample that were sometimes inappropriate. This was especially so when there was a variation in image quality across the photomicrograph. Image enhancement, although creating better contrast in some cell borders, resulted in inappropriate gaps between cells and thus errors in the analysis.

A question posed at the outset was, could increased variability arise from the tracing technique, particularly if individual cells had poorly defined borders? However, repeatability of the tracing method was also found to be good. This was determined by tracing a small cell from a photomicrograph on ten separate occasions which gave a mean area of 1.39 μm and a SEM of ±0.03. Tracing a large cell from the photomicrograph to give 10 repeated measures gave a mean of 5.55 μm and an SEM of ±0.12. These results agree with others who have evaluated their own measurement systems by tracing geometric figures of known area to assess repeatability and validity (Hirst et al. 1989).

A small cell tended to have well defined borders but indistinct apices, whereas large cells had wider out of focus borders but more definite apices. If some cell borders were slightly out of focus on a photomicrograph, the cursor dots to pinpoint the apices of the cells were placed so that the line drawn between two cell apices was placed in the middle of the border. The greater the magnification of the photomicrographs on the monitor, the greater was the potential error due to this phenomenon.

With some irregularly shaped cells which did not have straight borders it was possible to trace curved lines with the mouse rather than have a straight line joining two apices. This did not affect the analysis when the cell area was being calculated since it was simply the area within an enclosure that was determined. Some image analysis systems do not allow this facility and therefore introduce some inaccuracy in the measurement of the cell parameters when dealing with asymmetrical or irregular images, in only allowing straight lines to be drawn.

Calibration of the image analysis system was vital to ensure that valid measurements were taken from the photographs. The camera back on the specular microscope had a graticule fitted at the film plane so that each photograph has a superimposed scale.

Prior to a measurement sequence the VIDS system was always calibrated, which in fact was a programmed step on the software before it would operate in the measurement mode. To calibrate the system, the cursor controlled by the mouse was clicked on each of two points on the graticule scale which was equivalent to 0.1mm. Every subsequent measurement was then relative to this calibrated value until a further recalibration was necessary.

The pachometer reading on the specular microscope was determined by the difference in focus when viewing the epithelium and endothelium, the sensitivity of the measurement being to the nearest 10 microns. The distance the objective lens has to travel to view these two layers is automatically determined on an electronic scale within the instrument. Since the angle of view between the illumination and observation axes is very small with the specular microscope, a correction factor does not need to be applied to the pachometric measurement as it would on other non contact systems (Doughty and Fonn 1993).

Therefore an experiment was designed to determine the morphological changes in the corneal endothelium of a group of long term PMMA contact lens wearers, and compare this to a group of normal healthy controls. It is the long term lens wearers that are most likely to be at risk of corneal damage as a result of chronic hypoxia over many years of contact lens wear.

The lenses (PMMA) have zero oxygen transmissibility and during lens wear it can be assumed that the central cornea is continuously covered by the lens. Also the daily wearing times of these lenses has traditionally been long (>12 hours) since on removal of the lenses vision is often reduced due to spectacle blur, caused by transient corneal shape changes.

In addition, central corneal thickness in the lens wearing group was compared to the control group. If the stroma is affected by long term lens wear as others have suggested (Holden et al. 1985), then an overall decrease in corneal thickness in contact lens wearers might be expected.

As previously noted, any trauma or injury to the endothelial cell layer results in a decrease in the number of cells and thus a decrease in the corneal reserves. Although the cell count may be low, the remaining endothelial cells are often capable of preventing corneal swelling until a critically low threshold is reached. A cornea of normal thickness provides little information about endothelial cell density or reserve.

6.3 Results

The clinical profiles of the two population groups are shown in Table 7. The age distributions of the two populations of subjects were not found to be significantly different (t test, $p>0.05$; Fig.36). The number of years of lens wear in the lens wearing group ranged from 10-35 years (mean = 18.6 ±6.18; Fig. 37). All subjects wore their lenses for 7 days per week for a minimum of 12 hours per day, except for three subjects who indicated that they wore lenses typically for 6 days per week and spectacles on the 7th day, as a convenient alternative for vision correction.

The control group showed the expected trend of decreasing cell density with age ($r=-0.55$, $p<0.05$) (Fig.38). The increasing trend in COV with age (Fig.39) was not statistically significant ($r=.36$; $p>0.05$). This confirms the published endothelial data referred to earlier (Bourne and Kaufman 1976a), and would suggest that the control group was a representative sample of the general population.

Relative to published norms for age against cell density, and given that endothelial cell loss is apparently greater before the age of 10 and after the age of 75 years, it should be noted that the overall age range in both of the groups used in this experiment did not cover either the very young or the elderly. A more gradual decline in cell density might therefore be expected between the ages of 30 and 65 years.

	control group	**experimental group**
number	45	57
age(mean/range)	39.78 ±12.27	43.47 ± 8.89
lens wear (years)	0	18.61 ± 6.18
cell area (μm)	3.07 ±.347	3.35 ±0.85
cell density (cells/sq.mm)	3304 ±377	3104 ±529
COV in cell area (sd/mean)	.29 ±.07	0.40 ±.122
coeff. of skewness	.81 ±.63	1.14 ±.74
coeff. of kurtosis	4.29 ±2.57	5.72 ±4.19
corneal thick.(mm)	.545 ±.034	0.552 ±.05

TABLE 7

Clinical profile of the control and experimental groups in the specular microscopy study.

The mean cell density for the control group was 3304 cells/mm^2, and for the lens wearing group it was 3104 cells/mm^2. These values are not significantly different (t test, $p>0.05$). However the COV in cell area for the control group was .29 (95% CL .18 and .53), whereas for the lens wearing group it was 0.40 (95% CL .27 and .78).

These COV values are significantly different (t test, $p<0.05$), confirming the greater variation in cell area found by other studies. As pointed out previously, the COV in cell area as a index on its own may be ambiguous, and as such, a measure of skewness of the distribution of cell sizes may be helpful. The mean coefficient of skewness for the control group was 0.81 (95% CL .603 and 1.014) and for the experimental group was 1.14 (95% CL .94 and 1.332). These skewness values are significantly different ($t=2.33$, $p>0.05$), confirming the greater variation in the distribution of cell areas or polymegethism in the lens wearing group.

Observation of the distribution plots of cell sizes revealed interesting patterns. Fig.40(a) shows a normal control endothelium (patient aged 43 years) and Fig.40(b) a plot of the cell areas. The distribution is approximately normal. Fig.41(a) shows the endothelium of a subject with 20 years of contact lens wear (patient aged 44 years), and Fig.41(b) a plot of the positively skewed distribution of cell areas.

In general therefore, it appeared that the reason for an increase in COV in the contact lens wearers, was an increase in the number of bigger cells. However, in some cases, it was observed that clumps of small cells existed and contributed to the increased COV. This phenomenon has been observed previously, but not considered in detail.

The following case was the most extreme example of the presence of small cells found in the series of patients assessed by specular microscopy.

A 49 year old woman presented to our research clinic as part of the investigation into the effects of long term wear of hard contact lenses on the cornea. She had worn poly-methyl-methacrylate (PMMA) hard lenses for 26 years to correct myopia (-4.00D R/L) with no history of any ocular problem. Her medical history indicated hormone replacement therapy over the last 20 years for Sheehan's Syndrome.

Her corrected visual acuity's were right and left 6/6. IOP was normal and there was no evidence of angle or iris abnormalities, including no unusual pigment loss or dispersion and no abnormalities of the lens or its capsule. The vitreous was clear but degenerate, and the fundus showed signs of myopic chorioretinal degeneration around the optic nerve head.

Contact specular microscopy of the central corneal endothelium revealed the general appearance of large cells interspersed with clumps of small cells (Figs.42a, RE). (Fig.42b, LE).

Image analysis of the left eye pictures gave a cell density of 1213 cells/mm^2, a mean cell size of 8.24 x 10^{-4}mm and a coefficient of variation in cell size of 0.75. The corneal thickness at the first examination was RE 0.53mm and LE 0.54mm, as determined by the specular microscope pachometer and slit lamp examination showed slight central corneal oedema consistent with hard contact lens wear. The right and left eyes were re-photographed 6 months later and a similar appearance was noted (Figs. 43a and 43b).

From the image analysis of the LE photomicrographs, a plot of the distribution of cell sizes revealed a bimodal distribution (Fig.44) indicating two separate populations of cells. A skewness index is not valid with such a distribution (Bulpitt 1987) and the high value of COV is not particularly helpful to describe this particular endothelial morphology.

In an attempt to evaluate corneal function we used a 'stress test' which involved the patient wearing a thick soft lens (0.3mm Allergan Hydron pHEMA) for a one hour period (LE), corneal thickness being measured before and after the wearing period. The PMMA lens for this eye had not been worn for 24 hours prior to this test. The stress test is based on the method used by Polse et al. (1989).

The ability of the cornea to return to base line thickness following the contact lens induced swelling, is assumed to be a measure of the pump function of the endothelium. This assumption has still to be experimentally verified. Fig.45 shows the recovery of corneal thickness to base line values relative to an age and sex matched non lens wearer. The age matched control subject was selected from the larger group acting as normal healthy controls for the main experiment. She had a cell density of 2651 cells/mm^2, a mean cell size of 3.76 x 10^4mm and a coefficient of variation in cell size (COV) of 0.26.

A slower rate of recovery was indicated in the lens wearing subject suggesting a compromised endothelial pump function although the initial degree of corneal swelling was less than in the non lens wearing control. The swelling test with a thick soft lens was tried in five other subjects (long term wearers) each being matched with a control subject. In all instances the response was similar to that in Fig.45. That is, the rate of recovery in corneal thickness to base line levels was slower in the lens wearers but it was nevertheless achieved in all subjects. This indicates a modified pump function in the corneal endothelium in the long term wearers.

It is well established that the corneal endothelium is affected by ageing and disease processes and by intra-ocular surgery. More recently reports have been published suggesting that long term contact lens wear causes morphological changes in the appearance of the corneal endothelium which may not be reversible. The most likely cause of this is chronic hypoxia although the resultant effect on corneal function is controversial.

One earlier report presented data on the appearance of clusters of small cells after a corneal graft rejection in one patient, which the authors felt might have been a consequence of mitosis in the corneal endothelium (Laing et al. 1984). Whether it could be speculated that a similar situation was happening in this patient, is open to debate.

To the best of my knowledge, similar appearances have not previously been reported in an otherwise healthy eye. The cell density (1213 cells/mm^2) of this patient, being lower than expected for the age, may contribute to the reduced rate of recovery following the swelling test, but is still sufficient to maintain corneal transparency. Since PMMA contact lenses (haptics and corneals) were fitted in large numbers between 1950 and 1980, patients still wearing these lenses should have their corneal endothelium assessed for morphological changes.

Clinical tests to determine corneal function, if available, then need to be applied to evaluate the effects of such changes. When evaluating the dependent variable it is worth noting any degree of association between some of the parameters. If factors such as the central corneal thickness or the coefficient of variation of cell area could be determined to be predictors of corneal function then they would have some clinical significance.

Figs.46-49 show endothelial micrographs of examples from the control group with details of the results from the image analysis. Figs.50-53 show endothelial micrographs of examples from the lens wearing group showing the increased polymegethism found. Details of the results from the image analysis are also provided.

Figs.54a and 54b show the general effect of age on the endothelial appearance. Fig.54a is a young boy (age 6) and Fig.54b of his paternal grandmother (age 84). These are presented to show the effects of age on the endothelium in otherwise healthy eyes. The details of the image analysis indicate the changes with age in the cell morphology{Figs.54(a), 54(b)}. Interestingly the cell area distribution for the 6 year old shows a negative coefficient of skewness, and this agrees with previous observations on young eyes (Waring et al. 1982).

It would seem reasonable to hypothesise, that the longer PMMA lenses had been worn, the greater the effect they would produce, but regression analysis on the parameters of the long term PMMA wearers did not reveal any significant relationships or predictors of clinical features.

Fig.55 shows a scattergram of years of lens wear against cell density, Fig.56 against COV and Fig.57 against corneal thickness. None of these shows a significant linear correlation indicating that years of wear on its own, does not constitute a dose related effect in these corneal parameters. On the basis of these results, corneal function would seem to be unaffected in the specific group of wearers assessed. Fig. 58 shows a scattergram of COV against CCT for all the long term PMMA wearers. Again, the correlation was not statistically significant.

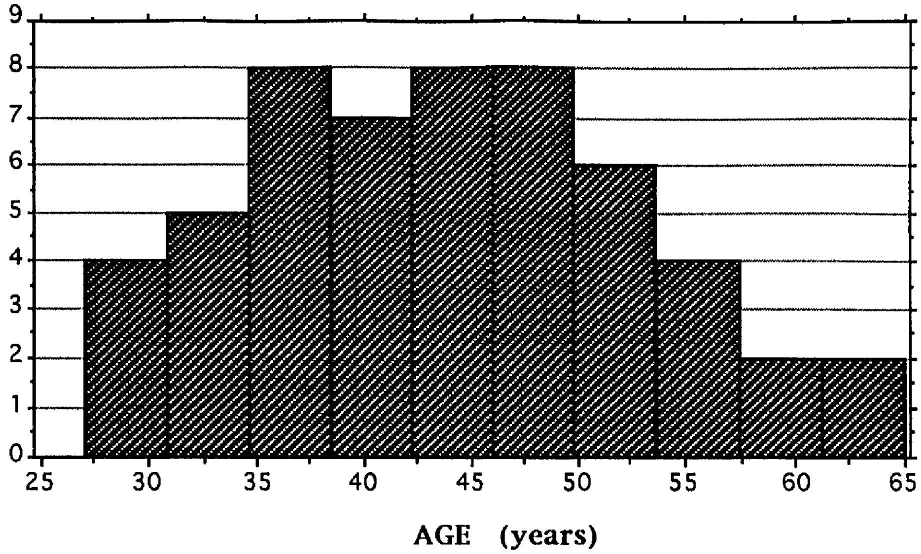


FIG.36

Frequency distribution of age within the experimental group of lens wearers.

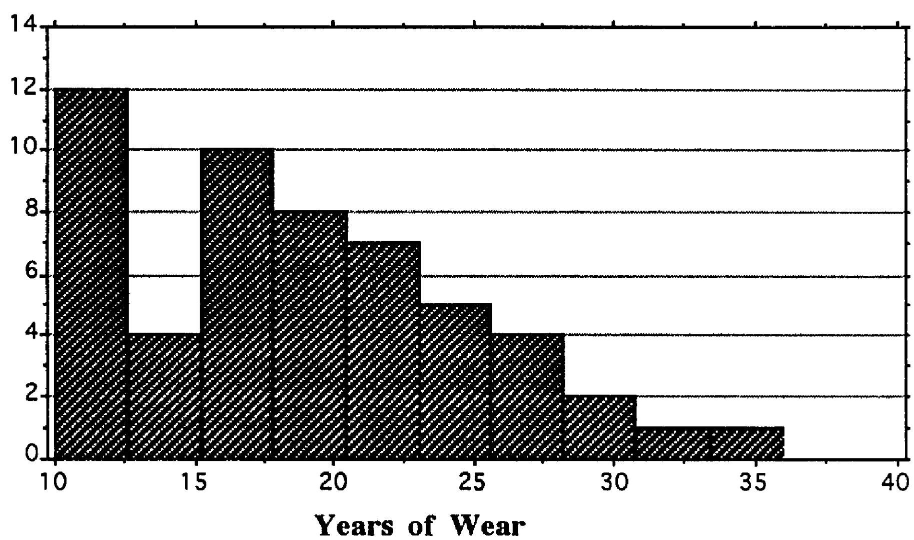


FIG. 37

The number of years of wear of hard lenses in the experimental group.

