A CLINICAL EVALUATION OF CORNEAL ENDOTHELIAL CELL DENSITY IN PATIENTS WEARING PMMA CORNEAL CONTACT LENSES

BY

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SUMMARY

A study was made to evaluate the corneal endothelial cell density in patients wearing PMMA contact lenses.

Forty subjects, 20 males and 20 females, aged from 16 to 38 years were included. Twenty of the subjects were non-contact lens wearers (control group). A modified photo-slit lamp was used for photographing and studying the corneal endothelium. Endothelial cell densities were calculated from the photographs obtained. Measurements of the corneal radius, corneal thickness and intra-ocular pressure were taken and their parameters statistically correlated.

A significant negative correlation (Pearson) was found between endothelial cell density and time of wearing contact lenses ($P < 0.05$). However, further investigation is suggested to determine the true rate of decrease in endothelial cell density with prolonged wearing of contact lenses.

A lower mean endothelial cell density was found in the contact lens wearers group compared with the control group.

A highly significant positive correlation ($P < 0.002$) between corneal radius and endothelial cell density was found. This correlation was lower for the contact lens wearers group ($P < 0.2$).

A slight gradual decrease in endothelial cell density due to increments in intra-ocular pressure was noted in both control and contact lens wearers group ($P < 0.25$; and $P=0.2$ respectively).

No correlation was observed between endothelial cell density and corneal thickness.

No correlation in cell density was found between males and females.

There was no significant difference in intra-ocular pressure, central corneal thickness and central corneal radius between wearers and non-contact lens wearers.

Key words: human corneal endothelium, cell density, contact lenses.

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1. INTRODUCTION

Since 1887 when the first contact lens was fitted there have been continuous efforts to develop new types of contact lenses. Many present-day hard contact lenses are made of Polymethylmethacylate (PMMA) which is an amorphous thermoplastic, composed of linear polymers of methylmethacylate and 70 - 75 percent syndiotactic (Fig. 1.1).

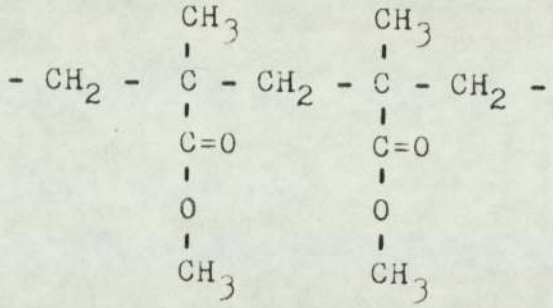


Fig. 1.1 Chemical structure of polymethylmethacrylate.

PMMA is a hard substance with many advantages. It has very good optical and other physical properties as well as being a proven non-toxic substance. The PMMA is highly transparent and transmits 92 percent of incident light of the visual spectrum in air, 8 percent being lost at the surfaces due to reflection. Infra-red radiation above 1200 mμ is not transmitted well but ultraviolet down to a wavelength of about 340 mμ is and can be absorbed by the plastic if an ultraviolet absorbing compound is incorporated into the polymer chain (Fig. 1.2). Pigments can be added to the PMMA polymer to obtain selective absorption.

Fig. 1.2

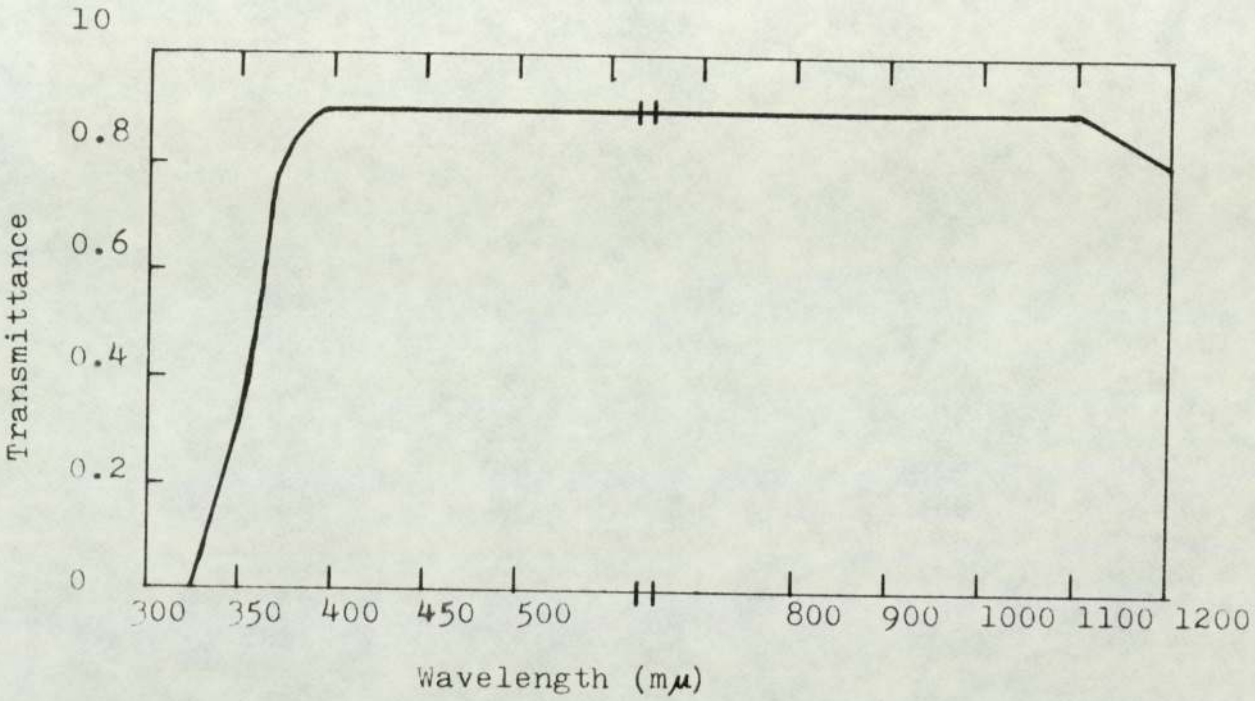


Fig. 1.2 - Transmission (transmittance) spectrum of polymethylmethacrylate (from Bier & Lowther, Contact Lens Correction).

The refractive index of standard PMMA with white light is 1.495. This varies as a function of the wavelength of the light used (Fig. 1.3).

The main drawback of the PMMA lens is its low oxygen permeability (0.1 to 0.4 percent water absorption).

When a PMMA lens is placed on the cornea, the oxygen reaches the cornea only through circulation of tear fluid under the lens. In order to diminish this problem, changes are made to the fit and design (e.g. smaller and flatter lenses) so as to permit greater tear circulation under the lens. Poor wettability is another disadvantage of the PMMA lens. The surface contact angle is 60 - 65 degrees. With the use of a wetting solution on insertion and proper spreading of the tear mucoid layer over the lens, an inadequately wetted surface has not been a major problem.

New gas permeable materials are now being developed from the polymerization of PMMA with other polymers in an attempt to find a further step forward. However, despite these new developments PMMA is still one of the most commonly used materials for hard contact lenses.

Contact lenses are, in fact, foreign bodies. As such it must be presumed that they may cause changes in all those structures related to them. The eyelids, tears and cornea are the most important structures to be considered for a contact lens fitting since the contact lenses may rest on, or affect all these structures.

Fig. 1.3

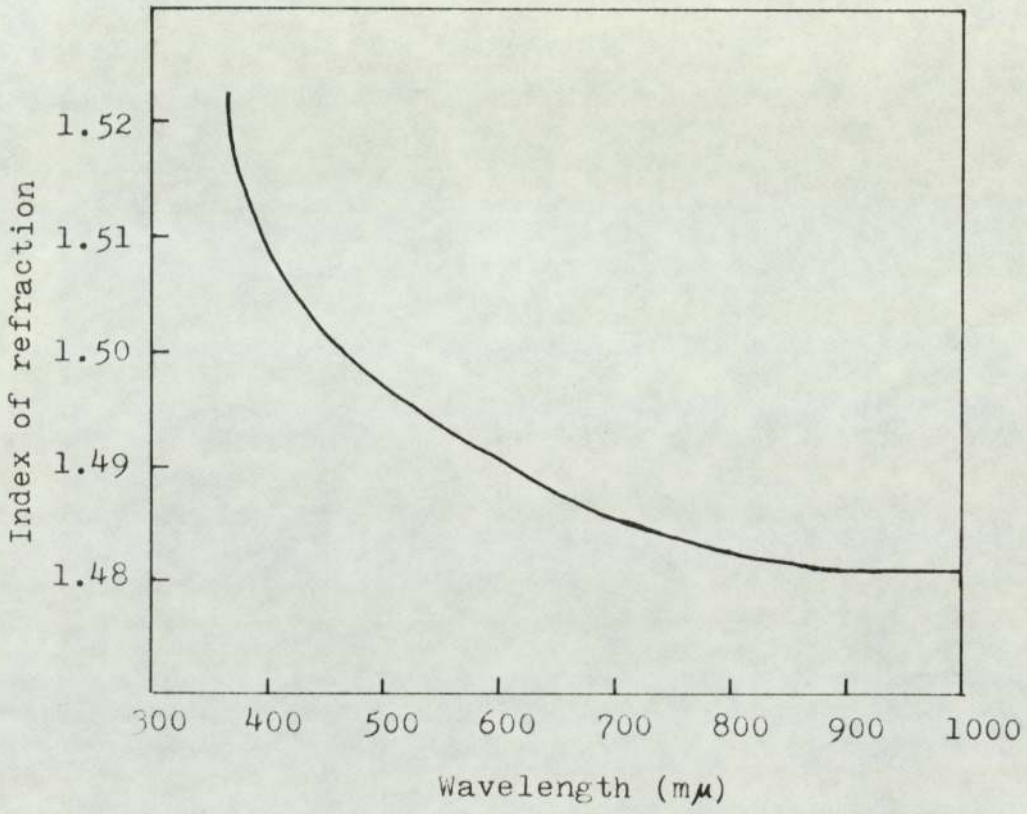


Fig. 1.3 - Refractive index variation with different wavelengths for polymethylmethacrylate (from Bier & Lowther, Contact Lens Correction).

2. ANATOMICAL CONSIDERATIONS AND IMPORTANCE OF THE EYELIDS, TEARS AND CORNEA AND ITS RELATION TO CONTACT LENSES

2.1 Eyelid - It consists of four layers:-

A. On the outer surface is a specialised skin, which is thin, loose and pliable. It consists of six or seven rows of epithelial cells with a keratinized surface, which ends near the meibomian glands. There are very fine hairs on the lids which have small sebaceous glands associated with them, also small sweat glands are present.

B. The underlying tissue is devoid of fat. It is a subcutaneous areolar layer of loose connective tissue, posterior to which is a layer of muscle - the orbicular muscle - which runs concentrically around the palpebral aperture. The action of the orbicular muscle is to close the lids. It is innervated by the seventh cranial nerve. In the upper lid, separated by a layer of areolar connective tissue, there is a portion of levator palpebrae superious which extends down just anterior to the fibrous layer and is attached through the orbicularis muscle to the skin. This muscle elevates the upper lid, however, there is not a corresponding muscle in the lower lid.

C. The fibrous layer, the tarsus, or tarsal plate, gives stiffness to the lids, but does not extend upwards and downwards the whole height of the lids, reaching, in the upper lid only about 8 mm. from the margin, in the lower

one about half that distance. Anterior to the tarsal plate lies the main nerves and blood vessels of the eye. The eyelashes emerge just anterior to the tarsal plate and the meibomian gland opening near the margin of the lid. Sebaceous glands (glands of Zeis) open into the lash follicles and some of the sweat glands (glands of Moll) which are also present in the region may do so as well.

D. The palpebral conjunctiva is posterior to the fibrous layer. It is a mucous membrane made up of a connective tissue layer, substancia propia and covered by epithelium. This is transparent and numerous vessels can be seen in it. These supply some of the oxygen to the cornea, via the tear film, on prolonged lid closure as during sleep. They produce a mucoid matter which makes up the deep layer of the tear film and is important in wetting the corneal epithelium. The accessory lacrimal glands of Krause and Wolfring are present in the deepest layer of the conjunctiva. These glands secrete the bulk of the tear film under normal conditions with the lacrimal gland proper only being active during profuse tearing such as emotional weeping. The conjunctiva of the lids is continuous with that of the eye-ball, the bulbar conjunctiva. This forms the upper and lower cul-de-sac or fornix with the conjunctival sac.