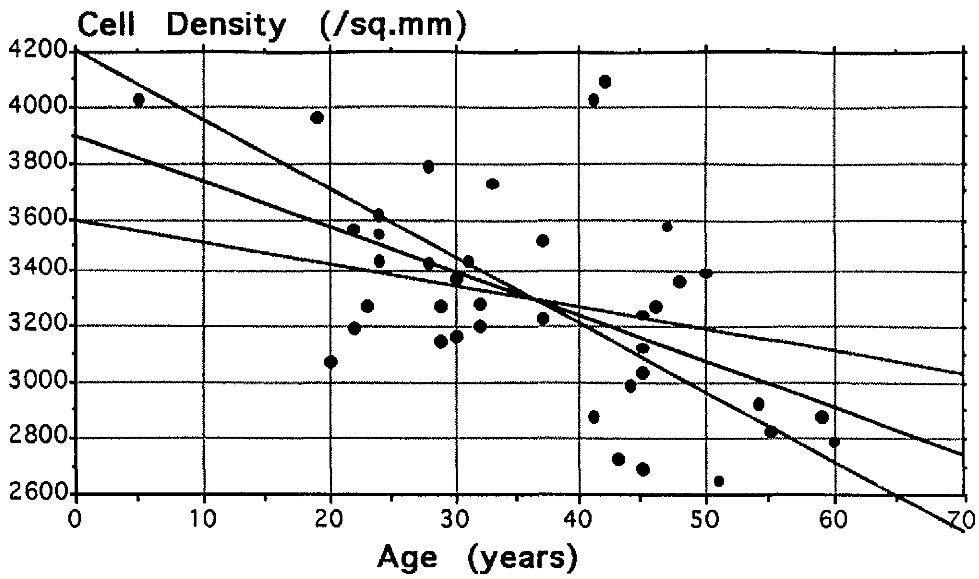


FIG. 38

Decrease in cell density with age for the control group (95% CI for the slope of the regression line are shown; r =-0.55).

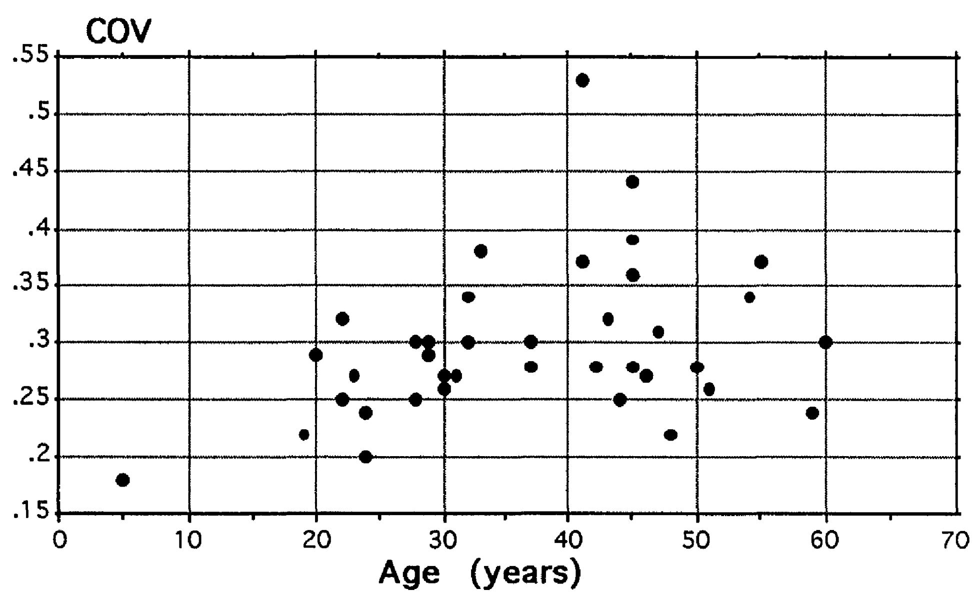


FIG. 39

Scattergram of COV with age in the control group (variance r^2=.133, not significant).

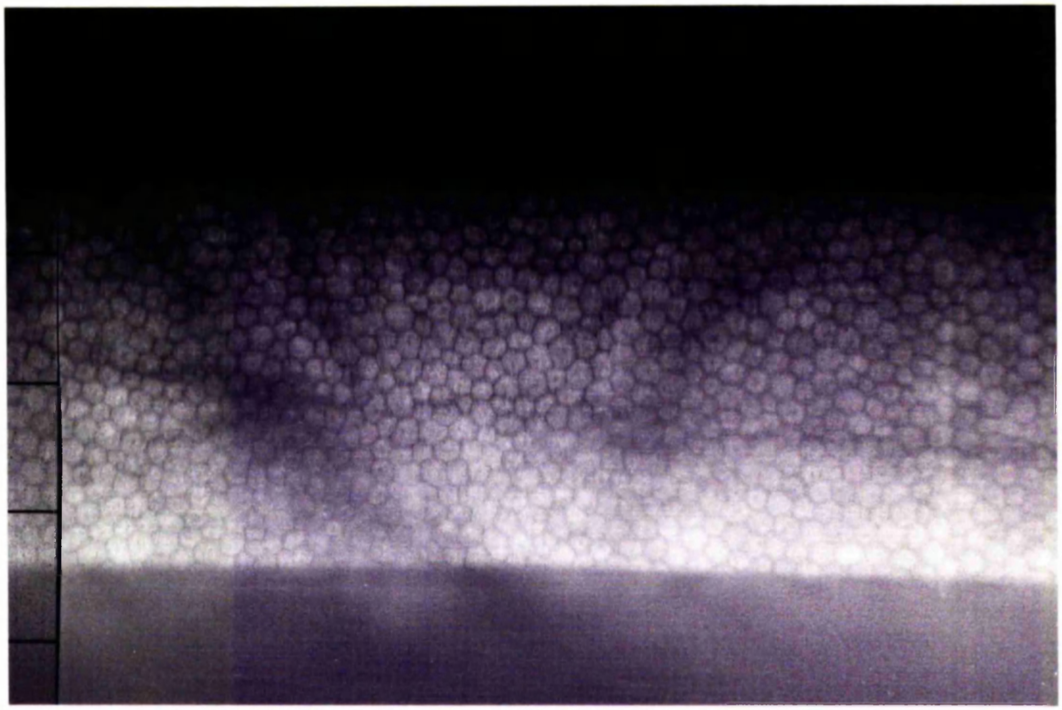


FIG. 40a

Specular photomicrograph from a normal control cornea

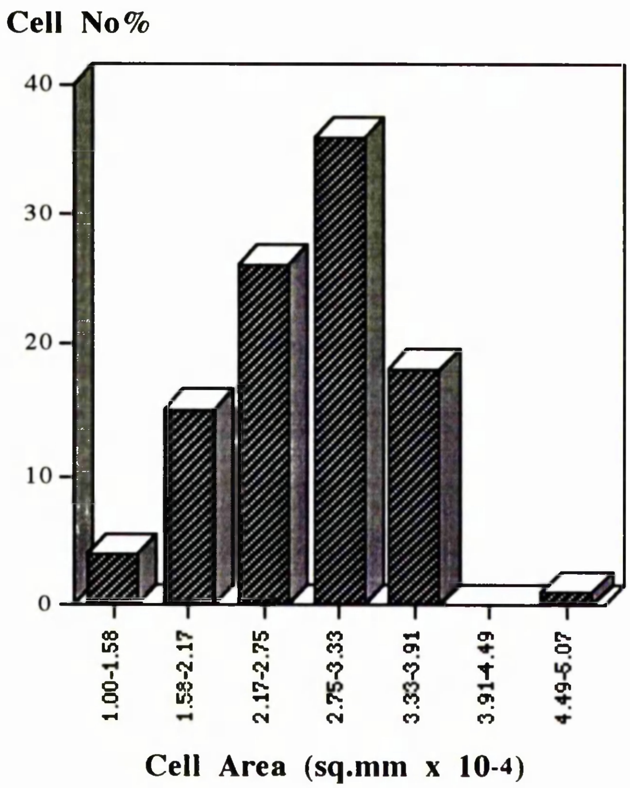


FIG. 40b

Cell area distribution for the above corneal endothelial photomicrograph.

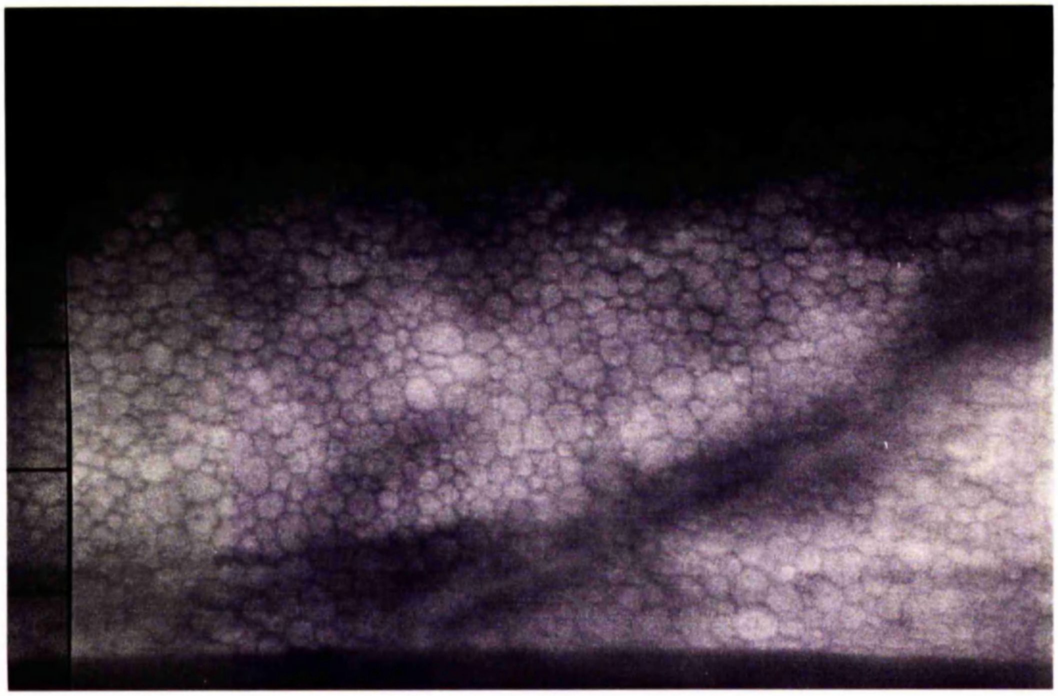


FIG. 41a

Specular photomicrograph of a long term PMMA contact lens wearer

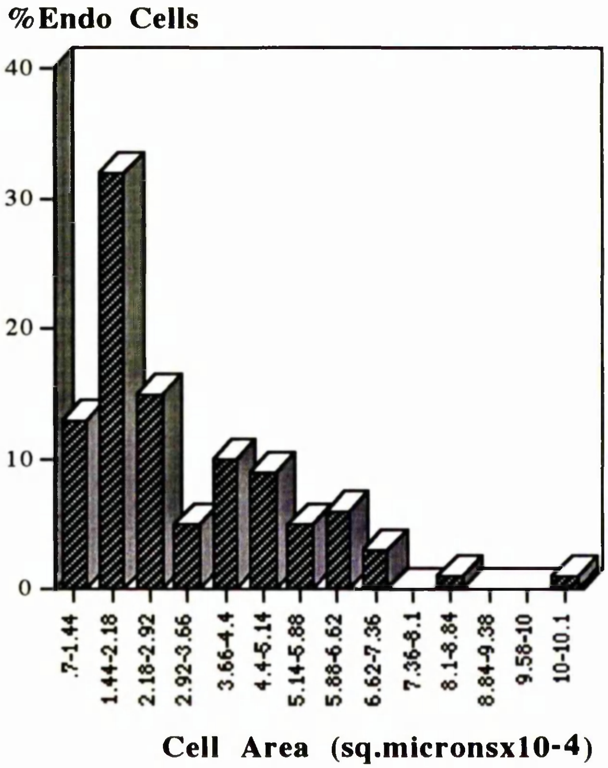


FIG.41b

Cell area distribution for the endothelial photomicrograph above showing a positively skewed distribution.

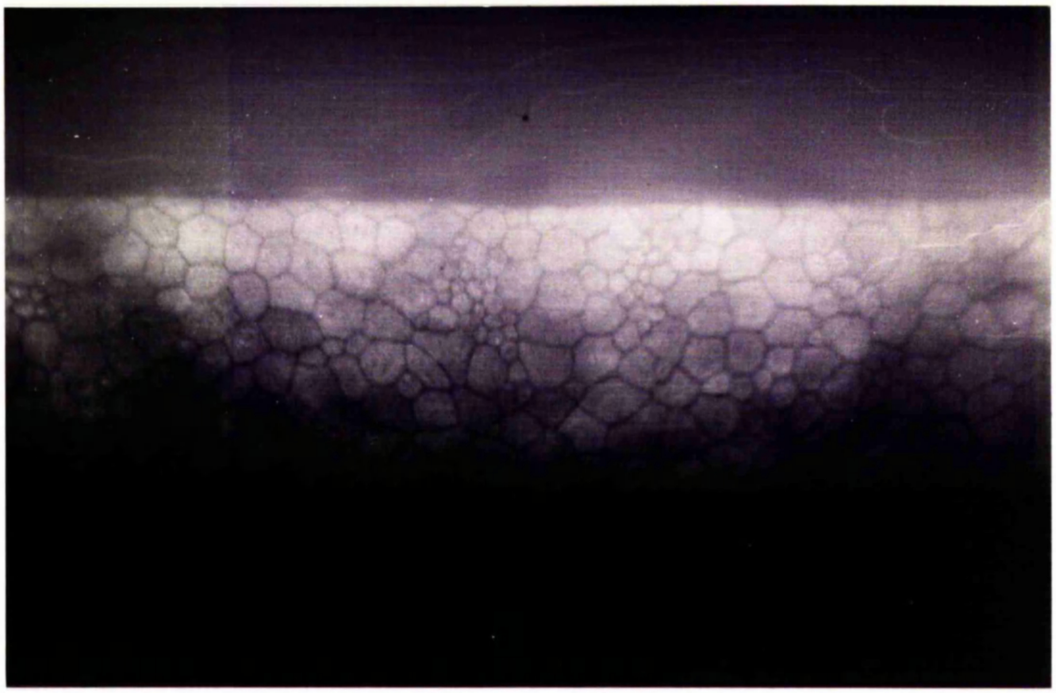


FIG. 42(a)

Subject (Mrs McQ, RE), showing clumps of small cells within a mosaic of larger cells .