Importance - The role of the eyelids is very important in meeting many needs of the eye. Amongst the functions

which have been suggested are:

a) Protecting the eye from the entrance of foreign particles,

b) Removing foreign matter,

c) Pumping of the lacrimal sac,

d) Blocking the light during sleep,

e) Supplying protection against trauma,

f) Distributing the tear layer over the cornea.

As far as contact lenses are concerned the eyelids play a significant role in the positioning of the contact lens on the cornea, in providing pumping action for lacrimal exchange, in the adaptation process and patient comfort. Tears are exchanged beneath the contact lens only by the action of blinking. Mandel (1976) demonstrated that the interchange of tears occur when the lens is driven downward by the upper lid until the lower lid or limbus is contacted.

The lids are very sensitive. During the first few hours of contact lens wear they may become even more sensitive (Lowther and Hill, 1968). Contact lenses with a too thick or poorly contoured edge may cause hyperaemia and oedema of the lid margins resulting in discomfort. There is evidence that keratinization of the lid margin epithelium increases with wearing of contact lenses (Seward, 1972).

2.2 Tear Film - This term is used to describe the tears covering the anterior surface of the cornea. It is approximately 7 mm thick. They are the result of the substances

produced by a group of glands; a) the primary and b) secondary glands. a) Primary glands - consist of the main lacrimal glands and the accessory lacrimal glands of Krause and Wolfring. They produce the central aqueous layer which makes up the major portion of the tear film. Under normal conditions a large part of this layer is produced by the accessory lacrimal glands. During times of excessive lacrimation (e.g. foreign body, a poorly contoured contact lens, etc.) the lacrimal gland is the source of the tears.
b) Secondary glands - consist basically of the meibomian glands in the lids and the mucous glands of the conjunctiva. The meibomian glands produce the superficial oily layer of the tear film. They secrete a lipid material which forms a thin layer over the entire free surface of the tear film. The lipids of this layer consist mainly of cholesterol ester and lecithin with lesser amounts of fatty acids, cholesterol and some phospholipids. The probable function of this lipid layer is to provide anterior hydrophobic limitation to prevent the tears from spilling out onto the lid, reduce water evaporation from the tear film and give a lubrication effect between lid and cornea. The mucuous glands of the conjunctiva produce the mucoid matter which makes up the deep layer of the tear film. The importance of this layer has been made evident in several studies and has been found to play an essential role in wetting the corneal surface. If the mucuous is experimentally removed from the epithelium, it is found that the cornea becomes

hydrophobic (Lemp, et al, 1970). This principle may play an important role in the formation of dry spots.

The lacrimal fluid is mainly water, 98.2 percent. However, it is also composed of many other components. Sodium ions have the highest concentration of all the ions with about 145 mEq/ml. Potassium (15 - 25 mEq/ml), chloride (125 - 145 mEq/ml) and Bicarbonate ions (20 mEq/ml) are also present. These components and their concentrations affect the osmolarity of the tear film. Concentrations of bicarbonate ion acts as a buffering system and is important in maintaining the proper PH of the tear.

Importance - the tear film is important in providing a good optical surface to the cornea. It covers microscopic irregularities of the epithelial surface and produces a smooth surface of the cornea. The cornea takes part of the oxygen required from the tears. If absent, the cornea would becomes opaque. The tears because of its antibacterial agents are also important as a defence mechanism for the cornea against infection. By the action of lacrimation a clean corneal surface is maintained by continuous renewal.

When a contact lens is placed on the cornea, there is usually excessive tearing. With increasing lacrimation changes in the constituents of the tear film occurs. There is increase in the water concentration in relation to other ions or molecules such as sodium, potassium, chloride, proteins, cholesterol and others. The increasing in water

concentration produces hypotonic tears with respect to the corneal epithelium so water will tend to be absorbed by the epithelium causing it to swell (Bier and Lowther, 1977a). Harris and Mandell (1969) isolated lacrimation as one of the factors contributing to corneal thickening during contact lens adaptation.

2.3 Cornea - It is an avascular tissue which constitutes the transparent anterior face of the eye. It forms the main refractive surface of the eye. The cornea is oval in shape being approximately 10 mm and 16 mm. It has an average anterior radius of curvature of 7.8 mm and a posterior of 6.5 mm, this produces a membrane which is thinner centrally. The average central thickness of the cornea is 0.52 mm increasing to about 0.65 mm at the limbus. The refractive index of the cornea is about 1.376, its concave posterior surface faces the aqueous has lower refractive index (1.336). The refractive power of the anterior surface is about 48.8 Dpt; of the posterior surface -5.8 Dpt., so the net power would be about 43 Dpt. The cornea is composed essentially of six layers: A) Epithelium, B) Basement membrane, C) Bowman's layer, D) Substantia propria or stroma, E) Descemet's membrane and F) Endothelium.

A) Epithelium - The corneal epithelium is composed of five or six layers of cells. Makes up 10 percent of the total corneal thickness. Three types of cells can be clearly distinguished in the corneal epithelium: a) squamous cells,

b) wing-shaped cells and c) basal cells. a) Squamous cells - The most superficial layer of cell is the squamous one. They are rather thin, flat polyhedral surface cells which will be sloughed off into the tear film. The squamous cells do not keratinize, or lose their nuclei or other organelles as epithelial cells do in other parts of the body. They must therefore retain some metabolic activity. b) Wing-shaped cells - They are called wing cells as they become flattened and send out lateral projections so they migrate from the basal layer. These cells are tightly bound to adjacent cells by desmosomes which prevent any significant inter-cellular space, so the passage of any substance through its surface is not easy. There are about three layers of wing cells. c) Basal cells - These form the deepest layer and consist of a group of columnar cells packed closely together forming a single layer of cell. These cells are the most active metabolically. They show mitotic activity and new cells are constantly being produced which later become wing cells. These cells contain only a small number of organelles in the cytoplasm.

Epithelial cells can be regenerated rapidly, although this varies according to the extent of the trauma, for example a 2 - 3 mm denuded area will need about three days to return to normal appearance. By means of the slit lamp examination of the epithelium appears as a dark line beneath the tear film.

B) Basement membrane - this layer is 200 - 300 Å thick and it is derived from the epithelium. Although it may be considered as a separate layer as noted by electron microscopic studies, this layer tends to separate from the Bowman layer and retain its relation to the epithelium. Regeneration of this layer takes place after injury and this may take up to six weeks to heal completely (Grayson and Keates, 1969).

C) Bowman's layer - it is composed of randomly oriented collagen fibrils, despite its apparent homogeneous appearance under the light microscope, with a thickness of 8 - 14 μm. This layer is basically a modification of the anterior stroma. It is acellular and resistant to trauma, offering a barrier to invasion of the cornea by micro-organism and tumor cells. If Bowman's is damaged it will not regenerate and scarring will occur (Grayson and Keates, 1969). On slit lamp examination it is seen as the most superficial surface of the stroma.

D) Stroma - it constitutes 90 percent of the cornea. It is composed of layers of lamellae which run parallel to each other and to the surface of the cornea. Each lamella runs the full length of the cornea and is made up of a multitude of collagen fibrils. The water imbibition of the stroma is brought by fluid absorption by the interfibrillar ground substance, causing a separation of the fibrils which themselves do not increase in size. Water constitutes 82

percent of the wet weight of the stroma and is therefore the substance in greatest abundance in the lining cornea. Collagen fibrils account for approximately 80 percent of the dry weight, interfibrillar ground substance 15 percent, and cellular elements only 5 percent. The main cells of the stroma are modified fibroblasts which lie parallel to the corneal surface and inter-connect with each other to form a synthesis network. The primary cellular element of the stroma is the fixed keratocyte. When the stroma is damaged scar tissue is formed during the repair process leaving a permanent opacity which could affect vision.

E) Descemet membrane - this structureless membrane bounding the surface of the stroma, is about 10μm thick. Although it is composed of collagen tissue, it has a certain elastic quality. This layer is considered to be a product of secretion of the endothelial cells and acts as the endothelial basement membrane. It is very resistant to damage in most corneal diseases. If a small area of the membrane is removed it can be reformed by the endothelial cells.

F) Endothelium - this is the most posterior layer of the cornea which involves the location of the primary active process that maintains corneal detergescence. The corneal endothelium is described in full in chapter two.

The cornea is a highly transparent tissue with less than 1 percent of light being scattered within it. There are several theories to explain this high transparency.

However, none of these theories are completely satisfactory. The most widely accepted theory is that of Maurice (1957) which postulates that the stroma consists of a two dimensional lattice of collagen fibrils, regularly and uniformly oriented and having a higher refractive index than the surrounding interstitial substance. The spacing of the fibrils of the lattice is less than that of the wavelength of the light. From the phenomenon of interference, waves of light striking the cornea are scattered from the individual fibrils and cancel one another in all directions except that of the beam which falls on the tissue. The lattice is thus transparent in the direction of the incident beam, and destructive interference eliminates any light scattered in other directions. Lack of regular lamellar structure and alteration of the crucial distance between the fibrils would alter the interference relationship and cause a decrease of transparency. Potts (1962) has pointed out that this theory explains only the transparency of the corneal stroma and cannot be applied to the other corneal layers, transparency of the epithelium and endothelium has only been explained by the relative small variation in refractive index of the different cell components and the minute size of many of these.

The cornea must retain its water content at approximately 78 percent to avoid the cornea becoming opaque. For this the metabolic processes of the epithelium and endothelium must be maintained. Experiments have shown that the

metabolic activity of the endothelium is the most important factor in maintaining corneal deturgescence (Dohlman et al, 1968; Mishima and Trenberth, 1968).

Normal metabolic processes may be disrupted when a contact lens is placed on the cornea. The existing deprivation of atmospheric oxygen in the cornea when wearing contact lenses would cause a decrease in the aerobic metabolism of the active epithelial cells. This could result in a corneal oedema affecting normal corneal transparency and thickness. In experiments with contact lenses on guinea pigs Smelser and Ozanics (1953) found that after the contact lens was worn for a short period of time the normal glycogen stores of the corneal epithelium were rapidly exhausted. Other investigations on guinea pigs (Smelser and Chen, 1955) found that the concentrations of lactic acid in the cornea increased rapidly after the contact lens was in place, indicating that there was a decrease in the aerobic metabolism.

According to Lowther and Hill (1973) a cornea depleted of glycogen will require between six and eight hours for the epithelium thickness to return to normal and eight to twelve hours for glycogen content to be fully restored under open eye conditions.

The temperature of the cornea is about 31.5 - 35^{o}C with the corneal apex being the lowest (Hill and Leighton, 1965a). Hill and Leighton found no increase in corneal temperature with a lens in situ under normal open eye

conditions as compared with the bare cornea, while with normal lid closure it increased by 2.5°C and with forced closure, as with blepharospasm, the temperature rise was 3.9°C. Decreasing palpebral aperture size (9 mm to 3mm) corneal temperature increased by about 1.7°C (Hill and Leighton, 1965b). It has been found no correlation between corneal temperature and the sensation of heat or cold (Hill and Leighton, 1965c). According to Bier and Lowther (1977b) this is most likely due to the lack of receptors for temperature change in the cornea, and the warm or hot sensation while wearing contact lenses may be due to a side effect created by a poor fit or a condition which causes corneal insult which secondarily results in engorgement of conjunctival vessels. Heat receptors are present in this tissue and lids which would give rise to this hot sensation.

Byron and Weseley (1961), reported a decrease in corneal sensitivity after four hours of contact lens wear. Hamano (1960) reported that after the patients had worn contact lenses for a month they showed a general overall reduction in corneal sensitivity, but after twelve months of wear sensitivity again approached normal.

It has been shown that corneal curvature change during adaptation to contact lens (Rengstorff, 1969; Brungardt and Potter, 1972), maximum increase (mean of 0.75 D) occurring 7 days after the beginning of lens wear. After maximum change at 7 days the corneal curvature goes back towards its original value and after six months wear

back to about 0.25 D steeper than K reading. A change in corneal curvature of over 0.75 (0.14 mm) may indicate a fitting problem.

A contact lens will tend to rest directly on the epithelial surface thus this limiting layer is commonly affected during contact lens wear. Clinical and histologic observations made by Dixon (1964) showed many alterations in the epithelium of corneas wearing corneal lenses, although most were transient.

Since contact lenses do not rest directly on the corneal endothelium changes in this layer would not be expected, however, recent investigations, with new specular microscopy techniques, found that morphological changes in the corneal endothelium occur due to contact lens wearing (Holden and Zantos, 1979; McMonnies and Zantos, 1979; Barr and Schoessler, 1980). Changes in this layer were also found under the influence of various conditions such as keratoconus, elevation of intra-ocular pressure and iridocyclitis (Laing et al, 1979a; Setala, 1979a and 1979b).

3. PURPOSE OF THE PROJECT

The aim of this work was to investigate the possible effect of hard contact lenses (PMMA) on the corneal endothelium cell population. Measurements of central corneal thickness, intraocular pressure and central corneal radius were also taken into account in an attempt to establish their possible correlation with corneal endothelium morphology.

CHAPTER II

1. THE CORNEAL ENDOTHELIUM
   - A. EMBRYOLOGICALLY
   - B. ANATOMY

2. IMPORTANCE OF THE CORNEAL ENDOTHELIUM
   - A. CORNEAL THICKNESS AND TRANSPARENCY
   - B. CORNEAL METABOLISM
   - C. OXYGEN UTILIZATION

3. ALTERATIONS OF THE CORNEAL ENDOTHELIUM

4. INFLUENCE OF SOME CONDITIONS ON THE CORNEAL ENDOTHELIUM

1. THE CORNEAL ENDOTHELIUM

According to Thomas (1955), Pappenheim is perhaps the first who mentioned the corneal endothelium in 1842. This is a single layer of cells lining the posterior corneal surface.

A. Embryologically

There are several opinions about the origin of the corneal endothelium. According to Seefelder (1926), and also Gluckmamn (1940); and Rones (1932), the epithelium and endothelium in the early stage form a compact layer and the stromal cells start growing between these two layers. Hagedoorn (1928), found in a human embryo of 22 mm that "indeed over the centre, the cornea apparently consisted of two very distinct layers: an epithelium and an endothelium, while the periphery the stroma-cells were sandwiched between these two layers". In this way Hagedoom believed the same as Seefelder two years before. Sondermann (1949) stated that the "so-called" corneal endothelium is of epithelial descent. The external and internal layers of the cornea, in the fifth week of foetal life are similar, and the inner lining regresses to one layer, in adult life. According to Thomas (1955), the differentiation of the endothelium takes place at about the 30 mm (two months) stage. He also noted with specimens that there is a condensation of the inner layers of the stroma which could be the initiation of the endothelium. Redslob (1935), stated that

the endothelium is the result of an independent cellular migration of connective tissue which occurs before the invasion of the stromal substance. Redslob also states that the endothelium is the result of the group of mesenchymal cells from which the muscle of Brucke develops. More recently Wulle and Lerche (1969) using an electron microscope noted that at the end of the seventh week of human embryo, mesenchyme cells, moving from the periphery into the space between the ectoderm and the lens, form an almost solid strand covering the posterior surface of the cornea. At the end of the eighth week the endothelium layer is entirely formed.

B. Anatomy

The corneal endothelium covers the complete posterior corneal surface. It is composed entirely of hexagonal cells about 5 μ high and 20 μ wide. This layer is very complicated in structure. The cell membrane facing the anterior chamber and the cell mambrane attached to Descemet's membrane show a rather flat surface, although some particular irregularities may be seen. The lateral cell membrane forms an intercellular border with the adjacent cells which is a paired membrane structure following a circuitous course. This intercellular border presents an irregular course which is greater in the limbal area. The posterior end of the intercellular border forms a terminal bar which is characterized by an extreme densification of the adjacent cytoplasm. The cytoplasm is rich in cell organelles.

Numerous mitochondria of various shapes and sizes are contained in the cytoplasm. Rough-surface endoplasmic reticulum, smooth-surface endoplasmic reticulum of vesicular type, well developed golgi apparatus, free RNP particules and centrioles are commonly found. The cytoplasm adjacent to the anterior chamber appears to consist of a complicated fine meshwork of filamentous components or occasionally fine tubules. This zone is almost completely free from cell organelles and is called by Iwamoto and Smelser (1965) "terminal web".

Anatomy of the corneal endothelium has received considerable attention by Iwamoto and Smelser (1965).

2. IMPORTANCE OF THE CORNEAL ENDOTHELIUM

It is well known that the endothelium of the cornea is vital to the maintenance of normal corneal physiology. Interruption of this cell structure can result in irreversible oedema leading to loss of corneal transparency.

A. Corneal Thickness and Transparency - The normal corneal thickness and transparency depend upon the regulation of its water content.

In rabbit, removal of the epithelium produced an average increase of 200% in the normal thickness in 24 hours (Maurice and Giardini, 1951). Harris (1960), suggested

that the main cause of hydration of the cornea following endothelial damage was influx of water from the aqueous humour rather than decreased transport of water out of the cornea. According to his view, the endothelium provides a barrier to the influx of water from the anterior chamber, and Descemet's membrane is the barrier across which outflow occurs. The role of the endothelium as a barrier is further supported by Dohlman, Busharat, Carroll and Allen (1968); a silicone rubber membrane was fixed behind the corneal endothelium of human corneal grafts. The physical barrier provided by the silicone membrane appeared to be enough to maintain the cornea in a state of dehydration. Mishima and Trenberth (1968), were able to show that the intact endothelium alone is able to maintain normal thickness of the cornea for a long period of time. It seems from this that the active cells of the endothelium are the site of a metabolic pump that controls the level of hydration of the cornea. The nature of this pump is still unknown, but an enzyme-sodium and potassium-activated AT Pase-is apparently an essential component of the mechanism. Hodson (1971) has shown conclusively that it is a sodium bicarbonate pump.

B. Corneal Metabolism - The energy by which the corneal metabolism maintains its activity is derived from the breakdown of glycogen and glucose. The supply of glucose to the cornea is possible by three ways:-

B.1 From the perilimbal capillaries: The glucose can diffuse from the perilimbal capillaries across their capillary net work, although because of the ease with which the glucose can be lost through the epithelium or endothelium and the lack of a significant centripetal flow of fluid in the stroma make it unlikely that the glucose reaches the centre of the cornea in considerable amounts (Maurice, 1968).

B.2 From the tear fluid: Glucose is also taken from the tears by the epithelium. However, the concentrations of glucose in the tear fluid is very small and the epithelium is relatively impermeable to glucose, therefore, the amount of glucose that comes via the tears is very small (Giardini and Roberts, 1950).

B.3 From the aqueous humour: Considering the problems of the limbus and tear fluid to supply glucose to the cornea it may be stated that the aqueous humour must be the main source of glucose to the cornea. The glucose passes across the endothelium cells which are permeable to glucose and actually facilitates its passage to the epithelium.

C. Oxygen Utilization - If the cornea is deprived of oxygen it becomes swollen, oedematous and translucent. Most of the oxygen consumed by the cornea is taken in by the epithelium and the endothelium. The corneal endothelium gets most of its required oxygen from the aqueous humour while the corneal epithelium gets much of its oxygen

from either the capillaries at the limbus or from oxygen dissolved in the pre-corneal film. Clinically this can be demonstrated when a tight contact lens is worn for a prolonged period of time, the cornea becomes oedematous (Fatt, Hill and Takahoshy, 1964).

Several methods to measure the amount of oxygen utilized by the cornea have been reported (Hill and Fatt, 1963; Fatt, 1978; Larke et al, 1979). The rate of oxygen uptake was found to be about 4.8 ml/cm^2 of corneal surface per hour, a greater amount of carbon dioxide, metabolic waste product was released into the atmosphere (Fatt, Hill and Takahoshy, 1964). Larke (1980) found values between 3.0 and 7.5 ml/cm^2 of corneal surface per hour.

According to theoretical calculation oxygen tension gradually drops from the anterior to the posterior surface of the cornea indicating that oxygen supply of the stroma and endothelium is via the atmosphere (Fatt and Bieber, 1968). Laboratories studies show, however, that oxygen tension in the mal-stromal region is less than that of the aqueous, indicating that the endothelium and posterior stromal regions are deriving their oxygen supply from the aqueous (Kwan, Niinikoski and Hunt, 1972).

3. ALTERATIONS OF THE CORNEAL ENDOTHELIUM

The corneal endothelium is susceptible to changes in its environment, cell loss as well as morphological

alterations are presented under certain conditions.

Most of the alterations in the corneal endothelium occur with age. In experiments on rabbits on the in vitro cornea, Oh (1963), found that the size of individual endothelial cells increased with age. Similar findings in human corneae were presented by Kaufman, Capella and Robbins (1966). They noted that in normal corneal endothelium of a very young person (one or two years of age) the cytoplasm of the individual cells was quite small, the cells were tightly packed together and the nucleous occupied most of the cell. These cells with advancing age appear to spread out with major cytoplasm. After the age of 40 the regularity of the cells starts to disappear, its hexagonal shape is no more distinct in all cells and the incident of central endothelial guttata increases. With advancing age degenerations of the cell also occur.

Capella and Kaufman (1967), Bourne and Kaufman (1976a) and more recently Lopes-Cardozo (1979) noted that there is a gradual decrease in the density of central cells with age (Fig. 2.1). Bourne and Kaufman also found that this loss of cells does not normally affect the barrier function of the endothelial layer. The space due to cell loss is filled by thinning and spreading of adjacent cells. Irvine and Irvine (1956), did not find any relationship between age and cell density. However, they noted that the lower mean cell count occurred in an individual 83 years of age.