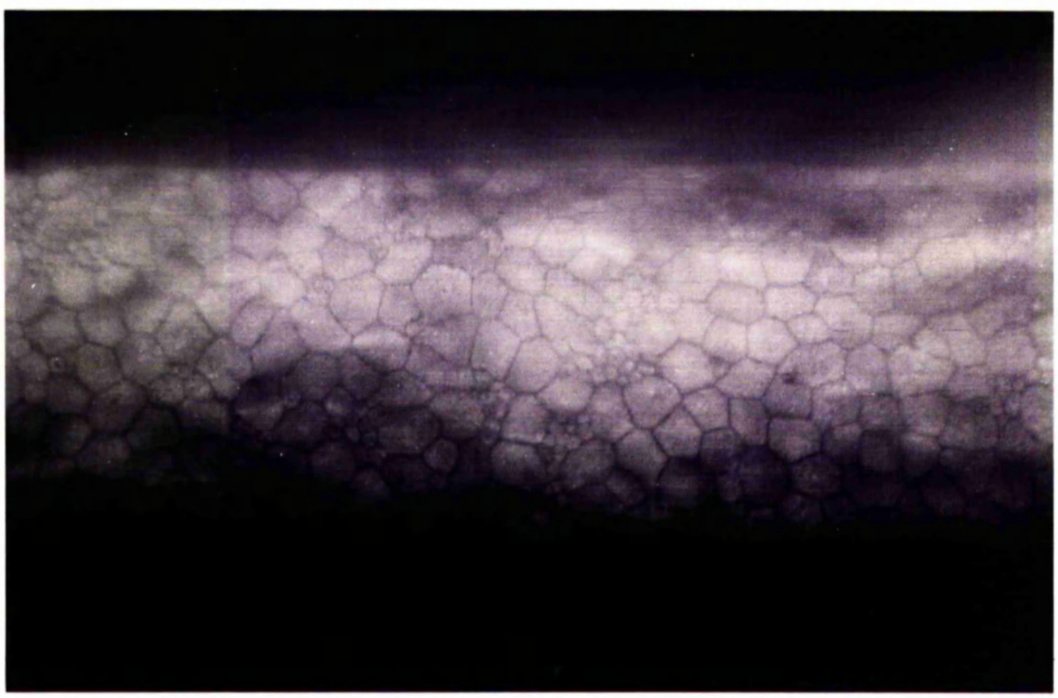


FIG. 42(b)

Specular photomicrograph of the central corneal endothelium of the LE (Mrs McQ).

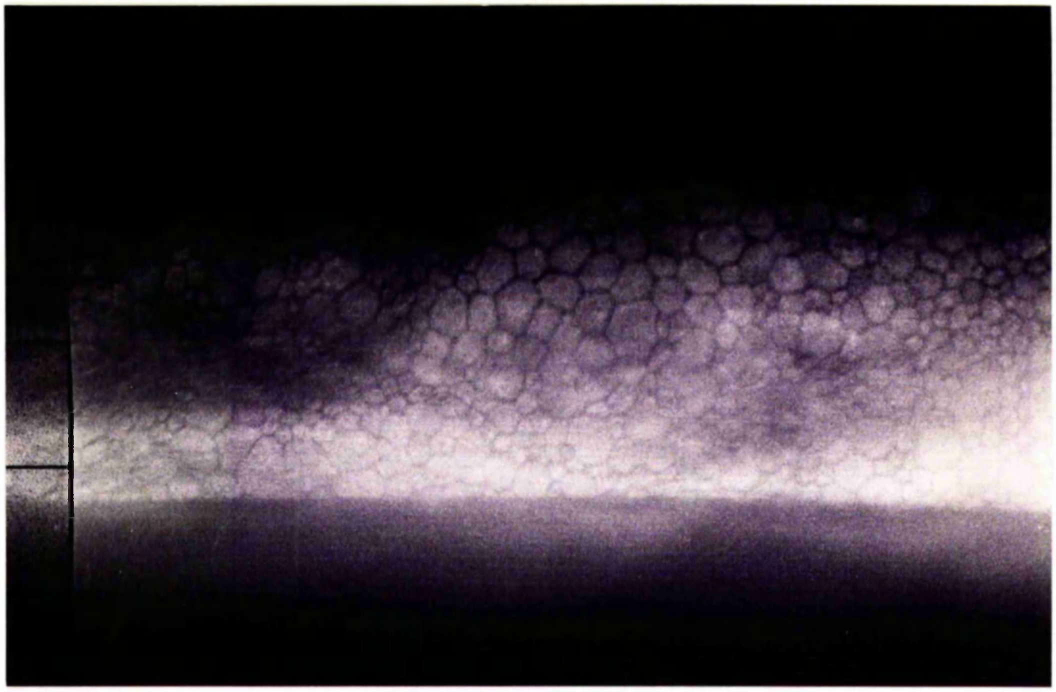


FIG. 43(a)
Specular photomicrograph of the RE (Mrs McQ) taken six months later.

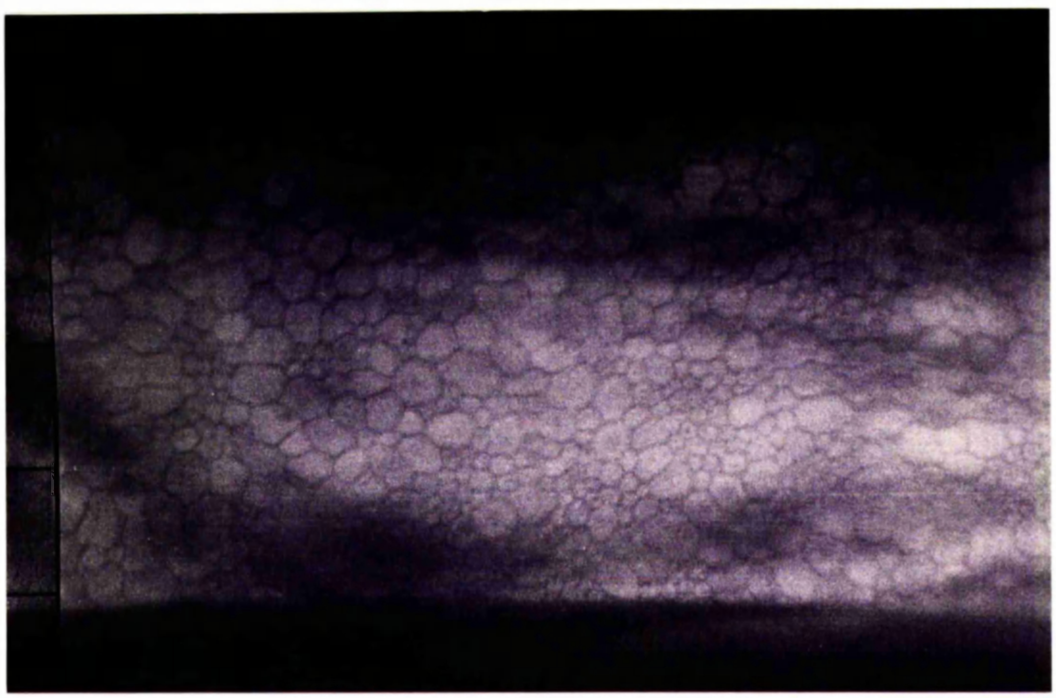


FIG.43(b)
Specular photomicrograph of the LE (Mrs McQ) taken six months later.

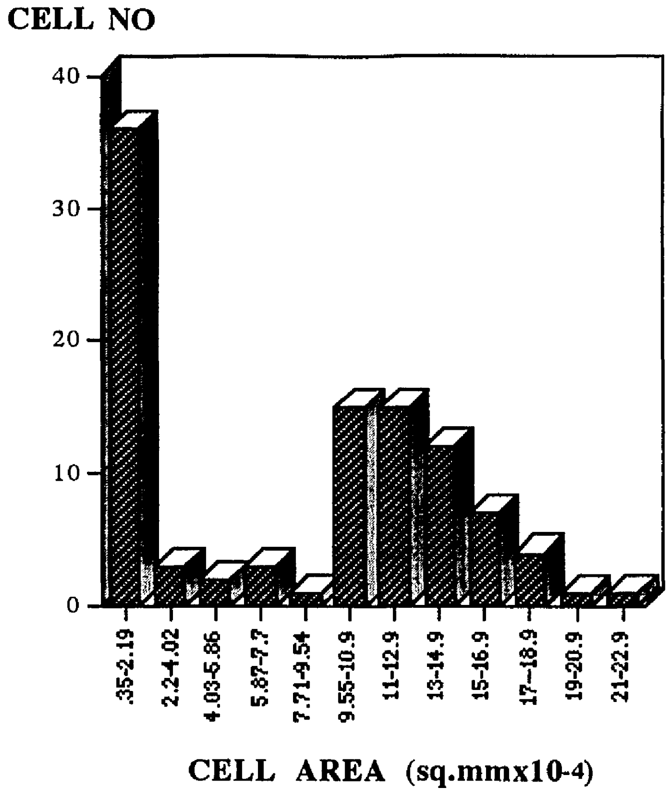


FIG. 44

Cell area distribution of Mrs McQ (RE) showing a bimodal distribution.

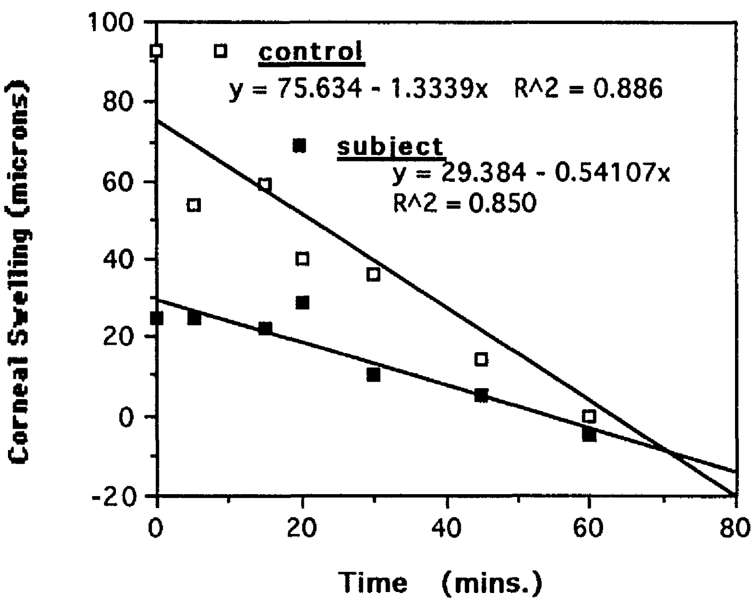


FIG. 45

The recovery in corneal thickness against time following induced oedema from a thick soft HEMA lens. An age matched control is shown for comparison.

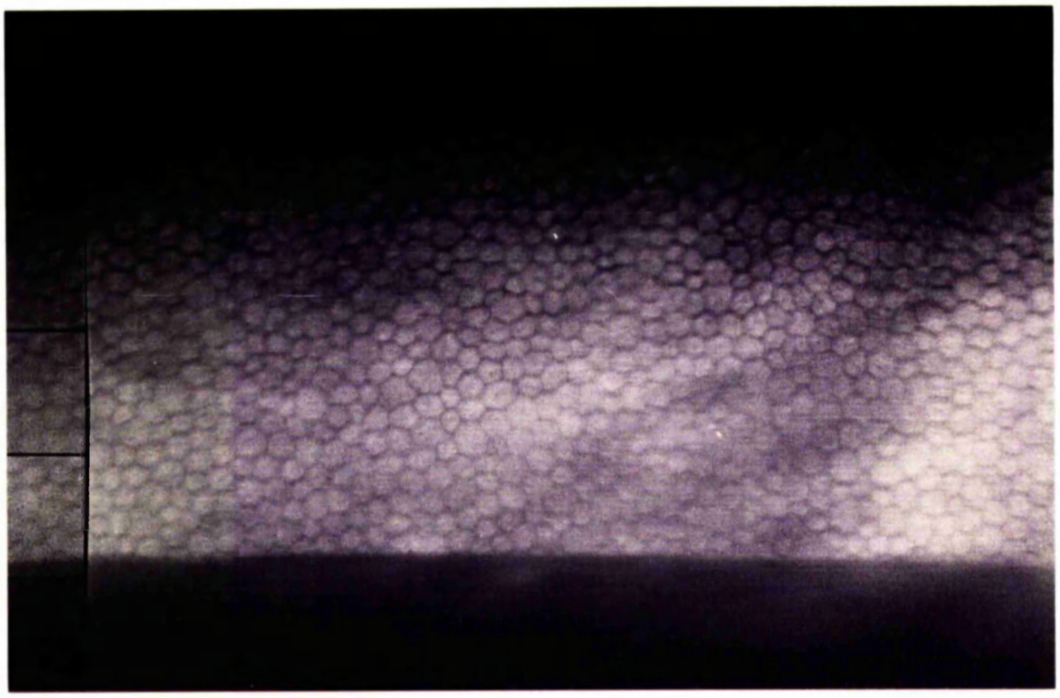


FIG.46a

Specular photomicrograph of a control cornea. Female (age 50), cell density 3401, COV .28, skewness 1.24.

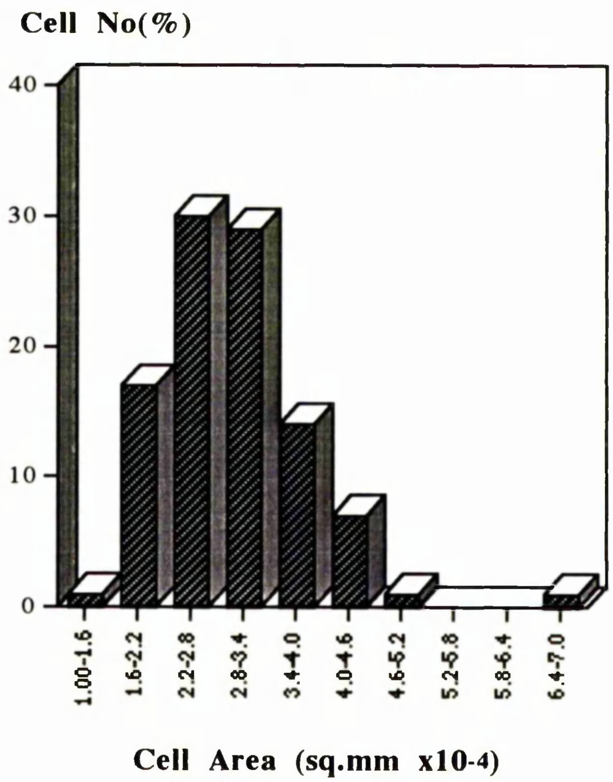


FIG.46b

Cell area distribution of the above photomicrograph.

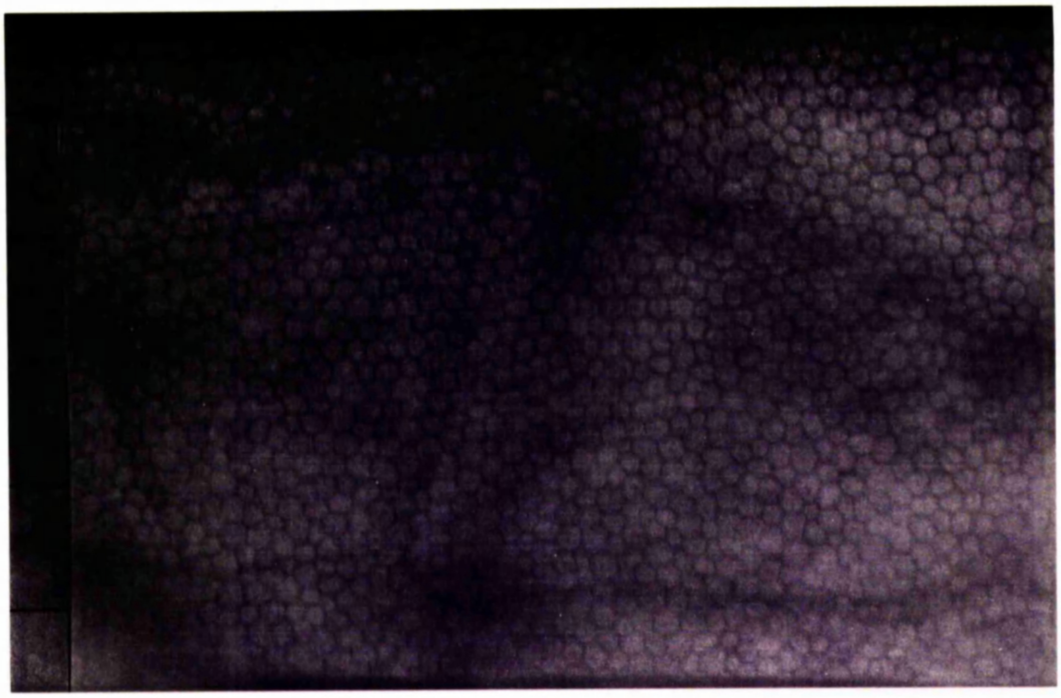


FIG.47a

Specular photomicrograph of a control cornea. Female(24) cell density 3610, COV .24, skewness .03.