Fig. 2.1

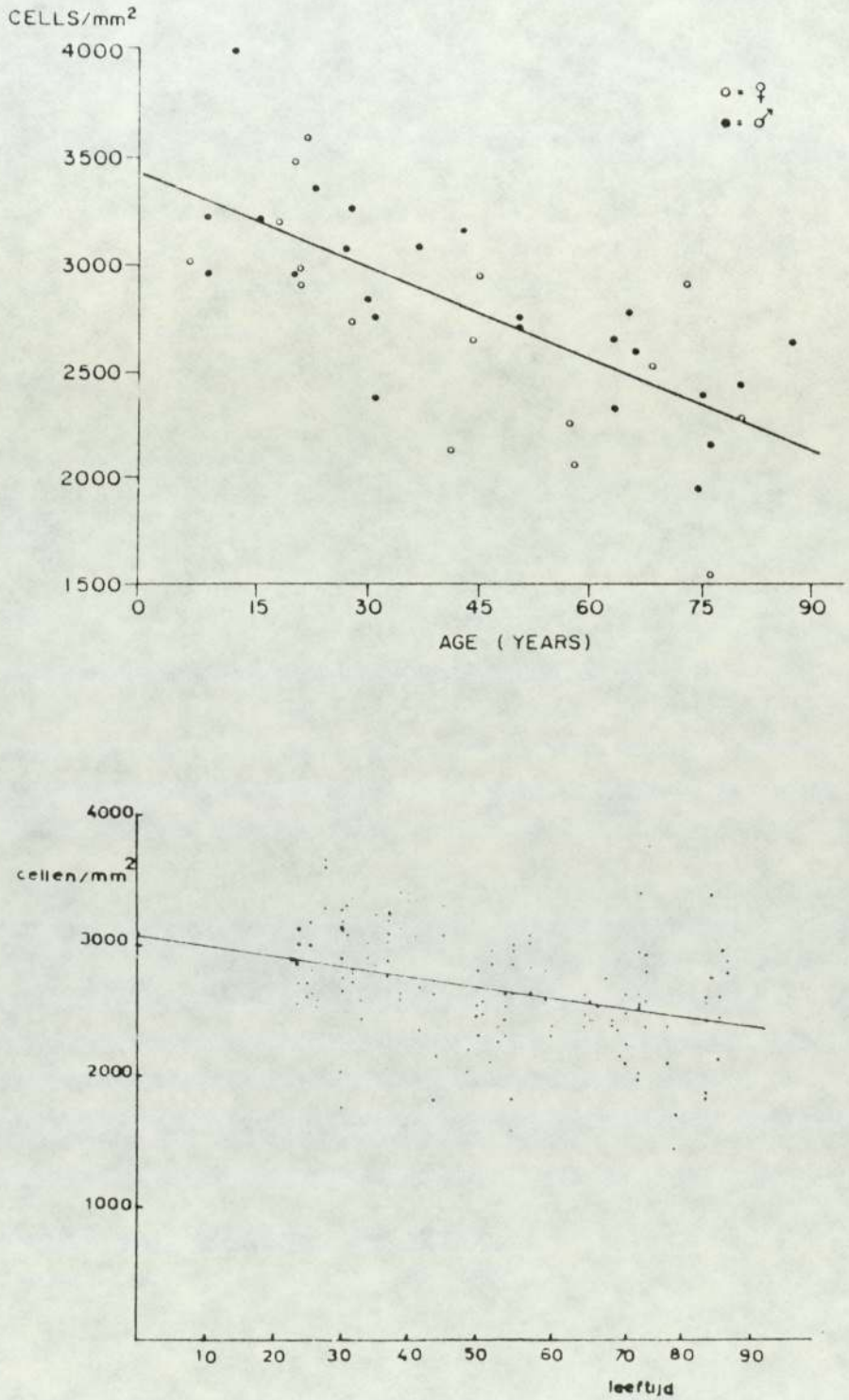


Fig. 2.1 - Endothelial cell densities according to Bourne and Kaufman (top); and Lopes Cardozo, presenting both a statistically significant linear regression of cell count with age.

Recently Rao, Shaw, Arthur and Aquavella (1979) followed by Blatt, Gullapally and Aquavella (1979), noted that there is a great variation in the endothelial cell morphology in normal human corneae. They divided the endothelium into two morphological categories: a) Homomegethous and b) Polymegethous.

a) Homomegethous - this kind of endothelium is presented when cells are uniform in size and arrangement.

b) Polymegethous - this is when cells are irregular in size and arrangement.

They also observed that when the corneal endothelium is homomegethous, the endothelial cells decrease with age, However, if the endothelial cells are irregular in size and arrangement (polymegethous), the endothelial cell density did not correlate with age.

Despite the mitosis shown in the endothelial layer of one normal rabbit by OH (1963), the regeneration of cells in humans rarely occurs according to Kaufman, Capella and Robbins (1966). However, regeneration of the corneal endothelium in organ culture has been demonstrated in human donor corneae (Doughman et al, 1976; Squires and Weimar, 1980). In rabbits Staatz and Van Horn (1980) found that aging reduced the rate of corneal endothelial regeneration. Regardless of age, they found that endothelial healing occurred by the division and migration

of newly divided cells onto the wound surface. Doughman et al (1976) and also Squires and Weimar (1980), also noted that the regenerative capacity of the human corneal endothelium has not been recognized in vivo . Squires and Weimar also found that human endothelium maintains the potential for mitotic cell division in wound healing. According to them the corneal endothelium healing occurred by enlargement and migration of cells surrounding the wound edge and by production of new cells by both amitotic and mitotic cell division. They also found that corneas treated with mesodermal growth factor (MGF) accelerated the healing response by a more rapid rate of repopulation of the zone of killed endothelium (dead zone) and by an increased width of the zone of activated cells peripheral to the dead zone.

Investigations in vitro carried out on human corneal endothelium by Irvine and Irvine (1956) gave values of endothelial cell density between 1,055 - 3,333 cells per square millimetre. The observations made in vivo corneae by Bourne and Kaufman (1976a)on 40 subjects and then by Lopes-Cardozo (1979) on 72 subjects gave values between 1,800 - 3,900 endothelial cells per square millimetre. Olsen (1979b)showed endothelial cell densities ranged from 2,200 - 3,200 cells per square millimetre.

Laule et al (1978) and Lopes-Cardozo (1979) found no significance difference in cell densities between left and right eye. However, Olsen (1979b)found that counts

from left eyes were found to be slightly lower (1.8%) than counts from right eyes ($P < 0.01$), with a standard deviation of 4.7%. He also found that the numerical relative difference between left and right eye was found to increase significantly with increasing age ($r = 0.34$, $P < 0.05$).

No significant difference in cell densities between men and women was observed by Laule et al (1978) and then by Lopes-Cardozo (1979) and Olsen (1979b).

Bourne and Kaufman (1976a) and more recently Ruben, Colebrook and Guillon (1979) found no correlation between cell density and corneal thickness, similarly no relation between endothelial cell size and stromal thickness was noted by Laing et al (1976).

4. INFLUENCE OF SOME CONDITIONS ON THE CORNEAL ENDOTHELIUM

It has been noted that the influence of certain stress and some pathological conditions affect the normal corneal endothelium.

Experiments in rabbits (Leibowitz, Laing and Sandstrom, 1974) showed that the replacement of aqueous humor with air for 30 minutes produced distinct morphologic changes in the cells of the corneal endothelium. These changes presented definite morphologic disruption of endothelial cells of sufficient magnitude to permit in situ documentation by photomicrography.

Sperling (1979) noted that an incubation temperature at or below the temperature in vivo is essential for endothelial survival in vitro.

The endothelial cells of both eyes of 60 unilateral iridocyclitic patients were studied by Setala (1979b). A lowered endothelial cell density was found in the affected eye. The diseased eye also had larger cells and showed a more irregular cellular pattern than the other eye (control eye) which showed a regular mosaic-like pattern.

Pleomorphic and enlarged endothelial cells were found to be typical for essential iris atrophy (Setala and Vannas, 1979).

Endothelial changes and no areas of normal endothelial mosaic were found in 17 patients with iridocorneal endothelial syndrome (Hirst et al, 1980).

Olsen (1980a)showed that only two patients (15%) presented a significant endothelial cell loss on the eye affected with acute uveitis.

Vannas, Setala and Jarvinen (1980) studied a family (25 patients) with autosomal dominant cornea guttata in three generations. It was found that the presence of the cornea guttata are consistent with autosomal dominant inheritance. The specular microscopic appearance of the endothelial cells was normal in the nonaffected areas between guttatas. There was no evidence of Fuchs' Dystrophy

in the whole pedigree, the corneal thickness of the patients being normal, indicating sufficient endothelial pumping activity. They came to the conclusion which supports a classification of cornea guttata in two entities. One is inherited as a dominant trait without cornealoedema and the endothelial cells appear normal in specular microscopy. The other form of cornea guttata is followed by Fuchs' Dystrophy.

Bourne and Kaufman (1976b) Forstot, Blackwell and Jaffe (1977) and more recently Sugar, Mitchelson and Kraff (1978a)found endothelial cell loss following intraocular lens implantation. Sugar et al (1978) established that there was a mean of 39.5% of cell loss in the operated eye when is compared with the unoperated eye. They also found that the loss of endothelial cells was roughly proportional to the endothelial trauma.

Bourne (1980) studied the central donor endothelium of 30 clear penetrating corneal transplants before and within one week after keratoplasty. The mean endothelial cell loss was 15.6% and there was no significant difference between the cell loss in phakic and aphakic transplant. He suggested that the decrease in endothelial cell loss in the phakic transplants was the result of the use of preoperative digital ocular massage, which softens the eye and decreases endothelial contact by recipientiris and lens during initial graft placement.

Sugar, Mitchelson and Kraff (1978b)observed that cataract extraction by phacoemulsification appeared to be more traumatic to the corneal endothelium than by intracapsular extraction. There was a mean decrease in endothelial cell density of 33.8% in the eye that underwent phacoemulsification when it is compared with the unoperated contralateral eye, while patients who had undergone unilateral intracapsular cataract extraction had a mean decrease of only 14.9%. A mean decrease in endothelial cell density of 36.8% in the aphakic eyes compared with normal eyes has also been reported by Olsen (1979c). A time analysis of the corneal endothelial cells after cataract extraction has been explained by Galin et al (1979).

Intraocular pressure (IOP) can alter the endothelium structure. Experiments in rabbits (Melamed et al, 1980) showed that increasing IOP induced corneal endothelium changes. In human subjects Irvine and Irvine (1956) reported that in two cases which presented a considerable lower density of cells than the average had been affected with glaucoma. Smolin (1968) observing the effect of numerous agents upon the delayed and immediate hypersensitivity reaction found that the deposition of inflammatory cells in the endothelium correlated with the severity of the clinically observable anterior chamber and corneal responses. His examinations confirmed the presence of lymphocytes and heterophiles during the delayed hypersensitivity reaction. Setala (1979a)found that a rise

in IOP of at least three days duration causes a decrease in endothelial cells of the eye affected by the acute glaucoma attack compared to the normotensive fellow eye. The mean difference was 9.7% ($P > 0.05$). If the pressure attack lasted less than three days the endothelial cells loss was only slightly lower than or about the same as in the control eye. Olsen (1980b) examined 23 patients with previous attacks of unilateral acute glaucoma. As compared to the healthy side, the endothelium of the affected side showed a mean decrease in cell density of 23.1% range -4.8 to 68% ($P < 0.001$). This cell loss was found to correlate significantly to the increase in corneal thickness measured during the acute attack on first day of admission. Olsen concluded that the IOP has a dual effect on the corneal hydration; if the endothelium is intact the IOP decreases corneal thickness, whereas an increase is seen only if the endothelium is acutely damage.

Pardos and Krachmer (1980) found no statistically significant difference in endothelial cell density between a group of diabetic subjects and normal subjects (control group).

Laing, Sandstrom, Berrospi and Leibowitz (1979a) observed the corneal endothelium in keratoconus. There appeared to be an increase in cellular pleomorphism.

Intraocular structures, presumably blebs or vacuoles were seen. They noted that these structures are not generally seen in the normal corneas. There were also many large, elongated cells with a definite tendency to assume a similar directional orientation. A great enlargement of cells (7 to 10 times larger than normal) was seen in a cornea with history of acute hydrops. This suggested that the endothelium and Descemet's membrane rupture in the area of enlarged cells and that the increase in the size of these cells reflects the changes necessary to repair the affected site. Ruben, Colebrook and Guillon(1979) examined a group of successful keratonic grafts (51 eyes) and found that cells counts a third of normal coexisted with normal acuity and normal thickness.

Zantos and Holden (1977) noted that soon after wearing soft contact lenses transient endothelial changes occurred. These changes or events were black lines, apparently between cells, and black spots obscuring or displacing cells (endothelial "blebs"). However, removal of the lenses results in a rapid return of the endothelial mosaic to a normal appearance. Barr and Schoessler (1980) also found endothelial blebs in patients who were adapted with hard contact lenses (PMMA). They found that the endothelial bleb response reached a maximum at 10 to 60 minutes after lens insertion in the unadapted eye. Endothelial bedewing was detected in patients who were chronically intolerant to contact lens wear (McMonnies and Zantos, 1979).

CHAPTER III

A. EQUIPMENT

1. PACHOMETRY - HISTORY - DESCRIPTION OF HAAG-STREIT PACHOMETER. MEASUREMENTS.

2. TONOMETRY. METHODS OF MEASURING INTRA-OCULAR PRESSURE (I.O.P.). DESCRIPTION OF GOLDMANN TONOMETER.

3. SLIT LAMP BIOMICROSCOPY. ILLUMINATION TECHNIQUES. PROCEDURE IN EXAMINATION BY SPECULAR REFLECTION.

4. KERATOMETRY. OPTICAL PRINCIPLES.

B. SOME OCULAR CORRELATIONS OF CORNEAL THICKNESS AND INTRA-OCULAR PRESSURE.

CHAPTER III

A. EQUIPMENT

1. PACHOMETRY - HISTORY

In living eyes, measurements of the corneal thickness were apparently first performed by Blix (1880). Using a modified ophthalmometer Blix found values between 0.482 - 0.576 mm. in 10 eyes. The apparatus consisted of two horizontally arranged microscope tubes with optical systems of equal power which converged at an angle of approximately 40^0, to a point in front of the tubes. One of the tubes contained an illuminated diaphragm adjusted to situate its image at the point of intersection of the axes of the microscopes. The tubes moved symmetrically along their axes. By simultaneous movement, the image of the diaphragm could first be observed as reflected from the epithelial surface, and then the apparatus was adjusted to cause the endothelial reflex to coincide with the point at which the epithelial reflex had been observed. The distance between the two points of focus is translated onto a scale, and represents the apparent corneal thickness (Fig. 3.1). To calculate the actual distance the radius of curvature of the anterior corneal surface and the refractive index of the cornea must be known.

After Blix, Gullstrand (1909) introduced a new method for the measurement of corneal thickness in living eyes. This method was considered to be accurate. However, it was not popular, evidently because it required the use of

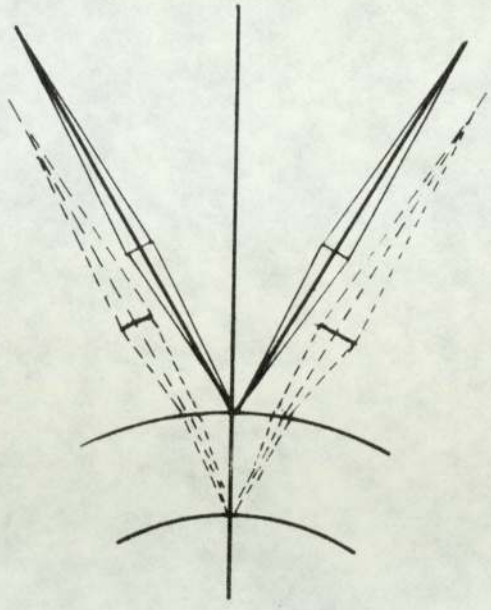


Fig. 3.1 - Diagram of light pathways in instrument designed by Blix.

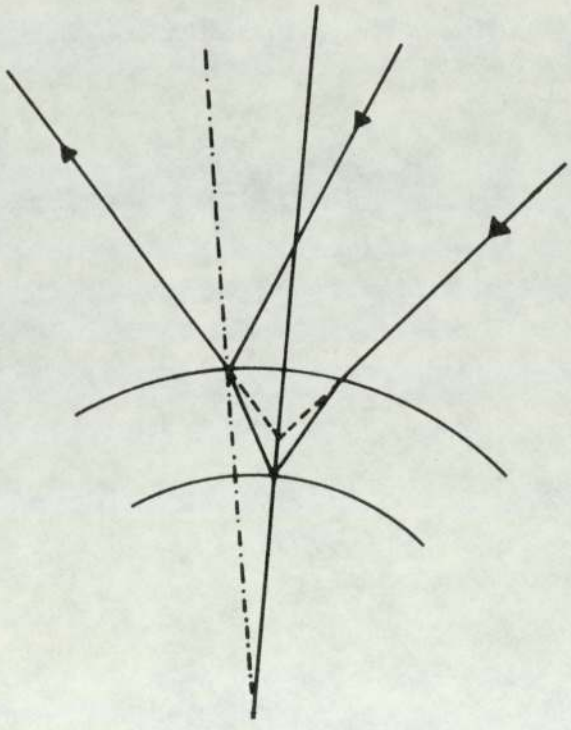


Fig. 3.2 - Diagram of light pathways in Gullstrand's instrument.

complicated equipment and was a time-consuming procedure. The principle of Gullstrand's method used reflections from the corneal surfaces. An ophthalmometric Nernst lamps with vertical slits were placed immediately above and below a horizontal telescope in front of the eye. Using a movable fixation target the eye was positioned so that the reflections of the slits observed from both the surfaces were aligned in the telescope. A theodolite instrument was set with its zero line against the endothelial focus. The first step in the procedure was to locate the posterior reflex. This lies at the basepoint of the perpendicular between the surfaces. Then a movable linear light source was adjusted so that the anterior reflex was seen in line with the posterior reflex. Thus the angle between the zero line and the movable linear source of light could be ascertained with the theodolite (Fig. 3.2). When the radius of curvature of the cornea and its refractive index were also known, the real corneal thickness could then be found.

Hartinger (1921) was the first worker to use the slit lamp beam for measuring corneal thickness. The beam was passed obliquely through an area of the cornea to be measured, and Ullbrich's measuring-drum was used to calculate the difference in position between the anterior and posterior surfaces of the cornea. Then knowing the radius of curvature of the cornea and its refractive index, the actual thickness could be calculated.

Juillerat and Koby (1928) also used a slit lamp to measure corneal thickness, with an eyepiece micrometer. This enabled measurements of the apparent length of the optical section to be made. They discovered that the best method was to set the axis of the microscope parallel with the optical axis of the eye, and the beam of light at an angle of 45^{o} to the microscope (Fig. 3.3). A formula is given for the calculation of the real thickness. Measurements on 20 subjects gave a value of 0.590 ± 0.014 mm, (mean $\pm$ standard error of mean). According to Ehlers and Hansen (1971) this high mean value was partly due to the assumption of a refractive index of 1.4, and the high standard error probably to the use of an eye-piece micrometer which required two successive readings to obtain the apparent thickness.

In 1948, Von Bahr introduced his new method of measurement. This was extensively explained in his work: "Measurements of the thickness of the cornea". The apparatus was based upon the principle used by Blix, but instead of involving successive adjustments of the point of convergence of the systems of illumination and observation to the two corneal surfaces, the new apparatus allowed simultaneous adjustments. The apparatus consisted of two rotating glass plates which were symmetrically movable by means of gearing. The glass laminae allow only the rays of the lower halves of the optical systems to pass. Thus, by proper rotation of these laminae, one could cause the specular reflection

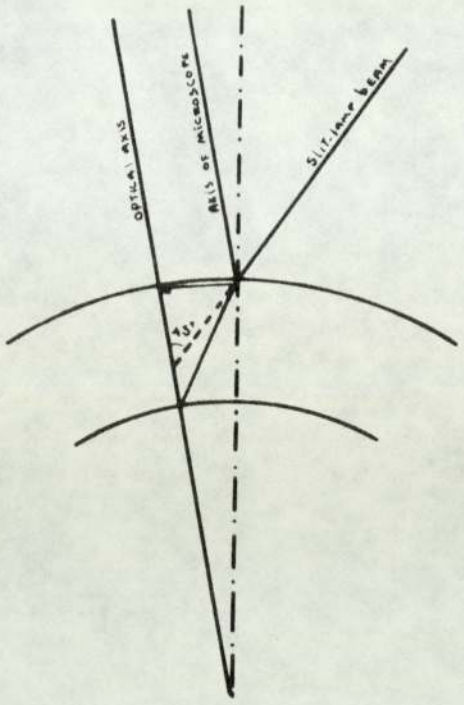


Fig. 3.3 - Diagram of light pathways as Koby and Juillerat's method.

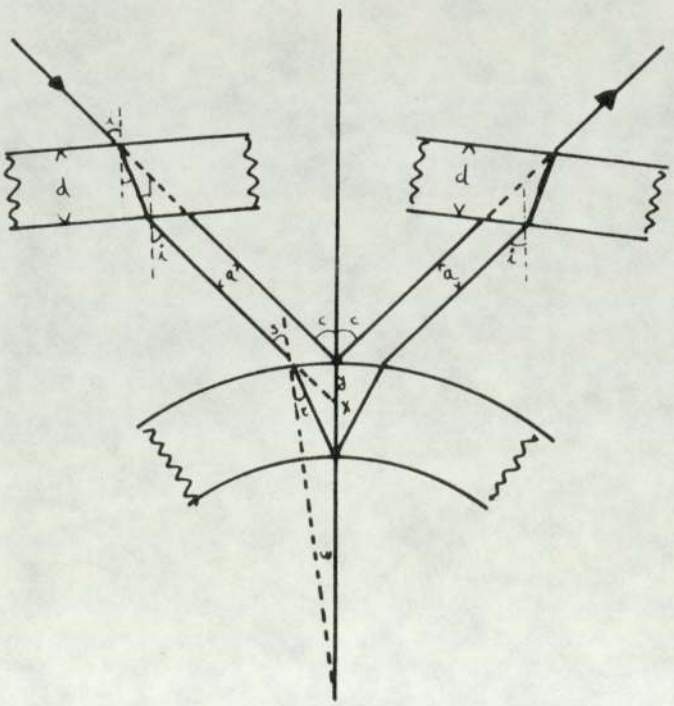


Fig. 3.4 - Diagram of light pathways in von Bahr's apparatus.

of the corneal surface to coincide with that of the endothelial surface. By observing the angle of rotation, the thickness of the cornea could be obtained from a curve which Von Bahr derived by calculation (Fig. 3.4).

Maurice and Giardini (1950) used the principle of Von Bahr's apparatus, but instead of two thin glass plates in front of the slit lamp there is only one thick plastic plate (approximately 4 mm thickness) mounted within a Haag-streit slit lamp, between the lens and the slit. A thin horizontal strip of coloured celluloid covered the central area of the plate. The plate was mounted so it could be rotated by means of a lever which moved over a fixed measuring scale. The plate was rotated until the white endothelial reflex was brought into alignment with the coloured epithelial reflex. The angle of rotation was a measure of the apparent corneal thickness, (Fig. 3.5). As a theoretical calculation of the real thickness was not possible, the apparatus was empirically calibrated using glass tubes of known thickness. The accuracy of the method was estimated by taking 25 readings in one position on one eye, resulting in a standard deviation of $\pm$ 0.011 mm. Maurice and Giardini (1950) on 44 subjects found a value of 0.507 ± 0.0042 mm (mean $\pm$ standard error of mean, N = 44).

Jaeger (1952) made an attachment fixed to the Zeiss slit lamp (Zeiss, Oberkocken) to measure the corneal thickness. The optical section of the light incident perpendicularly on the corneal surface was observed at an angle of

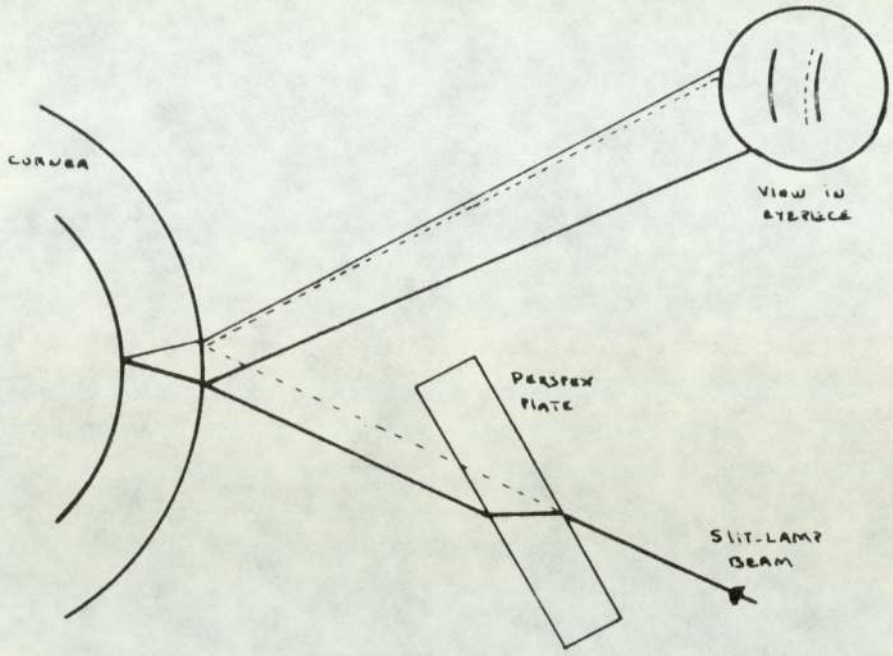


Fig. 3.5 - Diagram of light pathways in instrument of Maurice and Giardini.