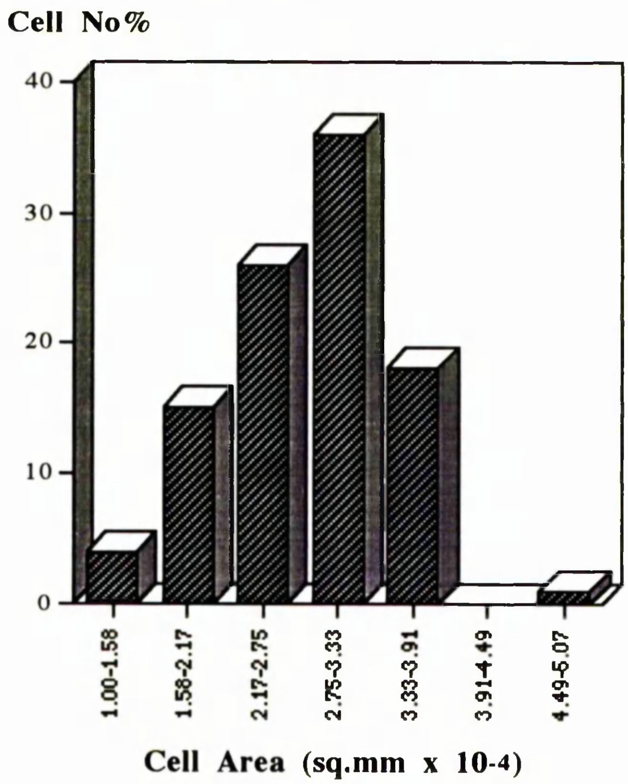


FIG.47b

Cell Area distribution of the above photomicrograph.

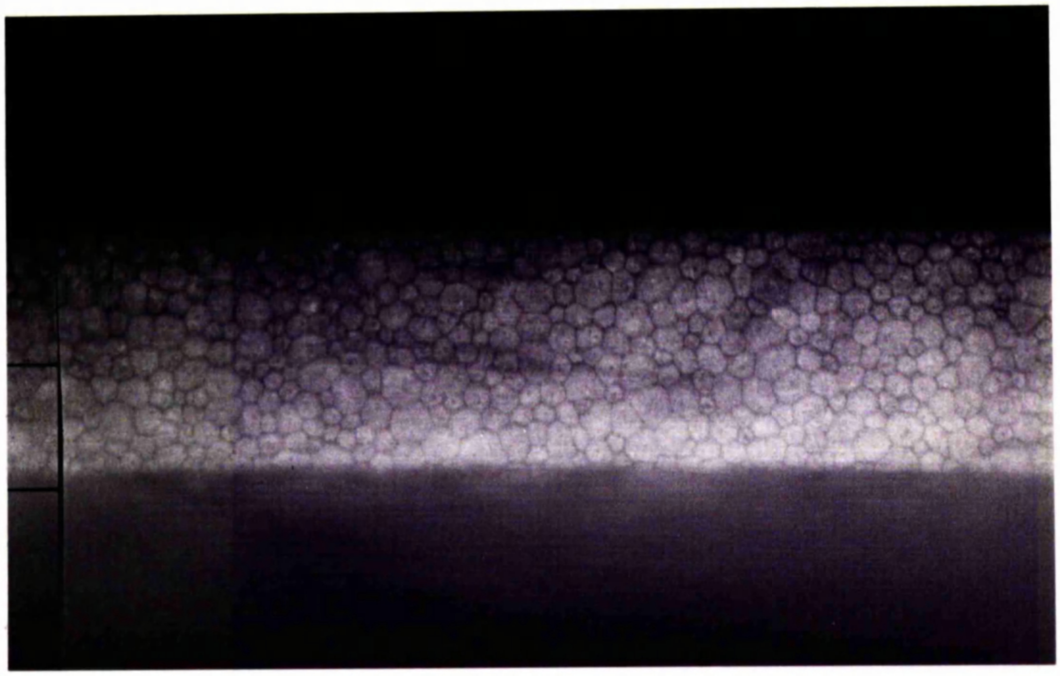


FIG.48a

Specular photomicrograph of a control cornea. Female (41), cell density 2881, COV .53, skewness 1.03.

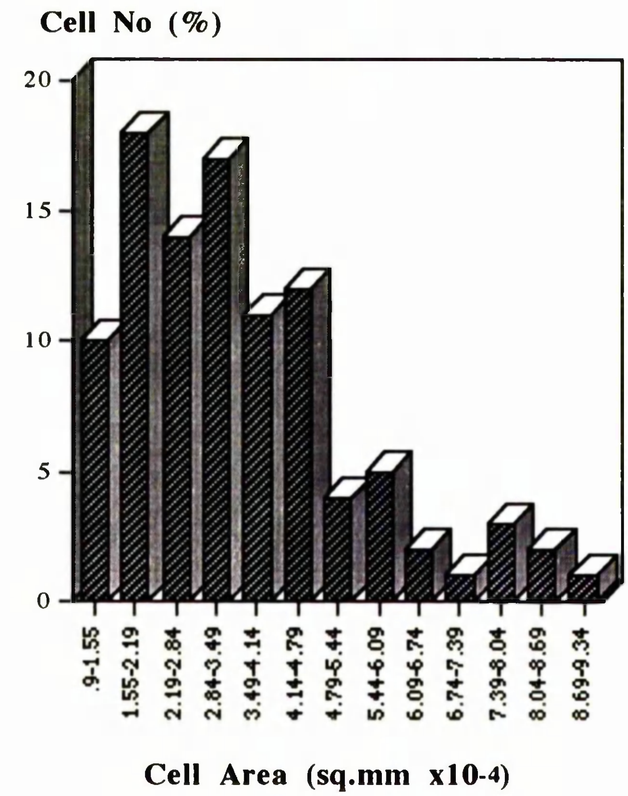


FIG. 48b

Cell area distribution for the above photomicrograph.

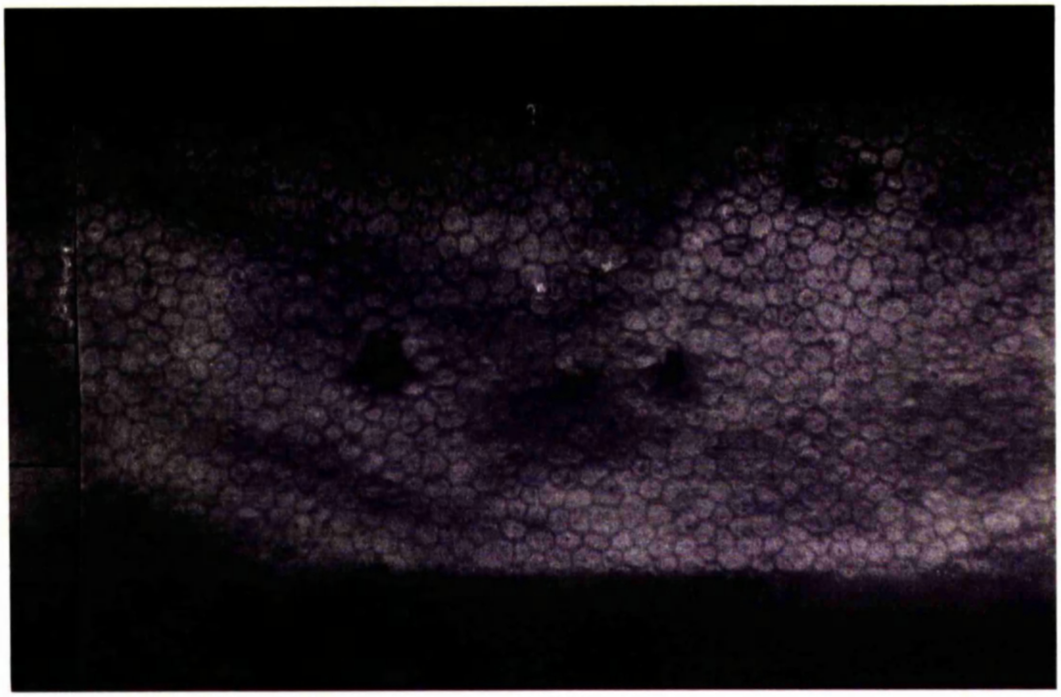


FIG.49a

Specular photomicrograph of a control cornea. Female(54) cell density 2923, COV .34, skewness 1.11.

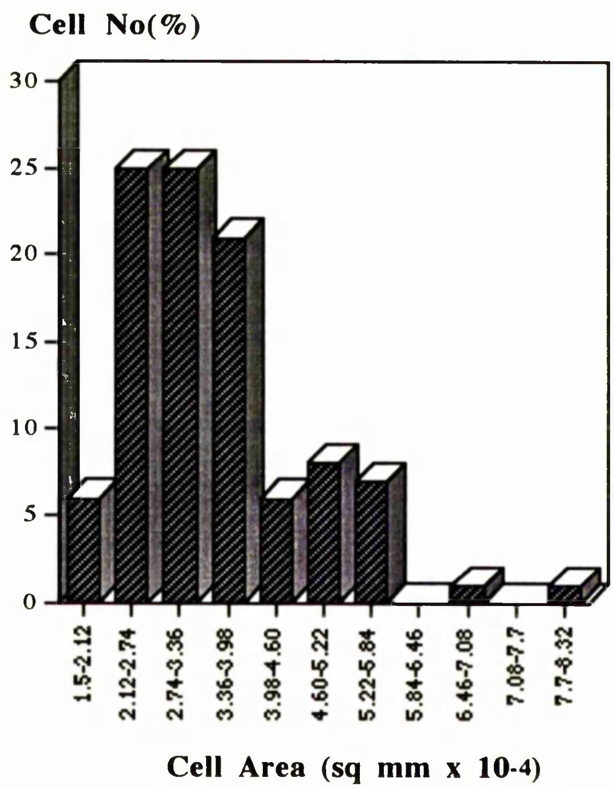


FIG.49b

Cell area distribution for the above photomicrograph.

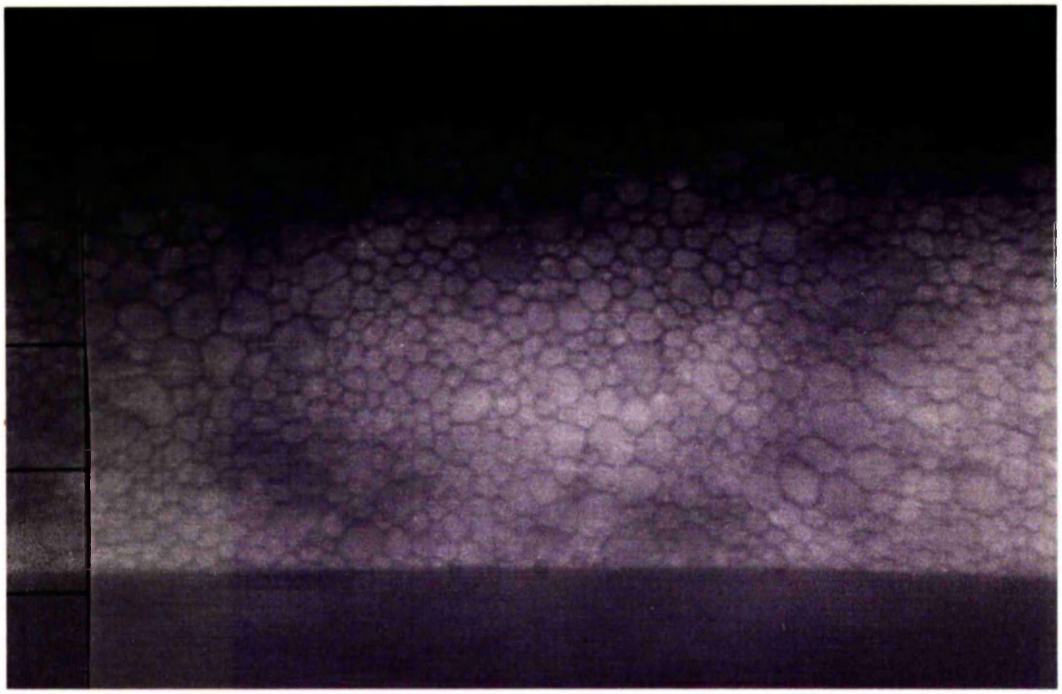


FIG.50a

Specular photomicrograph of a long term PMMA lens wearer. Female(57) 11 years wear, cell density 2739, COV .76, skewness 2.5.

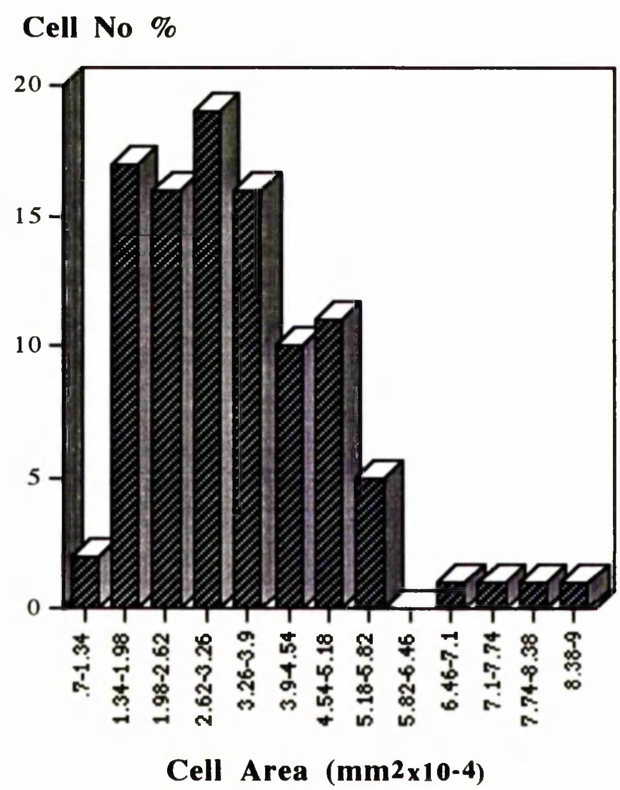


FIG.50b

Cell area distribution for the above photomicrograph.