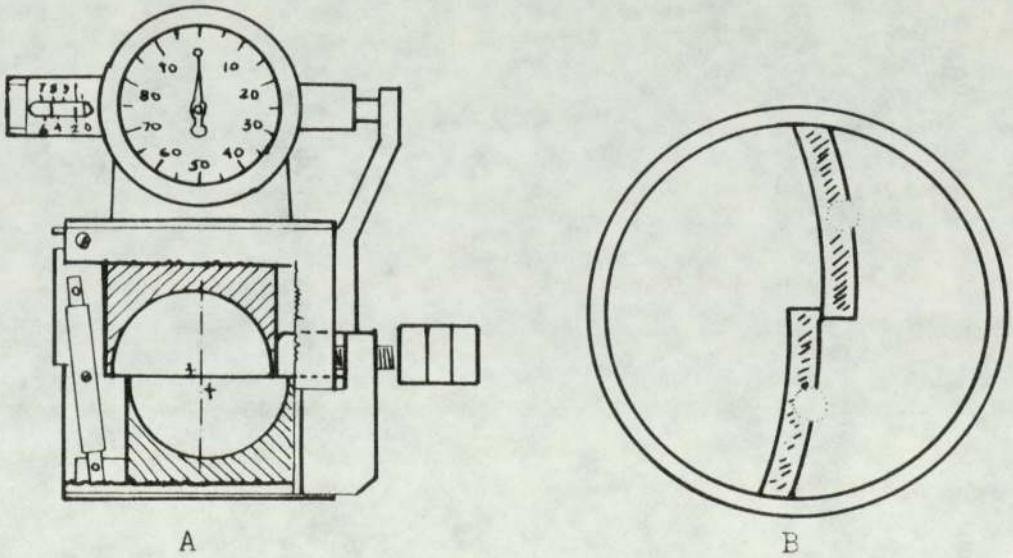


Fig. 3.6 - A. Diagram of Donaldson's split ocular. B. Appearance of slit lamp beam in cornea when observed through split ocular.

40^{o}. The image of the optical section was seen in the microscope, and the thickness was measured by aligning the anterior and posterior corneal surfaces by means of a rotating plane parallel glass plate covering the lower half of the reflected light. The calculation of the real thickness was found from a given formula. Jaeger (1952) stated that the angle of rotation of the glass could be read with an accuracy of $\pm$ 0.5^{o}, corresponding to $\pm$ 0.02 mm., in apparent thickness.

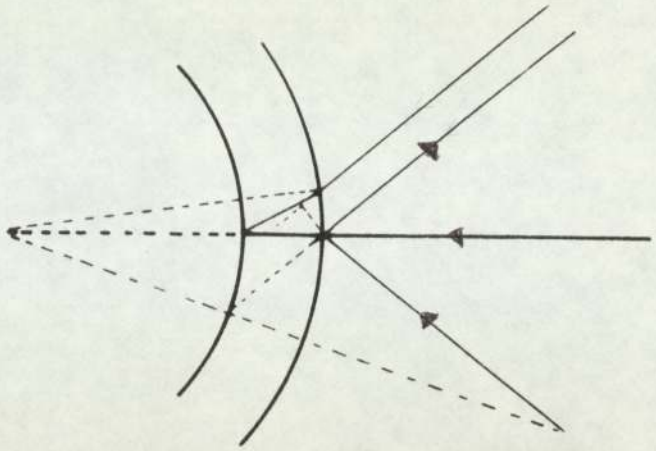
Donaldson (1965) made an image-splitting eyepiece (Fig. 3.6). The principle involved the production of a horizontal prismatic effect when one half of the lens was moved in relationship to the other half. This resulted in a doubling of the image seen through the ocular. The ocular replaced the standard ocular on the right-hand side and the slit lamp arm was fixed at 45^{o} in relationship to the microscope. The patient was asked to fixate a target provided on the slit lamp (Haag-streit), while the beam of the slit lamp was manœuvred as much as possible without reducing illumination. The beam was then positioned on the apex of the cornea in order to measure the central thickness. Here, the reflections of two small incandescent bulbs, located above and below the objective lens of the microscope, were seen one above the other. The slit lamp as a whole was rotated so that the beam as viewed through the split ocular, was seen to overlap the two corneal reflections of the small bulbs. By means of the Knurled

thumb-screw, the split image of the slit lamp beam was displaced to cause the edge of the upper half to touch the endothelial portion of the lower half. The resulting figure could be converted to actual corneal thickness by means of a graph conversion table.

Donaldson (1965), took measurements on 268 eyes taking five or more readings and determining the averages. He found a mean central corneal thickness of 0.522 mm, with an average deviation in consecutive readings of 1.2%. Martola and Baum (1968) using Donaldson's method found values of 0.504 - 0.540 for central corneal thickness, and 0.520 - 0.726 mm. for peripheral corneal thickness (temporal limbus) in 209 eyes. One striking advantage of Donaldson's apparatus was that it could be used with any slit lamp. Modifications to Donaldson's apparatus have been introduced by Mandell and Polse (1969) to measure corneal thickness in patients with keratoconus.

Using Jaeger's principle, Mishima and Hedbys (1968) introduced a new attachment for the Haag-Streit slit lamp. This involved a determination of the apparent thickness of the corneal optical section, when the slit lamp beam was passed perpendicularly through the tissue surface (Fig. 3.7a). This attachment secured the perpendicular direction of the slit lamp beam, overcoming one of the disadvantages presented by other methods. This apparatus is at present one of the most widely used in daily clinics.

A.



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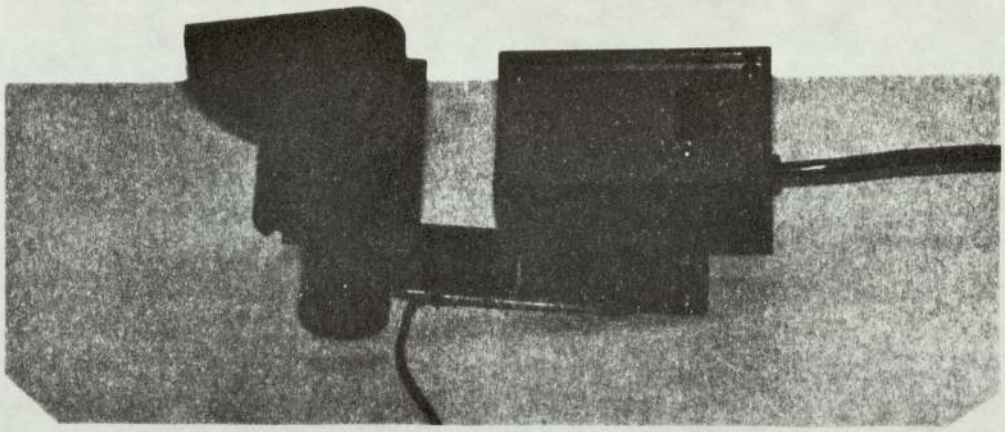

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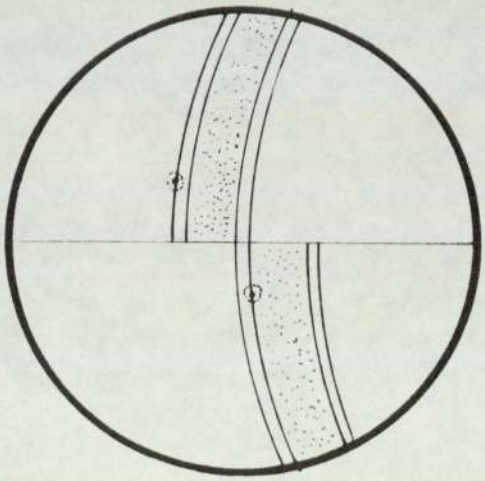


Fig. 3.7 - A. Diagram of light pathways for the measurement of corneal thickness (Mishima and Hedbys). B. The Haag-Streit depth measurement attachment 1 with Mishima and Hedbys modification. C. Alignment of the endothelium of the upper half and the epithelium of the lower half.

Description of the Apparatus (Haag-Streit Pachometry)

The apparatus consists of a main attachment and a split-image eyepiece (Fig. 3.7b). The main attachment contains two glass plates - the lower fixed and the upper rotatable - and these plates are placed in front of the right microscope. A metal diaphragm with a narrow vertical aperture extends from the main attachment. The extension of the diaphragm is twice as long as the early Haag-streit pachometer, and has attached to it two small lamps (pinlights Kay, type 15-45) on this extended diaphragm. These pinlights are placed about 10 mm. apart from each other on the vertical line, which is equidistant from the vertical aperture of the diaphragm and from the centre of the glass plates in the main attachment. When the attachment is set, the vertical aperture of the diaphragm makes an angle of 40^{o} with the right half of the microscope.

Measurements

The patient is instructed to look at a target that can be moved and fixed at any position, depending on which part of the cornea is to be measured. The slit lamp is adjusted so that the light beam is incident on the cornea almost perpendicular to its surface, and the blurred images of the pinlights are seen equidistant from the horizontal dividing line of the visual field. The microscope should be moved until the blurred images of the pinlights are seen on the corneal epithelium to ascertain the

perpendicular direction of the beam. By means of a sector-shaped disc placed at the top of the attachment, the image of the endothelium seen in the upper field is moved until it is in line with the image of the epithelium seen in the lower field (Fig. 3.7c). The corneal thickness can, therefore, be calculated from the angle of rotation of the upper glass plate using the thickness and refractive index of the glass plate, and the curvature and refractive index of the cornea. From a table the thickness of the cornea can be found using the scale reading of the attachment.

The average corneal thickness found by Mishima and Headbys (1968), was 0.518 $\pm$ 0.02 mm. (standard deviation), the standard error of the mean of their reading was found to be about 1%.

Cardona and DeVoe (1971) designed another attachment to be fixed on the Haag-streit 900 slit lamp. The instrument consists of a double mirror, one of which is movable. Thus, separate images are obtained from the epithelial and endothelial surfaces. These surfaces can be measured by means of a calibrated gearing system. The calibration is based on a chamber filled with a solution which has 1.376 index of refraction, similar to human corneae. Their measurements on 204 subjects gave an average value of 0.535 mm. for central thickness and 0.651 mm. for peripheral thickness.

Other methods of measuring corneal thickness, using

laser light interferometry, photography and ultrasound have been used. However, these techniques require sophisticated equipment, whose application would be more complex than the other methods. Its daily clinical use would therefore be more limited.

At present the most widely used and simplest pachometer is the Haag-streit depth measuring attachment I, as introduced in 1968 by Mishima and Hedbys (Fig. 3.7b). Since this device was introduced some improvement has been made by Ehlers and Sperling (1977) and subsequently by Hirji and Larke (1978a).

The following table shows the corneal thickness measurements obtained by various investigators:-

AUTHOR	DATE	CORNEAL THICKNESS	NO OF EYES
Blix	1880	0.482 - 0.576	10
Gullstrand	1909	0.46 - 0.51 RANGE	2
Juilleral & Koby	1928	0.466 - 0.703	20
Von Bahr	1948	0.565 $\pm$ 0.035	224
Maurice & Giardini	1951	0.507 $\pm$ 0.028	44
Donaldson	1965	0.522 $\pm$ 0.041	268
Martola & Baum	1968	0.523 $\pm$ 0.039	209
Mishima & Hedbys	1968	0.518 $\pm$ 0.02	NOT STATED
Lowe	1969	0.517 $\pm$ 0.034	157

Hirji and Larke (1978b)studied 8 normal patients to examine corneal thickness during waking hours. The rate of

change of corneal thickness (mm./hr) decreased linearly during waking hours. The results revealed a statistically significant ($p = 0.01$) change in corneal thickness. They also found no statistically significant relation between variation in corneal thickness and the menstrual cycle.

2. TONOMETRY

The pressure within the eye is maintained mainly by the circulation of aqueous humour, which is a transparent colourless fluid contained in the anterior and posterior chambers of the eye.

The production and secretion of aqueous humour by the cells of the ciliary epithelium is continuous, so it is obvious that there must be a continuous escape or drainage of aqueous humour, and this occurs by way of Schlemm's canal (Fig. 3.8). The aqueous humour, to enter the canal, must percolate between the trabecular meshwork and finally cross the endothelial wall, and then is incorporated into the general circulation via the intrascheral venous plexus. The rate of aqueous production is about 2 microlitres per minute in the human eye (Goldmann, 1950).

Methods of Measuring Intra-Ocular Pressure (I.O.P.)

There are basically two ways whereby the intra-ocular pressure may be measured: manometrically and tonometrically.

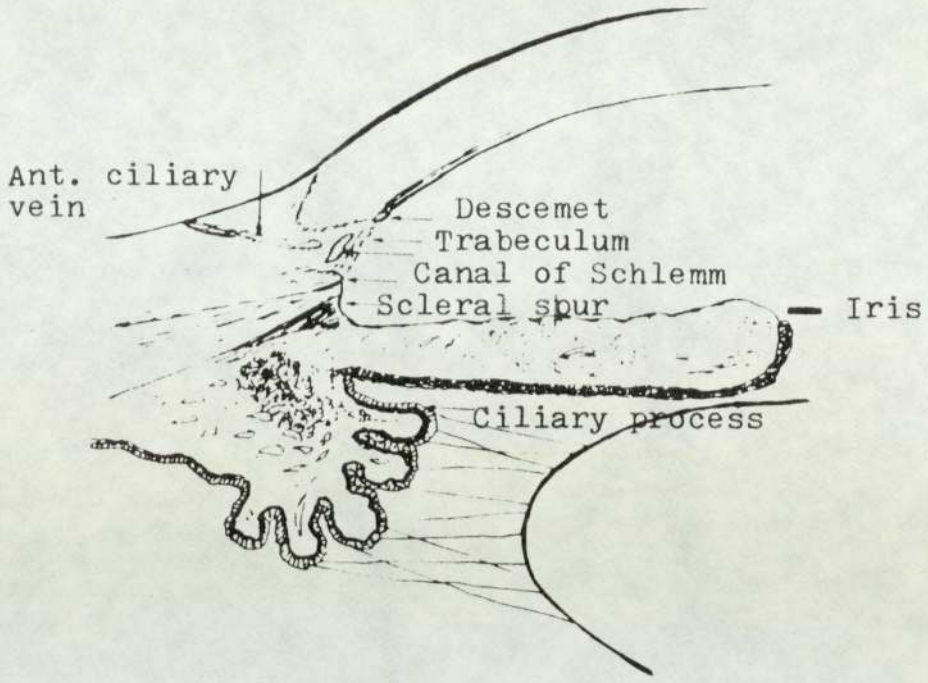


Fig. 3.8 - Schematic drawing of the chamber angle, illustrating the canal of Schlemm (Cordero- Moreno).

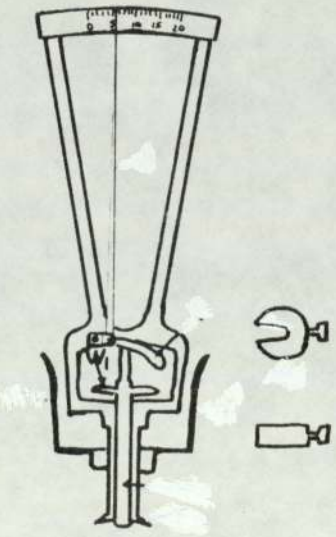


Fig. 3.9 - Diagrammatic drawing of the Schiotz tonometer (Davson, The Eye).

a. Manometry - This method is probably the most accurate, but also the least used in the daily clinic. The reason is that the eye has to be subjected to a surgical technique. This consists of introducing a cannula into the anterior chamber.

b. Tonometry - In daily clinical practice the measurement of the I.O.P. is most widely estimated by tonometry. Because it measures pressure indirectly the method is less accurate than manometry. However, tonometry gives sufficiently accurate results for clinical and some experimental purposes. The principle of tonometry is of applying a force to the eyeball and measuring the resulting deformation.

b.1 Digital Tonometry - Mackenzie (1830) seems to have been the first to recognise the relationship between "hard eyes" and vision-loss in glaucoma. High I.O.P. could be detected by applying a force to the tunics of the eye with the fingers. This technique requires a lot of experience to be reliable, and is not applicable to those glaucomatous eyes which have only marginally-increased pressure.

b.2 Impression Tonometry - These instruments are largely used, some possessing electronic adaptations, and consist of the application of a plunger of known weight to the cornea, so that the degree of indentation can be measured. According to Carter (1977), the impression tonometer of Von Graefe (1863) is probably the first "working" tonometer.

Three feet rested upon the orbital margin, while a weighted central plunger indented the cornea. The principle reason why the Von Graefe instrument was never used extensively, was probably that chloroform narcosis was necessary for it's use. The topical anaesthetic effect of cocaine was not recognized until years later, by which time more refined instrumentation was available. The most widely used impression tonometer is that of Schiotz which was first introduced in 1905, and then modified in 1924 and 1926 (Schiotz, 1905-27). The Schiotz tonometer (Fig. 3.9) consists of a foot-plate in its lower end, which is curved to fit an average corneal curvature. A weighted plunger runs through the axis. Movements of the plunger operate a hammer which converts vertical movements into a reading of the pointer on the scale. The scale reads from 0 to 20, and the lower the I.O.P. the greater the indentation and the greater the scale-reading. The instrument is held upright on the eye by means of a frame which supports the foot plate and can move freely within the cylindrical part of the frame. The actual weight resting on the eye is that of the plunger, foot plate, hammer and scale (17.5 gr.). The observer holds the instrument by the frame. Using the smallest weight on the plunger, the total effective thrust on the eye amounts to 5.5 gr., and the reading is referred to as the 5.5 gr. reading. With the heavier weights the thrust may be increased to 7.5 or 10 gr., thereby allowing the instrument to give a more accurate scale-reading at

higher levels of I.O.P. The accuracy of the instrument would increase if the weights of the plunger and foot-plate were decreased and the protusion of the plunger were limited (Majzoub, 1967). The instrument must be calibrated. The calibration is based upon measurements made on enucleated human eyes, cannulated and connected to a reservoir and manometer, so that the I.O.P. can be set to various known levels. The most striking disadvantage of the Schiotz tonometer is the weight of the instrument (17.5 g.) which, when applied to the eye commonly elevates the pressure within it by 50% to 100% of its initial value (Gloster, 1966). To make allowance for this the tonometer scale reading and plunger load must be referred to a graph or table to estimate the true I.O.P.

b.3 Applanation Tonometry - This consists of the determination of the amount of flattening of the surface of the eye by the application of a given force. The earliest applanation tonometer was the Maklakoff (1885) which consisted of a cylindrical piece of metal with a flat base weighing 5 - 15gr. After a dye-solution had been spread over the cornea, the foot of the instrument was allowed to rest for a moment on the cornea, and then transferred to a piece of paper to obtain a print of the area of contact between them.

The film of tears covering the surface of the cornea has a surface tension which tends to attract any object

applied to it. Thus, any force applied to the cornea will be supplemented by an unknown force. With human corneae using very small areas of applanation, the effect of surface tension makes the flattened area greater than would be expected conversely, with large areas of applanation the force required to bend the corneal tissue makes the flattened area less than would be expected. For this reason, the earliest applanation tonometers were not very accurate. Goldmann introduced in 1955 a refinement of the applanation tonometer (Fig. 3.10). With Goldmann applanation tonometry the degree of applanation is kept fixed and the instrument indicates the force required to produce the given applanation.

Description of Goldmann Tonometer

The ocular stresses caused by an indentation instrument, the effect of ocular rigidity and the necessity to calculate the value P_T (I.O.P. with the tonometer at rest upon the eye) into terms of P_O (I.O.P. prior to tonometer contact of the eye) are eliminated or minified by Goldmann tonometry.

In the formula: Pressure = Force/area, if the area of contact is kept small (diameter 3.06 mm.), the volume of fluid displaced by the flattening is 0.5 $mm.^3$ in a cornea of average radius. So the pressure is raised only a tiny amount by the flattening and the resultant elevated pressure

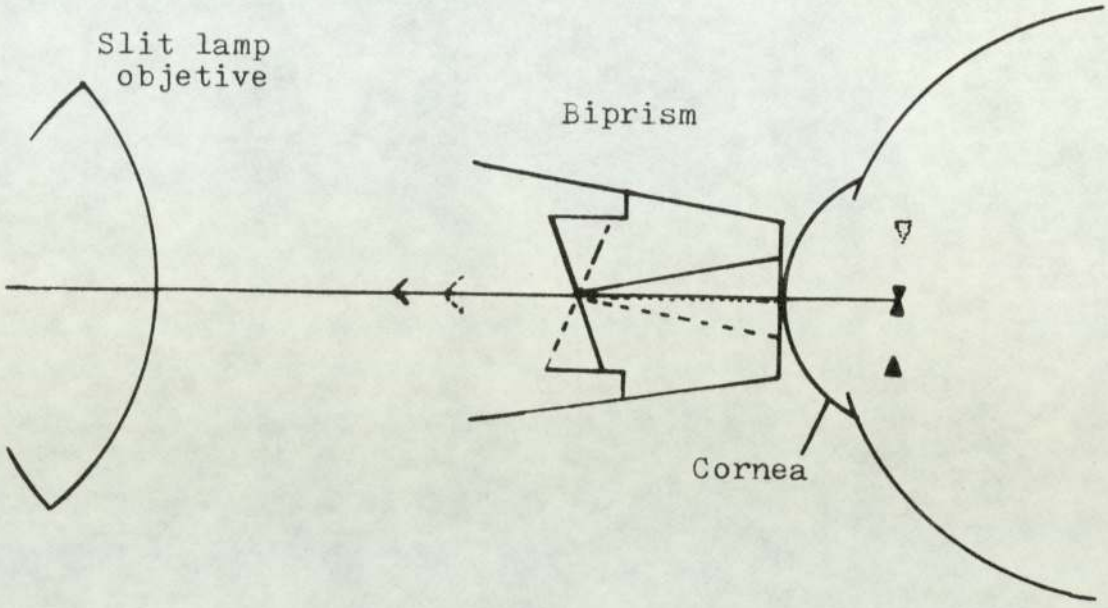


Fig. 3.10 - Diagram showing the contact element as in Goldmann tonometer (from Campbell et al, Physiological Optics).

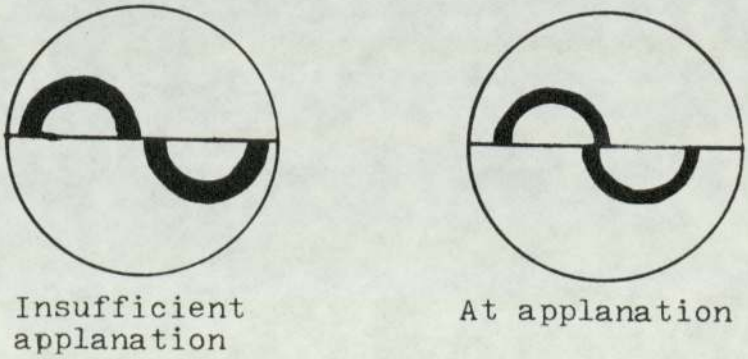


Fig. 3.11 - Images of fluorescein ring when using Goldmann tonometer.

P_T is very close to the initial I.O.P. (P_O), so $P_T = P_O$. Variations in corneal curvature and scleral rigidity make no appreciable difference. Since the surface tension of the capillary liquid film between the cone surface and the cornea does make a measurable difference, it has to be taken into consideration in the calibration. A circular applanation area, with a diameter of 3.06 mm. when flattened by 1 gram of pressure, corresponds to an I.O.P. of 10mm.Hg.