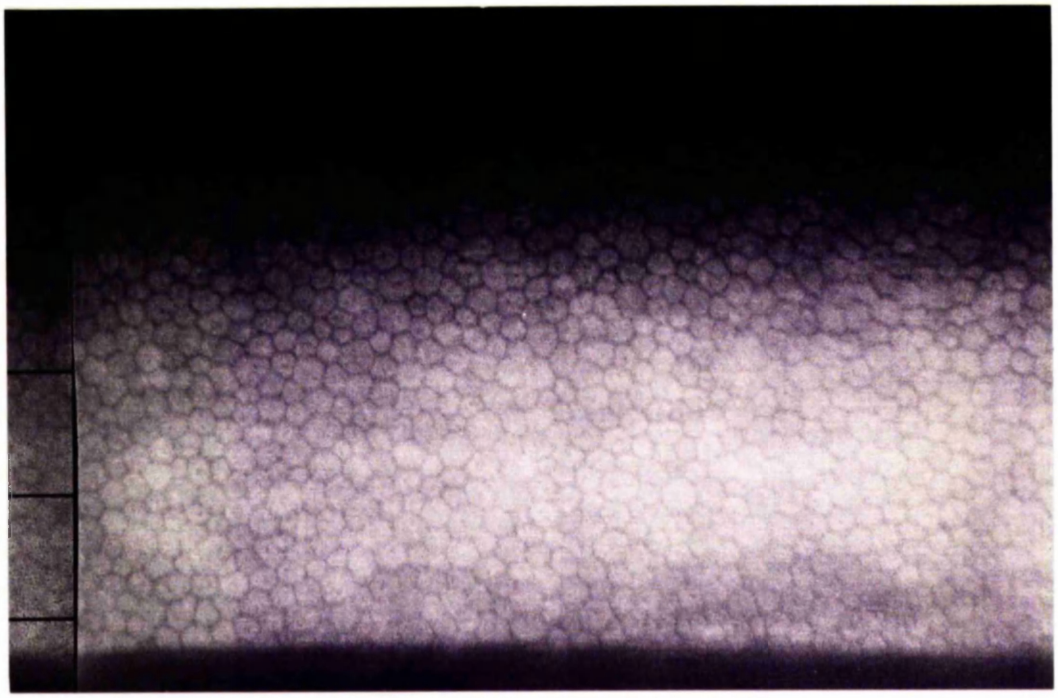


FIG.51a

Specular photomicrograph of a long term PMMA lens wearer. Male(47) 16 years wear, cell density 2898, COV .34, skewness .91.

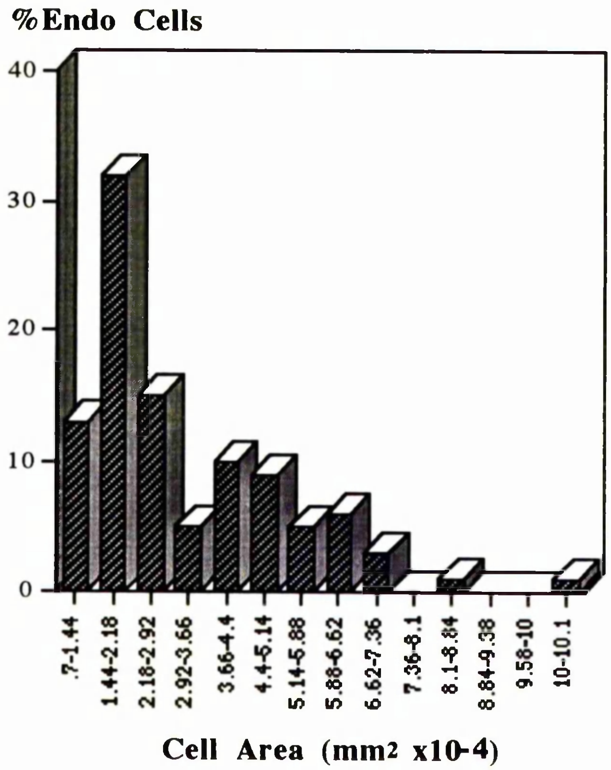


FIG.51b

Cell area distribution for the above photomicrograph.

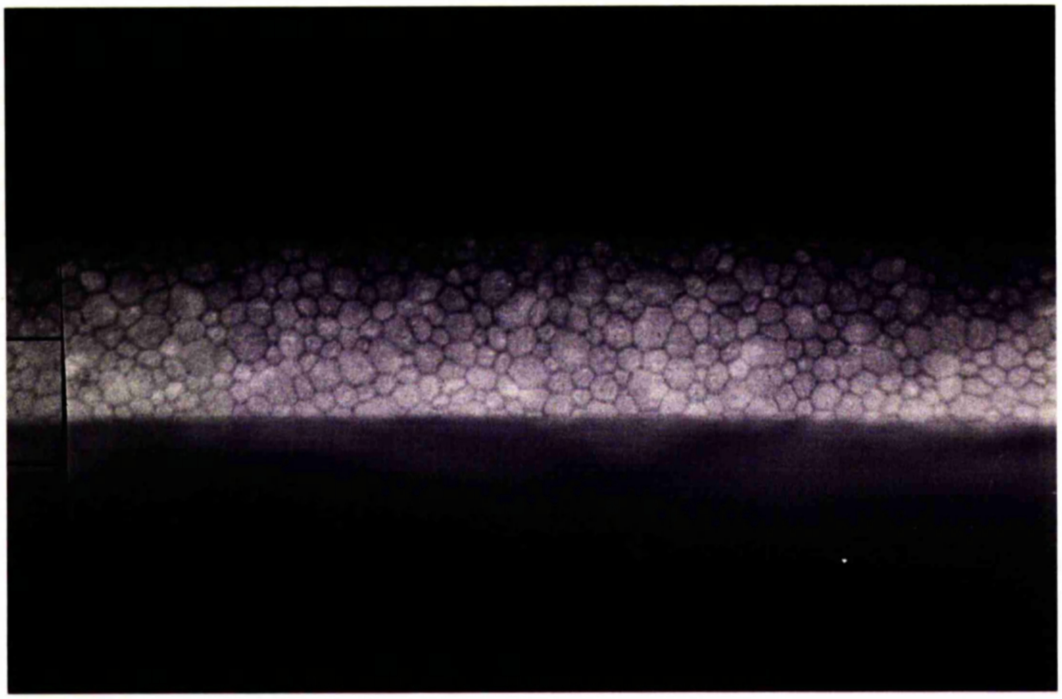


FIG.52a

Specular photomicrograph of a long term PMMA lens wearer. Female(30), 10 years wear, cell density 2673, COV .49, skewness .91.

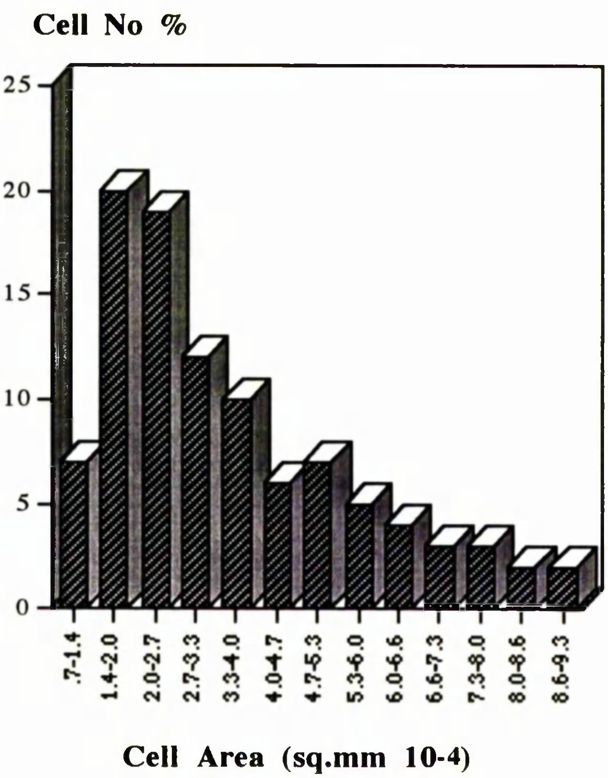


FIG. 52b

Cell area distribution for the above photomicrograph.

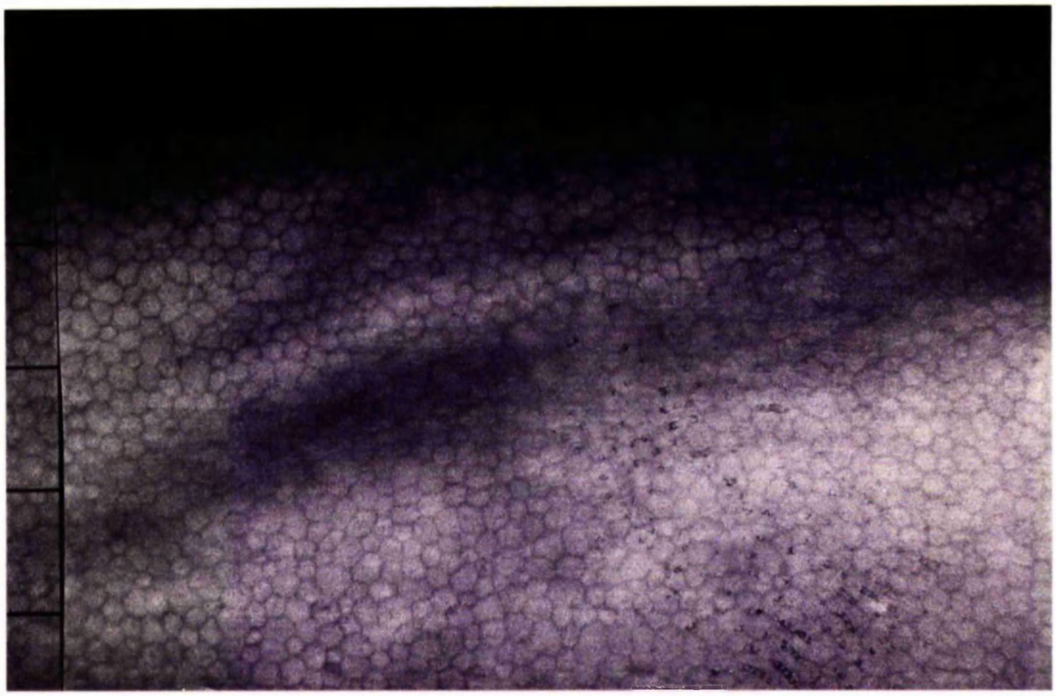


FIG.53a

Specular photomicrograph of a long term PMMA lens wearer. Male(45), 23 years wear, cell density 3436, COV .39, skewness 1.61.

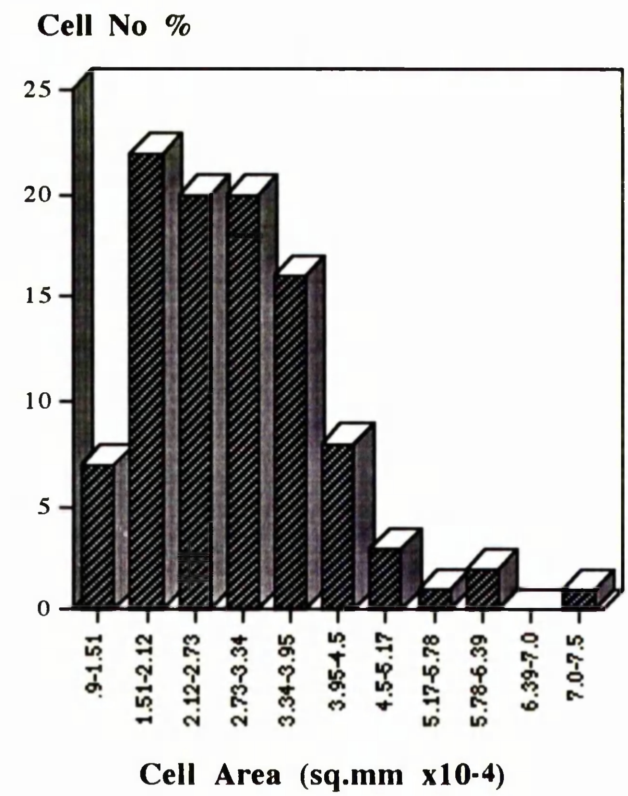


FIG.53b

Cell area distribution for the above photomicrograph.

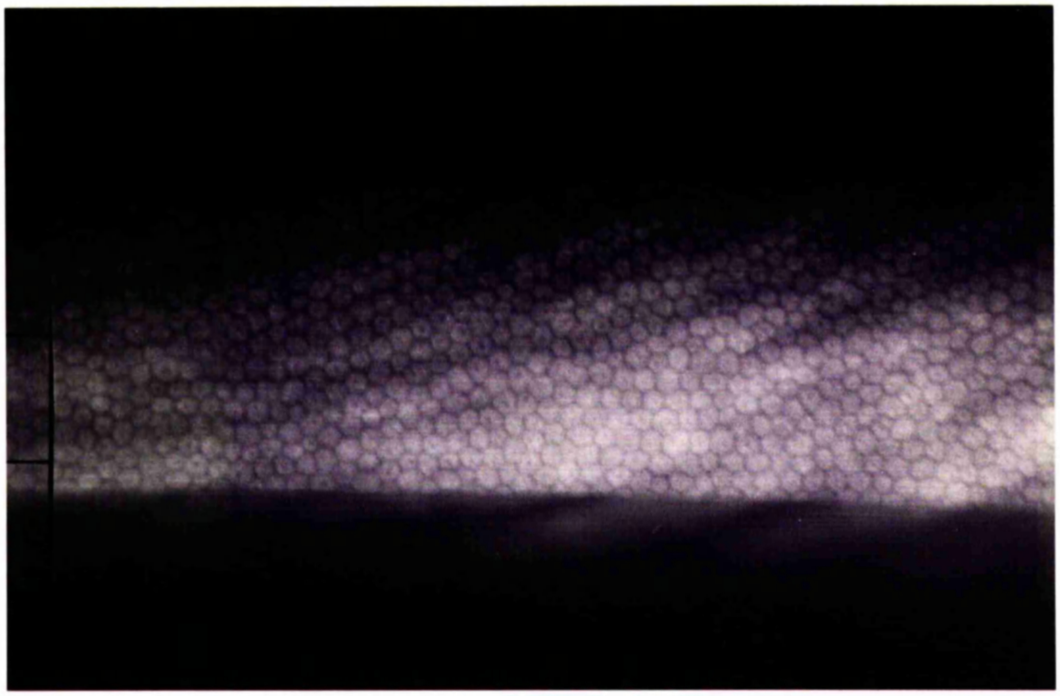


FIG.54a

Specular photomicrograph of a 5 year old boy (4500 cells/sq.mm, COV 0.18).

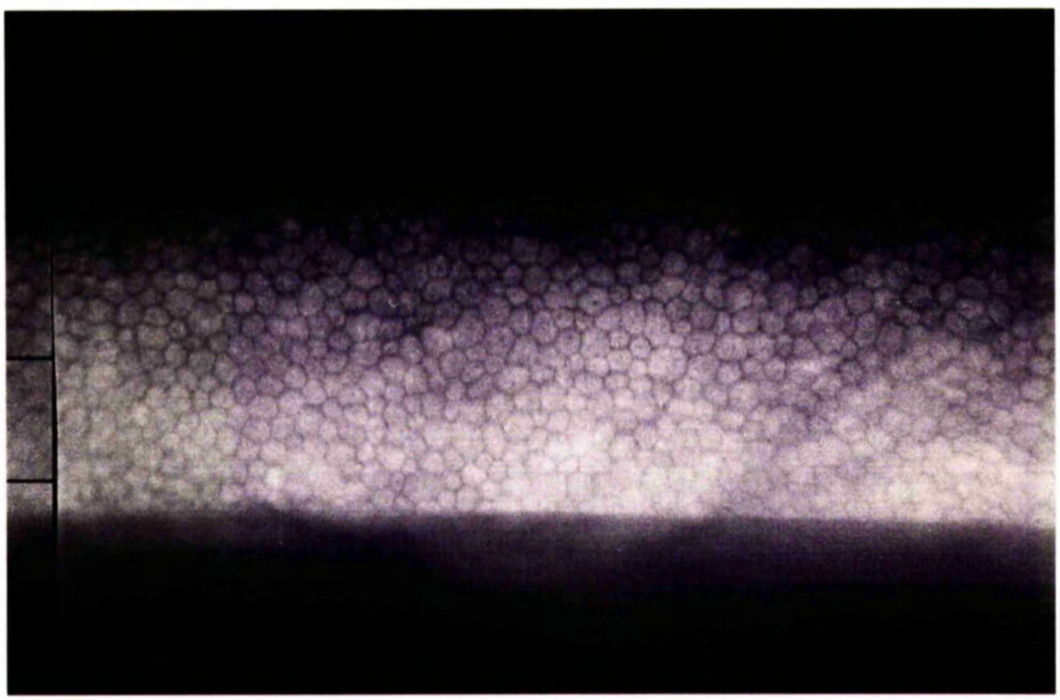


Fig54b

Specular photomicrograph of a 85 year old lady (2800 cells/sq.mm, COV 0.31).

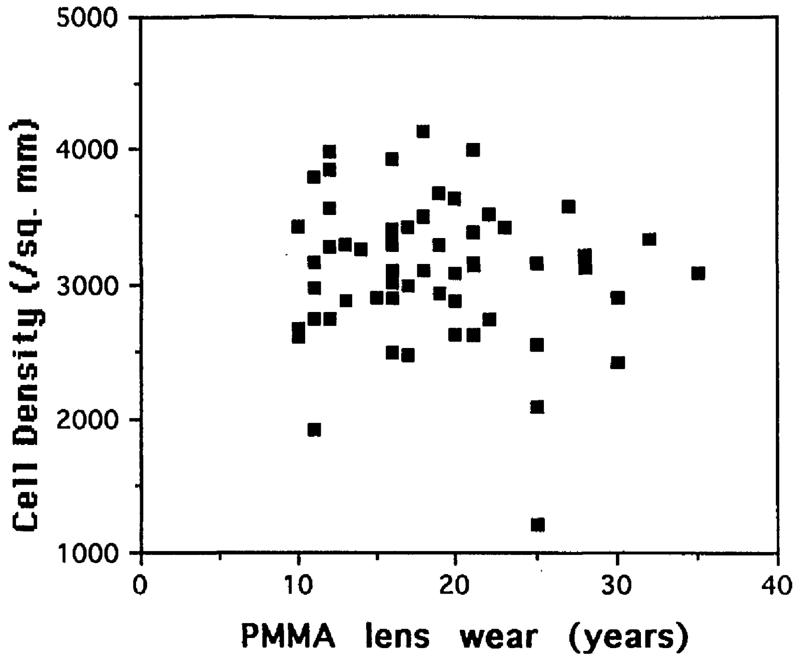


FIG. 55

Scattergram of years of lens wear against cell density for the PMMA group.

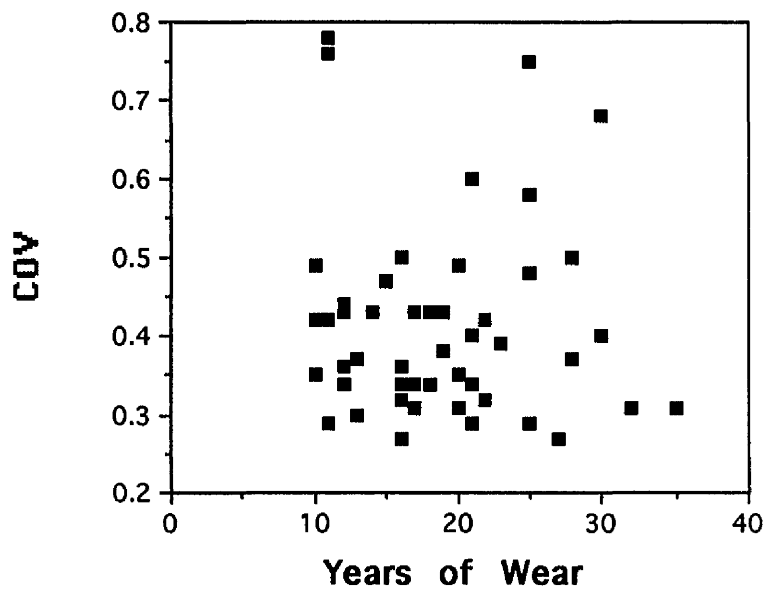


FIG. 56

Scattergram of years of lens wear against COV in cell area for the PMMA group.

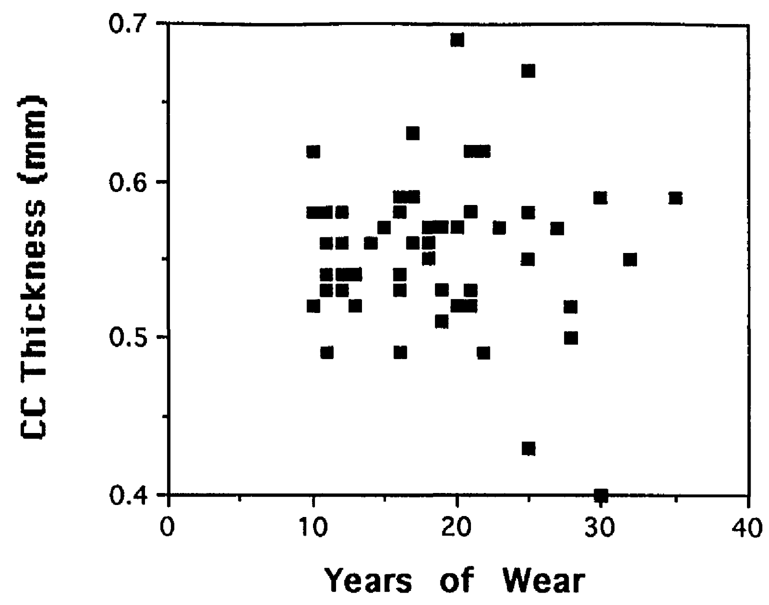


FIG. 57
Scattergram of duration of lens wear against central corneal thickness in the PMMA group.

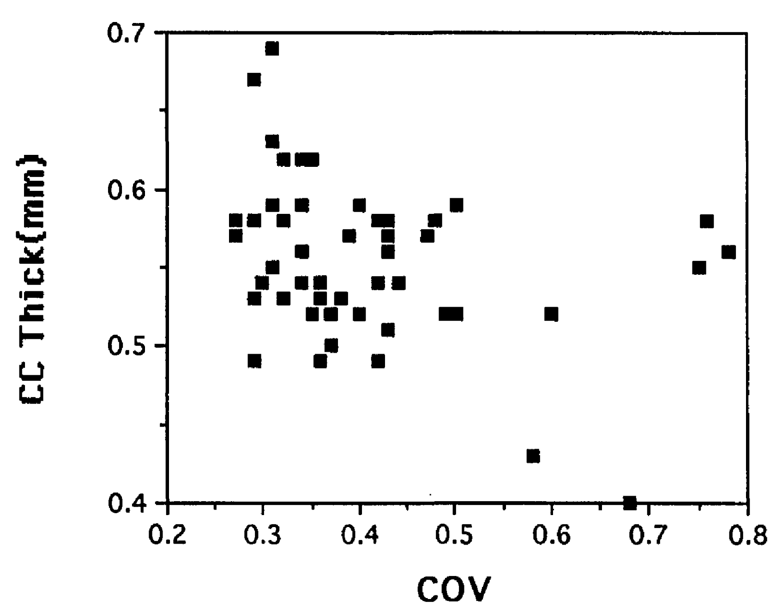


FIG. 58
Scattergram of the COV in cell area against central corneal thickness in the PMMA lens wearing group.

6.4. Discussion

The aim of the experiment was to provide answers to the vital questions on contact lens induced endothelial changes, and their significance relative to the physiological changes that may occur normally with age. With the appropriate patient groups and statistical analysis, it should be possible to quantify the effects of long term contact lens wear, and assess the significance of any difference in the endothelial morphology found between lens wearers and non lens wearers.

This results of the specular microscopy study demonstrate that long term PMMA hard lens wear does affect the corneal endothelial morphology. The major difference found between lens wearers and non lens wearers was the increased polymegethism (COV) in the lens wearing group.

This confirms the results of other investigators in showing that, although the mean endothelial cell size of long term wearers was within the normal range, there was a wide variability of individual cell sizes that is out of proportion to age matched controls. The polymegethism seen in some of the lens wearing group was similar or even greater to that seen in older non contact lens wearing populations. This gives the impression of an accelerated ageing process.

Most reports highlight that although cell density is unaffected, there is a significant increase in the degree of polymegethism and pleomorphism with PMMA contact lens wear of greater than 10 years (MacRae et al. 1985, 1986, 1989). The exact mechanism by which these changes take place is not fully understood.

The validity of some statistics used to quantify differences between contact lens wearing subjects and control non lens wearing subjects has been questioned, particularly in respect of the coefficient of variation in cell area which is commonly used as an index of polymegethism (Doughty 1988). Plotting the distribution of cell areas as shown in Fig.44 gives a clearer understanding of the proportion of both small and large cells and therefore a good graphical interpretation of the cell population.

However, despite the endothelial changes found, the mean corneal thickness of the lens wearing group was not shown to be significantly thicker or thinner than the mean of the control group. Although no difference was found between the cell density of the two groups, this cannot be taken as a predictor of corneal health, or an indication of the risk of corneal decompensation (i.e. an irreversible increase in corneal thickness).

However it can be assumed that in the group of patients tested, no evidence of chronic oedema existed (increase in CCT) or a significant degree of stromal thinning occurred (decrease in CCT). Despite the decline in cell density with age in the normal population, there is not an equivalent increase in corneal thickness.

Although to date, only weak correlations have been established between corneal thickness and any endothelial morphological parameter, the recommendation has been made that contact lens wearers should have pachometry performed routinely. This concept may now be more realistic with the introduction of ultra-sonic pachometers for clinical use. They have been shown to be reliable and valid in comparison with optical pachometry (Wheeler et al. 1992).

Hence, there is a need to examine what relationship might exist between endothelial polymegethism and central corneal thickness. There do not appear to be many studies where both corneal thickness and endothelial morphometry have been performed. In this study, no significant linear relationship was found between polymegethism (COV) and central corneal thickness (CCT). Fig.58 shows a scattergram of COV against CCT for all the long term PMMA wearers.

The effects of intra-ocular surgery on the corneal endothelium are usually a reduction in cell density, an increase in cell size, and an increase in the coefficient of variation of cell area as the repair mechanism of the endothelium responds to localised damage. This has been very clearly explained by Leisgang (1991) when describing the regional variation in the corneal endothelium after cataract surgery when an incision has been made at the superior limbus.

The complete procedure of sampling cells from specular photomicrographs has been questioned by Hirst et al. (1984, 1989). Using large composite photomicrographs these authors calculated measurement and sampling errors of up to 10% when considering the mean cell size within the central 4mms of the cornea.

However, they were not using current image analysis systems and the specular microscope had a narrow field. With the mask and graticule methods they described, errors were shown to be greater and sampling accuracy reduced. Matsuda et al. (1989), in a well controlled study on specular microscopy of the endothelium in post keratoplasty cases, analysed 100 cells per patient. They calculated a cumulative inherent error of less than 5%.

It would appear that the main reason for an increase in the COV in the contact lens wearers was an increase in the number of larger cells giving a positively skewed distribution. However it was observed that in a number of instances, (approx.10% of the experimental group) examples of clumps of small cells were evident. This has been observed and commented on by other workers (Hodson and Sherrard 1988), but the phenomenon has never been fully explained.

However it is an observation that has not been associated with other situations affecting the endothelium, such as ocular surgery. These small cells tend not to be so predominant and of a size which can give a negatively skewed distribution, but it can lead to a cell distribution which in the more extreme cases is bimodal in nature. Doughty and Dilts (1993) have shown that in the rabbit cornea, loss of homogeneity in the endothelial mosaic can result from increased size variation in cells that do not have 6 sides. This suggests that polymegethism without cell loss, is the result of cell reorganisation (mediated by cell fusion) into smaller and larger cells, rather than cells sliding into areas vacated by damaged cells.

There is a also need to examine what relationship might exist between endothelial polymegethism and central corneal thickness. There do not appear to be many studies where both corneal thickness and endothelial morphometry have been performed. In this study, no significant linear relationship was found between polymegethism (COV) and central corneal thickness (CCT).

Further attempts to look at 'on line' analysis of video images should be done so that even larger numbers of cells can be counted and measured with automated systems (Nishi and Hanasaki 1989). In this way, valid results of the endothelial morphology could be obtained even more quickly, which would provide clinical information about a patient that would enhance the usefulness of specular microscopy as a clinical tool. The impression gained from this study, was that the human eye can interpret the image variability on a photomicrograph better than a computer. Automated image analysis systems need to be assessed with this in mind.

Numerous corneal insults such as infection, trauma, or intra ocular surgery can damage and destroy endothelial cells and therefore hasten the 'ageing' process (Leisgang 1990). In normal healthy eyes, there is a tendency for the COV to increase with age. This has been explained by the decrease in cell density with age resulting in the remaining cells becoming more irregular as they spread to cover the total area. This means that carefully controlled studies are necessary to differentiate real differences in experimental groups from this normal physiological change.

Two very recent papers extensively address the problems of cell analysis and provide some interesting discussion on polymegethism and pleomorphism (Doughty and Fonn 1993, Doughty et al. 1993). They present an analysis of the average area of different cell types (number of sides) and showed that the greater the variation in the number of cell sides, the steeper the slope of a plot relating cell areas to the number of cell sides. This indicates that the remodelling of the endothelium when cell loss is not evident, may follow some ordered process.

On morphometry, Doughty et al. (1993) show that significant uncertainty exists concerning how many cells should be measured. Their results show that for normal (homomegethous) and irregular (polymegethous) endothelia, even cell counts as low as 50 cells can usually provide average cell area values that are within 1 to 2% of the values estimated from larger groups of cells. This agrees with the arguments put forward in Chapter 6.

A similar reliability was observed for estimates of COV for normal endothelia. However for polymegethous endothelia, even with 100 cells analysed, the estimates of COV only approach ±4% reliability. Doughty et al. (1993) concluded that this uncertainty in COV estimate should be considered in both comparative studies and regression analysis of COV changes over time, or with other variables.

Some of those cell parameters which were not considered to be appropriate in this work (Table 6), may have application to future research projects. These would include the ratio of major to minor axes of cells to indicate regular or irregular deformation, and the long axis orientation which may have relevance to the response of endothelial cells to stress. Another example of a feature that could be studied in more detail, is the movement of cells within the endothelium as part of the repair mechanism following ocular surgery. The orientation of each cell is demarcated by its long axis and therefore, directional changes in cell alignment can be quantified.

Although no changes were made to the VIDS image analysis programme during the project, it may be worthwhile modifying the existing image analysis software to obtain the number or percentage of six sided cells in the total sample. Since each border junction or apex is defined in the tracing technique this information could be used to count the number of cell sides. A histogram of the number of cell borders could then be drawn to indicate the variability in shape within a particular endothelial sample, and therefore the pleomorphism at a glance. Such a measure would be preferred to the term 'hexagonality' (Doughty 1992) as outlined earlier in this chapter.

The lack of any relationship between COV and CCT in the normal eye and in the contact lens wearers (Fig. 58) contrasts with that in the surgical eye (Liesegang 1991). This can be hypothesised to be due to at least two factors.

Firstly, the surgically traumatised endothelium simply responds to the stress in a different fashion to that seen as a function of age or contact lens wear. Secondly, the index of assessment (the COV), may not be reporting the same sort of changes in the two situations. It should be noted that cell density values as in this experiment, are simply calculated arithmetic mean values and do not reflect the range of cell area values. Since the variance in cell areas in some cases of contact lens wear has been reported to increase, the validity of the mean cell density values as the sole measures of cell areas, that are then used in statistical comparisons, needs to be questioned.

A criticism of constructing the type of regression estimates used in this study, has recently been described by Doughty et al. (1993) pointing out that the measures of the variance in endothelial cell area (COV) have been treated as point estimates without consideration of the reliability of these measures.

This may be a valid argument in very polymegethous corneas, particularly where cell clusters exist, but as pointed out in the material and methods section of this experiment, a careful measurement technique was applied to minimise the error. Studies where a descriptive statistic such as the COV is used as an index of polymegethism, should always quote the confidence interval for the mean values (Bulpitt 1987).

The distributions of cell areas, the possible changes in these distributions, and their consequences on the COV are more important questions. In the simplest situation from a mathematical perspective, the COV value will be expected to increase in a population sample if there is an increase in the range of values about an arithmetic mean.

Three different variations for a change in the endothelial cell population have appeared in the literature. It has been shown in some contact lens wearers that there can be no significant change in endothelial cell density despite an increase in the range of cell area values (Osborn and Schoessler 1988). Alternatively there have been reports in which the calculated mean endothelial cell area increases along with the range of cell areas (Doughty 1990). This implies a corresponding decrease in cell density.

In these two instances the increased COV results from the appearance of additional larger endothelial cells. That is, the distribution has become skewed to the right or towards larger values. This is typical of the distributions found in the PMMA wearers in this study. Very few examples have been published where increases in mean cell area are not accompanied by significant increases in COV. It has been reported for the endothelium of some older patients, patients with congenital glaucoma, in unilateral bupthalmus, in unilateral perforating injury, and following cataract surgery (Liesegang 1991).

More commonly however, age dependent increases in cell area are not proportionate, but are accompanied by the appearance of a greater number of large cells and the distribution becomes skewed. This is why it is important to report the distribution of area values along with a measure of skewness of the distribution as highlighted in the case (Mrs McQ) illustrated earlier (Fig.44).

In this study, as in other reports (Garsd et al. 1983, Hodson and Sherrard 1989), distribution profiles as well as specular photomicrographs, reveal the presence of numerous rather small cells with areas that are substantially smaller than the average control cornea values. Since the increase in the number of very small cells produces an increase in the COV, the question can be posed as to whether all reports of polymegethism are equivalent.

Based upon the cell area distributions evident in this study, a case can be made that the endothelium of an eye following ocular surgery responds differently to the contact lens wearing eye. Unlike post surgical eyes, the contact lens wearers do not seem to have cell loss and in some cases at least, respond with the appearance of very small cells. Following ocular surgery the endothelium has to cope with the underlying stress of acute postoperative inflammation.

Therefore, there are two consequences of the consideration of what the coefficient of variation in cell area (COV) reports. The first is that the contact lens wearing case, although different from the control, appears also to be different from the surgical case. This may appear to be an obvious conclusion, but part of the concern over polymegethism has arisen as a result of comparisons made between reports of polymegethism post-operatively and corneal thickness, and the appearance of polymegethism especially following long term PMMA lens wear or extended wear.

Secondly, it has been reported that corneas which showed lesser degrees of polymegethism showed less post operative corneal oedema (measured as increased central corneal thickness) than those with marked polymegethism. The apparent resolution of the postoperative oedema was considered more complete in those corneas with more uniform endothelia (Rao et al. 1984).

As indicated by Doughty (1990) in illustrating the ambiguity of the COV, four points need to be made resulting from previously published studies before surgical data is used to support a case for substantial concern over the contact lens associated polymegethism.

Firstly, the increased corneal oedema reported in polymegethous corneal endothelia was based on a subjective assessment rather than an objective measurement (Rao et al. 1980). The division of polymegethous from homomegethous was based on a subjective judgement rather than morphometry of the specular micrographs.

Secondly, in the same study, the rates of recovery from oedema were actually faster at days 1 to 3. Thirdly, the cornea has been reported to thin post-operatively when no change in COV was observed (Bourne et al. 1985). Also, other authors have found no morphological difference between corneas having an uncomplicated postoperative course and those developing bullous keratopathy (Murata et al. 1979).

Therefore, although there is some concern that the polymegethous state is a predictor of increased CCT, it was not confirmed in this experiment. Whether significant polymegethism does mean that the cornea will not recover rapidly from stress induced oedema, needs further investigation. Fourthly, the best correlation to date between COV and corneal thickness recovery after a stress test in non surgical eyes has a variance (r^2) value of only 0.38 (Ikeda et al. 1982).

Evidence that polymegethism may be accompanied by functional impairment of the cornea, comes primarily from the work of Rao et al. (1984). More recently Lass et al. (1988) and Dutt et al. (1989), used fluorophotometry to demonstrate that extended wear and long term PMMA contact lens wearers with polymegethism, had a decrease in endothelial barrier function and an increase in endothelial pump rate.

Other studies have shown that in individuals who had worn (EW) lenses for several years, the central corneal thickness returned to normal levels about 2 days after lens wear was discontinued (Holden et al. 1985), a feature which would be unlikely if a functional deficit was present in the endothelium.

The lack of a dose related effect on the endothelial parameters measured, highlights the importance of clinically assessing each individual long term wearer, without preconceived ideas of what is likely to be found. In looking at the correlations, spurious results can of course arise if the variables in question are not independent. e.g. years of wear is likely to be related to the age of subjects and it has already been pointed out that cell density normally decreases with increasing age. However, since none of the relationships which were examined in this study were significant anyway, a type 1 statistical error, that is rejecting the null hypothesis when it is true, is not likely to have been made (Bourke et al. 1985).

Therefore the questions that arise are;

(1) Is there a structural and physiological response in the corneal endothelium from chronic hypoxia?
(2) If there is an effect, does it depend on the degree, duration and timing of the low oxygen dose?

Corneal endothelial structure can be studied by an analysis of endothelial photographs but 'in-vivo' assessment of corneal function has generally not been possible. However a measurement of corneal hydration control (CHC) such as that proposed by Polse et al. (1989) may provide some important answers, and studies need to be done on long term PMMA contact lens wearers on whom the lens wearing history is known.

Again, Polse and colleagues (1990) have attempted to get some preliminary data on corneal hydration control in long term PMMA lens wearers, but their results were inconclusive on a very small sample, and the overall question remains unanswered. The work conducted by Polse and colleagues (1990), although not definitive, does however suggest that hypoxic exposure alters endothelial morphology and reduces corneal function. In this respect, their results are sufficient to indicate the need for further investigation of the structural and functional changes that occur as a result of wearing contact lenses that cause corneal hypoxia.

Investigating the relationship between pH and hypoxia, McNamara et al. (1993) found that human eyes exposed to a mixture of CO_2 and nitrogen, produced less corneal swelling than nitrogen alone. This means that reduced pH levels in the stroma due to the CO_2, affects corneal fluid flux only under non steady state conditions, and this interesting paradox suggests that stromal pH may modulate hydraulic conductivity across the corneal endothelium. This again needs further experimental investigation.

As most contact lens practitioners are aware, problems with respect to hypoxia related to PMMA lenses can be alleviated with the use of gas permeable materials. The preferred method of refitting long term PMMA wearers, is to immediately cease wear of the PMMA material, and refit with gas permeable lenses without allowing a delay between the cessation of PMMA and the resumption of wear with a gas permeable material. Some adjustment of the lenses may be necessary as the cornea continues to rehabilitate under the gas permeable material, to optimise the fit of the lens while at the same time, maximising the vision.

6.5. Summary

Changes in the corneal endothelium associated with long term hard poly-methyl-methacrylate (PMMA) contact lens wear have been documented of which polymegethism is the most significant. Few previous studies however, have considered the cumulative changes seen after prolonged long term wear.

The endothelial specular appearances of 57 PMMA contact lens wearers (age range 29-64) who have worn PMMA lenses regularly on a daily basis for 10-35 years were compared to 45 age matched controls (age range 5-84). The endothelial cell density (ECD), coefficient of variation in cell size (COV), and coefficient of skewness as a measure of asymmetry of the cell population, were the main parameters assessed.

The central corneal endothelium of all patients was photographed using a Keeler-Konan contact endothelial specular microscope and the photomicrographs were analysed using an Optomax Image Analysis system (AMS, Cambridge, England). Central corneal thickness was also determined using the specular microscope pachometer in both the control and experimental groups.

The degree of polymegethism (mean COV) was significantly greater in the contact lens wearing group (t test, $p<0.05$) than in the control group. No significant relationship was found between COV in cell area and central corneal thickness in either of the groups. Central corneal thickness was not related to the number of years lenses had been worn and the mean corneal thicknesses for the control and the experimental groups were not significantly different (t test, $p>0.05$).

The coefficient of skewness in the experimental group showed that in some instances the COV of cell area was increased by the presence of groups of small cells rather than the more usually reported increase in larger cells. An important finding was that the cell densities of the two population groups were not significantly different (t test, $p>0.05$).

Though no cornea of the contact lens wearing group showed evidence of decompensation, the widespread endothelial changes suggest that this might be a risk following intra-ocular surgery.

The management of long term contact lens wearers, including corneal physiological and refractive requirements, needs careful control.

SECTION 4

GENERAL SUMMARY AND SUGGESTIONS FOR FURTHER RESEARCH

Chapter 7. Overall Conclusions from this Study

7.1 Properties of Lens Materials

The overall work done in this project has revealed a number of interesting aspects about lenses, materials, and the corneal response to long term wear of PMMA contact lenses. From the vast bulk of literature on corneal physiology, it is now clear that oxygen transmissibility is of major importance with any contact lens. With new developments in computerised interfacing of measurement apparatus, the principles developed in this study could be enhanced to produce an acceptable laboratory method of measuring oxygen permeability of materials without the need for more complicated and expensive methods such as coulometry.

To further strengthen this suggestion, a range of calibration materials have been produced for such polarographic measurements (Fatt, personal communication 1993) and the International Standards Committee (ISO) have indicated that they will adopt polarographic oxygen measurements of such materials as standards.

Six RGP materials have been put into a repository for testing. The range of materials consists of Menicon EX, Menicon SFP, Boston Equalens, Quantum II, Fluoroperm 30 (all fluoro-silicone acrylates), and Polycon 11 (silicone acrylate). This approach should clarify many of the confusing issues relating to oxygen permeability measurements which currently exist in the contact lens industry.

Following the experiments described in Chapter 3.4, work is underway to computerise the interfacing of the recording system of the polarographic method described earlier. It is clear however that although it is possible to show that one material may be more oxygen permeable than another by a factor of for example, 25 to 30 times, the 'in-eye' performance of a lens with respect to the oxygen transmissibility cannot be equated. Therefore, clinically it is still necessary to develop a useful measure of 'effective oxygen transmissibility' if this can be met with universal approval.

Although it was possible to measure the elasticity or flexibility of gas permeable materials in laboratory experiments, contact lens practitioners would probably prefer to assess the effects of lens flexure by looking carefully at the refractive result when fitting astigmatic corneas. It is likely that research will pursue the goal of finding the gas permeable lens material that is highly oxygen permeable, flexible enough to be comfortable in the majority of eyes, can be machined easily, and can also maintain its shape on an astigmatic eye to correct any corneal astigmatism. In effect, the ideal combination of the desirable mechanical and physiological properties needs to be determined. A further series of laboratory experiments should consider;

1. Computerisation of oxygen permeability measurements using a polarographic cell.
2. Test methods for mechanical properties for all contact lens materials; e.g. flexibility, hardness, scratch resistance, fractural resistance.
3. A model for induced and residual astigmatism for all contact lens types.

7.2 Corneal Response to Contact Lens Wear

More studies need to look at the long term ocular response to wearing contact lenses on a daily and extended wear basis and although this present study has looked at the effect of long term hard lens wear on the corneal endothelium, there are aspects of soft lens wear which need to be assessed. This applies not only to the endothelial morphology but to the vascular response of the eye on which only anecdotal reports exist in the literature of limbal blood vessel changes as a result of soft contact lens wear over a number of years.

An assessment of the limbal vascular response to contact lens wear was initially considered for this study, and preliminary experiments were conducted. Difficulties however were encountered with the experimental techniques of red free video-angiography and fluorescein video-angiography, and this meant that the studies to evaluate the vascular response to contact lens wear could not be completed in the time course of the project. This important work therefore remains to be done as a future experiment.

Probably the chief complaint or symptom of the lens wearers in the long term PMMA study was that of spectacle blur, caused mainly by corneal distortion. With the introduction of the sophisticated corneal modelling systems, allowing detailed topographical corneal analysis it will now be possible to investigate the degree of corneal distortion produced by contact lens wear, and the time course of its reversibility.

The optical methods used to assess the cornea such as keratometry, rely on the corneal surface to reflect light in a regular manner, but when the corneal surface warpage changes are more than the tear film can correct, then apparent distortion of a reflected image results. It is an area that requires more research, particularly as both rigid and soft lens materials are developed for extended wear.

The detailed use of the topographic corneal modelling systems will allow much greater information to be gained about contact lens induced topographic changes and their reversibility. To clarify this point, and to show the potential of this area of study, Figs.57 and 58 show a normal eye with regular astigmatism and a long term PMMA lens wearer with the lens just having been removed.

Compared to the normal control, the astigmatism in the contact lens wearer is irregular and asymmetrical, and explains the reduced visual acuity obtained with spectacles. 'With the rule' regular astigmatism demonstrates the typical reversed 'bow tie' effect whereas irregular astigmatism such as that found in long term PMMA lens wearers shows an irregular pattern. These patterns have been described previously (Bogan et al. 1988) and suggest interestingly, that 7% of 'normal' eyes demonstrate an irregular corneal pattern.

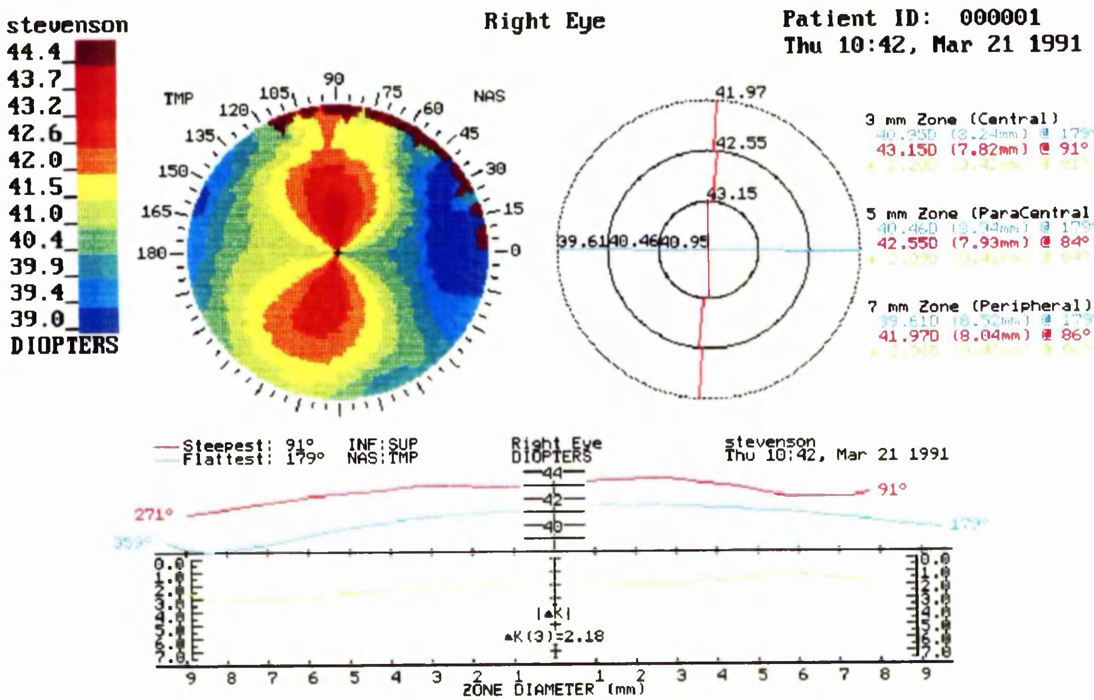


FIG.59

The corneal topography shows 'with the rule' corneal astigmatism commonly found in the normal eye.

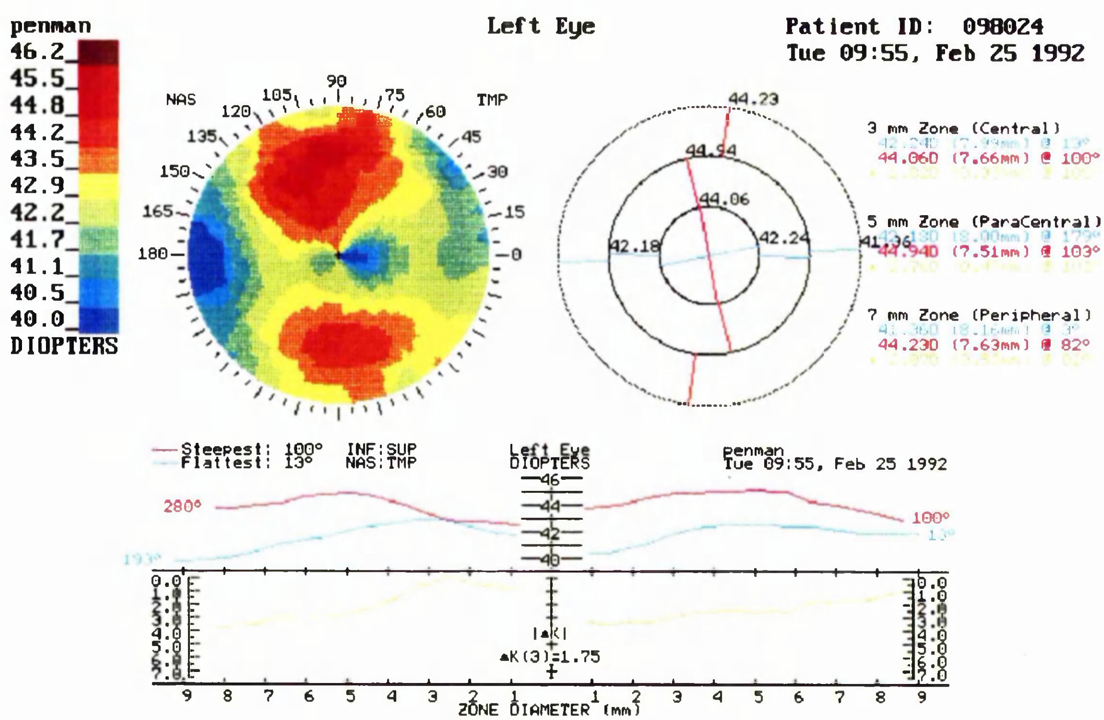


FIG.60

The corneal topography shows irregular corneal astigmatism or 'corneal warpage' from long term PMMA lens wear.

Also whether gas permeable lenses or extended wear of soft lenses has any effect of corneal topography needs to be investigated. The spectacle blur associated with hard lens wear has the effect of increasing lens wearing time on a daily basis since vision with a spectacle correction is often unacceptable to the wearer.

The refractive management of these patients offers a significant challenge to the contact lens practitioner and with the advent of laser refractive surgery, a detailed knowledge of contact lens wearers corneal topography will be necessary when such patients seek surgical correction of their refractive error. Equally important will be the measurement of corneal topography after excimer laser surgery, particularly if contact lenses need to be fitted to correct a residual refractive error.

The polymegethism found in the long term wearers is of fundamental interest. Further work will need to be done to look at the role of specular microscopy as a valid technique of photographing the polymegethous endothelium, and how the results compare to laboratory electron microscopy studies. This would only be possible if specular microscopy was carried out on eyes which are due to be grafted or enucleated, with specimens subsequently becoming available for pathology laboratory analysis and investigation.

Once the technique of laser confocal microscopy becomes readily available for clinical use, further insight into the morphological changes in the corneal endothelium should be possible. The early reports of the technique are impressive (Ichiyima et al. 1992) with the instruments having the ability to 'section' thin slices through the cornea, even to the extent of imaging the anterior side of the corneal endothelium.

Epithelial desquamation changes have been detected in the rabbit eye which were related to the oxygen transmissibility of contact lenses worn, and the conclusion was that confocal microscopy is a good way to evaluate the contact lens safety and efficacy 'in vivo' at the cellular level non-invasively (Ichiyima et al. 1992). Cavanagh et al. (1993) have described excellent results with a tandem scanning confocal microscope in a range of clinical conditions including acanthamoeba keratitis, contact lens abrasion, post excimer ablation and corneal dystrophy's. The ability to evaluate these conditions at the cellular level, using a non invasive technique, provides a much enhanced means of clinical evaluation.

It is clinically easier to detect epithelial and stromal oedema than endothelial changes which require either excellent biomicroscopic technique or specular microscopy.

Although contact specular microscopy is unlikely to become a routine procedure in contact lens practice, the role that the endothelium plays in the maintenance of corneal integrity and in the success or failure of contact lenses needs to be carefully considered. Now that long term wearers of soft lenses do exist (>10 years), it would be interesting to evaluate the associated endothelial changes.

Contact lens practitioners are now also seeing former PMMA wearers now having been wearing RGP lenses for a period of years. Conducting specular microscopy on a group of these individuals similar to the experiments done in this study, could determine whether some reversibility of polymegethism had occurred. If the lens parameters could be determined it may be possible to correlate oxygen transmissibility with specific endothelial features.

Also, if contact lens related research could consider longitudinal studies of the endothelial response to different modalities of lens wear in large sample groups a greater understanding of the changes would develop. Improved instrumentation at more realistic cost levels would assist this development. Consideration should also be given to developing an animal model to investigate polymegethism if such endothelial changes could be produced in the short term.

If for example, polymegethism could be induced in the perfused rabbit corneal endothelium 'in vitro' the biochemical, physiological and morphological changes could be evaluated. Laboratory confocal microscopy would then offer the most likely means of obtaining the serial sections of the endothelium to allow the shape of individual cells as a function of thickness to be evaluated assuming the same cells could be followed through the full thickness of the cell layer. Previous difficulties in using TEM and SEM to view both sides of the endothelium have been outlined by Sherrard and Yew (1990).

Corneal function tests such as those suggested by Polse and his colleagues (1989, 1990) offer the best possibility of further investigating the clinical significance of contact lens induced polymegethism. Experimental techniques such as fluorophotometry although interesting, are complex, difficult to control, and require expensive apparatus. Although the soft lens swelling or 'stress' test can be time consuming, it can at least be used experimentally, to investigate the more significant cases of polymegethism.

There are also now a number of comparative results available in the literature on the technique allowing some understanding of the range of responses likely to be found.

In Polse's experiments (1989,1990), corneal hydration control (CHC) is defined as the percentage rate per hour (PRPH) that characterises the exponential deswelling of the cornea following induced corneal oedema. CHC is calculated from two sets of corneal thickness data obtained from separate test procedures. One test involves making a series of corneal thickness measurements for 1-2 hours to determine base line corneal thickness.

The second test monitors corneal thickness recovery after corneal swelling is induced by a thick soft lens (stress test). Data from these two tests are combined to give a composite exponential model that provides an estimate of the deswelling rate which is expressed as the percent recovery per hour (PRPH).

Preliminary data (Polse et al. 1990) from experiments on corneal function tests on previous lens wearers, suggests that hypoxic exposure alters endothelial morphology and reduces corneal function. However an assumption was made by Polse and his co-workers (1990) that the hypoxic dose was related to years of lens wear, and although this seems a reasonable assumption, a significant correlation between years of lens wear and corneal thickness was not confirmed in the group of long term PMMA lens wearers, in the present study.

Overall therefore, it is important to remember that although it is often assumed that the deswelling function is a measurement of an endothelial pump function, this has never been proved. Doughty (1989) reported an experiment where corneal deturgescence occurred without the apparent participation of the endothelium. There may be other factors involved in corneal hydration control and it should not be assumed that the endothelium is solely involved in this process.

After ten years of research on the corneal endothelial response to contact lens wear the significance of the findings is still in question. It will be fascinating to see in another 10 years if the recent statement (Bergmanson and Weissman 1992) that "*****no scientifically proven loss of endothelial function has been demonstrated in the contact lens wearers with polymegethism***" will still be applicable.

A future series of experiments should therefore address;

1. Automated image analysis of endothelial photomicrographs.
2. Correlation of specular microscopy with electron microscopy.
3. Specular microscopy of former PMMA wearers refitted with RGP lenses.
4. Use of confocal systems to view sections of the endothelium.
5. An animal model for endothelial polymegethism.

In conclusion, contact lenses are generally very safe devices to correct refractive errors, provided they are used as recommended. However, too many wearers do not comply with appropriate lens maintenance programmes, and as such, lenses can become dangerous.

However, the fact that contact lenses have optical, occupational and cosmetic advantages for millions of individuals in developed countries is undisputed, and perhaps rather than optical or medical science, it will be the art of patient management by practitioners that will prove to be the main limitation in contact lenses achieving their full potential in the future.

We know that hypoxia, carbon dioxide, lens deposits, bacteria and other organisms, tear exchange and the environment, are very important factors in the ocular response to lens wear. It can therefore be predicted, with some confidence, what is needed to achieve long term success with contact lenses without adverse effects on the eye. Minimising hypoxia and contact lens induced inflammation should be the immediate physiological goals in attempting to meet the challenge of extended wear.

It is estimated that currently 1% of the world's population wear contact lenses. Solving some of the above problems could optimistically increase this figure to 10% and allow contact lenses to achieve their full potential. Further studies are undoubtedly necessary to better define the risk to the patient from extended wear of contact lenses. Although not apparent in the United Kingdom, the demand for extended wear does exist, particularly in the United States, and at the present time, no contact lens has been shown to be suitable for such wearing patterns.

For the present, it is important that individuals desiring extended wear be advised of the potential risks, in particular, corneal ulceration. The risks and benefits of extended wear must be thoroughly discussed so the patient can make an informed, intelligent decision. Daily wear of oxygen permeable contact lenses remains a much safer option.

APPENDICES

1. When discussing the measurement of corneal thickness, the term pachometer has been used throughout the thesis. Etymologically, pachometer comes from the Greek terms "pachy" (thick) and "metron" (to measure). Pachometer can also be spelled pachymeter and both spellings are acknowledged by the Oxford English Dictionary. The spelling pachometer was introduced into ophthalmology by Maurice (Waring 1992).

It is reported that this was on the advice of Duke-Elder who preferred to derive the term from the Greek noun for thickness rather than the Greek adjective for thick. In considering the term "pachometer" it is not the meter that is thick but rather the meter that measures the thickness. Therefore the noun form pachometer seems preferable as pointed out by Maurice (quoted in Waring, 1992).

Polymegethism has been incorrectly spelt *'polymegathism '* in many occasions in the ophthalmic literature. Where the spelling *'polymegathism"* has been used in references, it has been maintained for accuracy. However, ***'polymegethism '*** meaning multi-sized cells is the correct term whereas *'polymegathism '* from the Greek "mega" meaning magnitude, suggests many large cells only, and is therefore incorrect in this instance.

2. **Material Properties: Oxygen permeability.**
In Chapter 7.1 it was mentioned that 6 contact lens materials had been placed in a repository for standardised measurements of oxygen permeability by polarography. This, in theory, would eliminate much of the confusion created by the lack of a standardised approach to the measurement of oxygen permeability over the last few years. Further information has just very recently appeared on this matter (Fatt and Ruben 1993).

The International Standards Organisation (ISO) has issued ISO standard No. 9913, "**Optics and Optical Instruments-Contact Lenses-Determination of Oxygen Permeability and Transmissibility**". This standard is applicable to both hydrogels and RGP's and recommends measurements on a series of samples of different thicknesses of a given material. The least squares regression method is used to fit a straight line to a plot of the reciprocal of the measured transmissibility versus sample thickness.

According to Fatt and Ruben (1993), Standard 9913 does not offer a procedure for statistical evaluation of the permeability, as calculated from the reciprocal slope although such methods are known and were described in Chapter 3.4. A correction for the 'edge effect' is recommended by Standard 9913. It does not however mention standard reference RGP materials for measurement, which is why Fatt (1993) has set up the single lot of materials (Dk range 14 to 159) in a repository to allow inter laboratory comparison of results.

Although the ISO Standard for measurement, and the range of reference materials should remove a great deal of uncertainty and confusion surrounding the Dk of RGP materials, it has to be remembered that, as pointed out by Fatt and Ruben (1993), much more than Dk is involved in satisfactory performance of an RGP lens on a patient's eye.

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GLOSSARY OF ABBREVIATIONS

Dk	Oxygen permeability diffusion coefficient
Dk/t	Oxygen transmissibility (t=average lens thickness)
EOP	Equivalent oxygen percentage
RGP	Rigid gas permeable
PMMA	Poly-methyl-methacrylate
pHEMA	Poly-hydroxyethyl-methacrylate
PVP	Poly-vinyl alcohol
RA	Residual astigmatism
IA	Induced astigmatism
FSK	Front surface keratometry
ECD	Endothelial cell density
EGF	Endothelial growth factor
CCT	Central corneal thickness
COV	Coefficient of variation
VIDS	Visual information display systems
SPK	Superficial punctate keratitis
EW	Extended wear
EWSCL	Extended wear soft contact lenses
CHC	Corneal hydration control
PRPH	Percent recovery in corneal thickness per hour
RE	Right eye
LE	Left eye
BOS.EQ.	Boston Equalens, rigid gas permeable material, Polymer Technology
FLU30/F30	Fluoroperm 30, rigid gas permeable material, Paragon Optical
FLU60/F60	Fluoroperm 60, rigid gas permeable material, Paragon Optical
FLU90/F90	Fluoroperm 90, rigid gas permeable material, Paragon Optical
FLUO 700	Fluorex 700, rigid gas permeable material, Fused Kontacts
SGP/2	Standard gas permeable, Permeable Contact Lenses
OC 18	Optacryl 18 Dk, gas permeable material
OC 32	Optacryl 32 Dk, gas permeable material
OC 84	Optacryl 84 Dk, gas permeable material
PP EW	Paraperm extended wear, gas permeable material

SD/sd	Standard deviation
CL	Confidence limits
CI	Confidence interval
SE	Standard error
SEm	Standard error of the mean
ISO	International Standards Organisation
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
AAO	American Academy of Optometry
IOP	Intra ocular pressure

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