The instrument probe that contacts the eye is shaped in the form of a truncated cone with transparent plastic plate surface of 7 mm. diameter. The device is mounted on a lever with a Haag-Streit slit lamp, which is pivoted so it can be swung out of the way. The cone consists of two prisms with their apices in contact. This causes the field viewed by the observer to be doubled allowing a comparison of the two images. A force is applied to the device by using a coil spring under tension. This force can be read from a rotating dial calibrated in centimetre of mercury (cmHg.) pressure. A sodium fluorescein solution customarily is instilled in the eye when the cornea is anaesthetized. This, when viewed under cobalt blue light renders the limit of the applanation zone easily visible. The so-called fluorescein ring corresponds to the meniscus of fluorescein and tears. The applanation zone lies just within the innermost border of this fluorescein ring. Doubling the image causes the fluorescein ring to divide optically into two equal semicircular halves (upper and

lower) whose respective centres are separated by a fixed amount. With increasing applanation, the area of measurable size is reached when the inside edge of one semicircle relates to that of the other in an essentially "S-shaped" configuration (Fig. 3.11).

Where a highly toroidal corneal surface is present, the operator can set an axis control on the applanating prism to cause doubling to take place in either principal meridian.

A reading of 22 to 24 mm.Hg. is considered suspicious. The average variation during the day is 3 to 4 mm.Hg. for a healthy eye. Ninety percent of the people showed a difference of only 3mm.Hg. between their two eyes, and only 3% showed a difference of over 4mm.Hg. (Borish, 1970a).

Other applanation tonometers such as the Mackay-Marg and the non-contact tonometer (N.C.T.) are also worthy of description.

The Mackay-Marg tonometer (Mackay and Marg, 1959) ensures the elimination of the forces of surface tension and corneal bending. The instrument has a central plunger which has a flat end surrounded by a circular flat annulus or "guard ring", out of which the plunger projects a few millimetres. The area of the plunger is small and the forces of surface tension and corneal bending act upon the guard ring without affecting accuracy of the measurement.

The I.O.P. acts on the plunger, causing it to move a minute distance relative to the guard ring. The extent of the excursion is measured by a pressure-sensitive device coupled to the plunger and connected electrically, so as to give a continuous record of the variation in force.

The N.C.T. (Grolman, 1972) measures the I.O.P. accurately without physical contact with the eye and therefore requiring no anaesthesia. This instrument utilizes a "puff" of air to flatten a circular area. The instrument lies at a controlled distance from the corneal surface, so the axis of the air-nozzle is coincident with the cornea. Then, a stream of air "flattens" an area of cornea and the I.O.P. is inferred from the force of the airstream against the eye at the instant of corneal flattening.

There have been several studies on normal I.O.P. in man. Sorsby (1972) quotes a mean value of 15.4 mm.Hg. with standard deviation of $\pm$ 2.5 mm. for applanation tonometry; and 16.1 mm.Hg. $\pm$ 2.8 mm. with Schiotz tonometry.

Graham and Hollows (1964) noted that intra-ocular pressure fell as the day advance. Gloster (1966) also reported a fall after 9.30 a.m. and after the mid-day. In normal eyes the diurnal intra-ocular pressure fluctuates on average by 5.9 $\pm$ 3.0 mm.Hg., according to de Venecia and Davis (1963).

Harrison (1964) found that the incidence of higher

intra-ocular pressure increase with age.

3. SLIT LAMP BIOMICROSCOPY

The slit lamp is one of the most important diagnostic instruments for examination of the anterior and posterior segments of the eye. Alivar Gullstrand won the Nobel prize in medicine and physiology in 1911 for his work on the dioptrics of the eye and the development of the bio-microscope. Since then there have been many refinements of the illumination system, and mechanical convenience of operation. Every model of the slit lamp, however, is based on the principles of the original Gullstrand instrument. The optical principles of the slit lamp biomicroscopy are described in Fig. 3.12.

Illumination Techniques

Various illumination techniques can be utilized to observe ocular structures (Borish, 1970b; Walker, 1977; Kercheval and Terry, 1977):

a. Direct Illumination - This technique permits a direct visualization of the structure to be examined. It is accomplished by having the light beam at an angle of about 40 - 50^{o} to the microscope, both being placed in coincident focus. Several types of direct illumination can be utilized depending upon the beam size and microscope magnification.

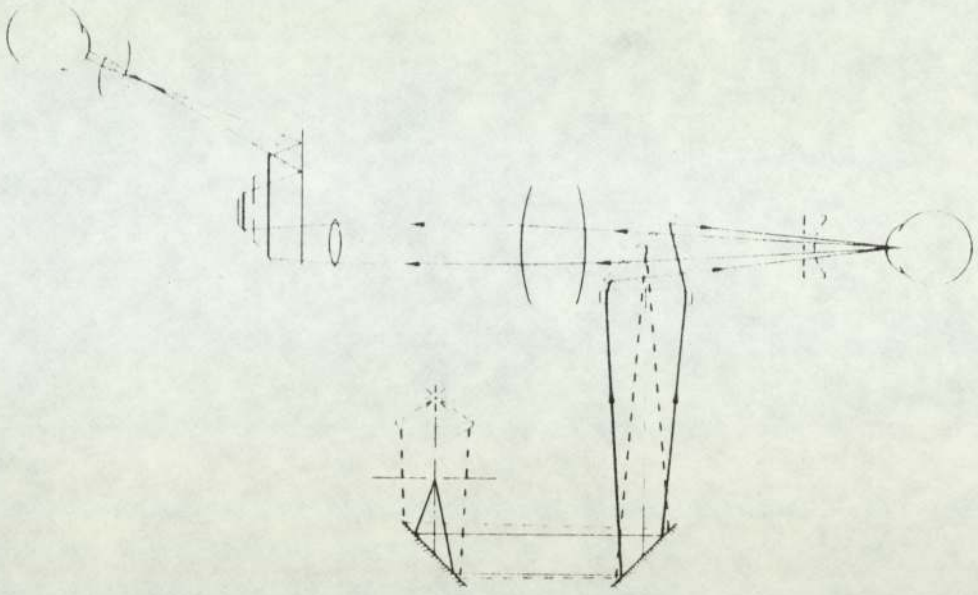


Fig. 3.12 - Optics of the slit lamp. Illumination and observation systems (from Campbell et al, Physiological Optics).

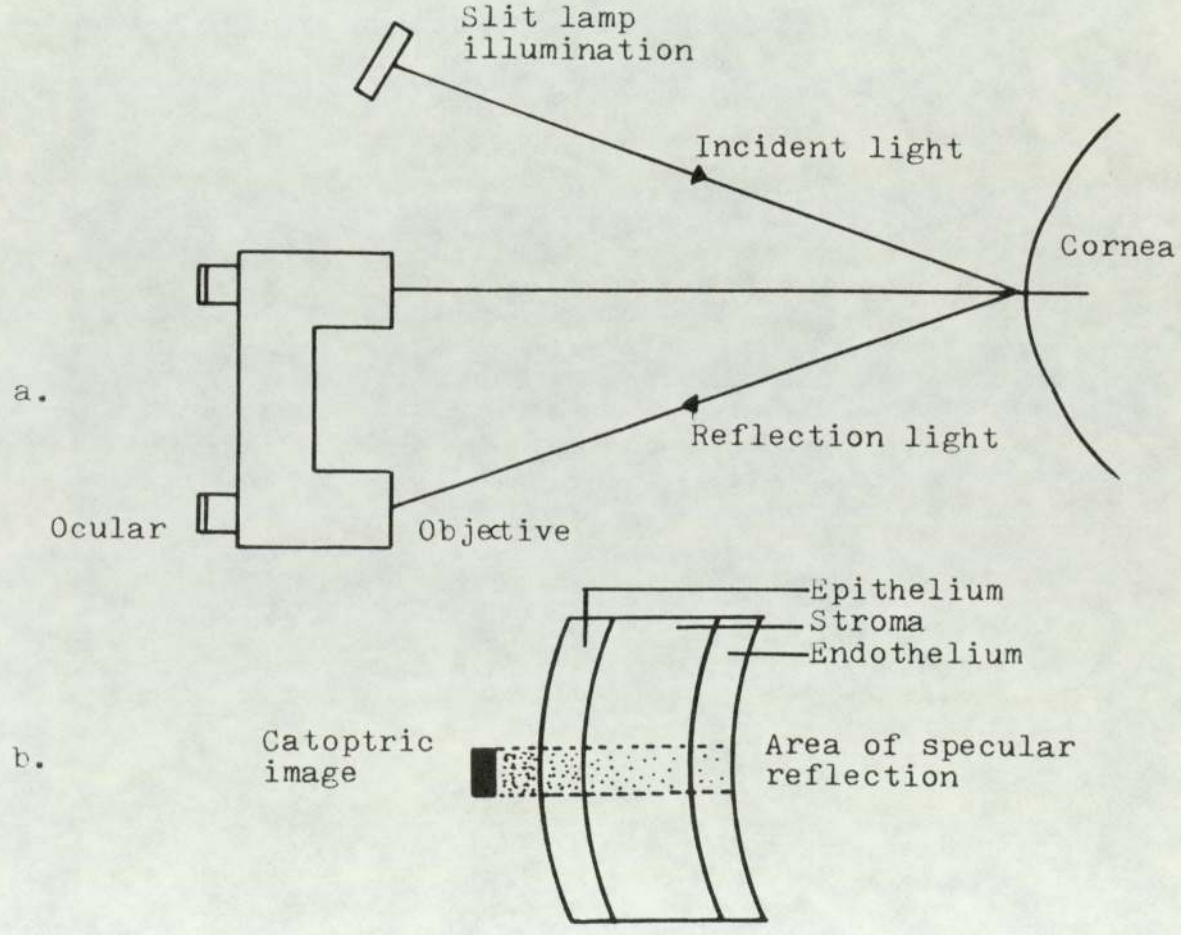


Fig. 3.13 - a. Diagram showing the slit lamp during specular observation. b. Specular reflection as seen in the central part of the parallelepiped.

a.1 A preliminary large area evaluation is normally carried out with a wide beam direct illumination.

a.2 A vertical beam of one or two milimetres on the cornea will form what is called a "parallelepiped prism". This provides a sectional view of the cornea and lens, so that the areas of discontinuity become observable. Using high magnification the depth and extent of a corneal abrasion, scarring, opacity, or foreign body, etc., can be detected.

a.3 An optic section is achieved when the parallelepiped width is further narrowed down to a slit beam. The very narrow beam is primarily used to differentiate the structural layers of the cornea and lens, and is best utilized when estimating the depth of an abnormality or the thickness of optical media and cataract position.

a.4 A square spot can be formed from the very narrow beam to examine the transparency of the anterior chamber.

b. Indirect Illumination - This technique of indirect illumination allows the examiner to observe a non-transparent structure while the light source is focussed on an adjacent area. A beam of light of intermediate size (3 mm.) is focussed to one side of the area to be observed. The slit lamp is rotated (30 - 60^{o}) to either side of the microscope. This illumination is useful in examining non-transparent or translucent structures. Lesions of the lids,

sclera and iris are examples of structures that may be examined under indirect illumination.

c. Retro-illumination - This illumination is used to observe transparent or semi-transparent media by reflecting light from tissues posterior to the media. There are two types of retro-illumination:

c.1 Direct retro-illumination. In this type of retro-illumination the tissue observed is in the direct path of the reflected light.

c.2 Indirect retro-illumination. Here the tissue observed is not in the direct path of the reflected light.

A slit beam of 2 to 3 mm. wide is normally used with the slit lamp at about 45^{o} to either side of the microscope. The mirror of the slit lamp is moved from a centrally fixed position enough to posteriorly illuminate the observed structure.

The technique of retro-illumination is the technique of choice in searching for keratic precipitates and other deposits on the corneal endothelium.

The lens can also be retro-illuminated in order to view the presence of water clefts, and vacuoles of the anterior lens. In addition to the transparent structures of the eye, retro-illumination may also be used to view the opaque iris (Walker, 1977).

d. Sclerotic Scatter - In this type of illumination the beam of light is focused on the corneo-scleral limbus. A bright but narrow slit (1 mm.) is focused on the limbus with the microscope at low magnification. The slit source is placed at a wide angle (40 - 60°), so as to produce an internal reflection in the cornea. Since the light is being internally reflected, no light emerges directly toward the examiner. If there is any area of reduced light transmission within the cornea, it will appear light grey or brighter depending on its degree of opaqueness. Minute corneal abrasions, stromal and epithelial oedema, corneal nebulae and maculae and corneal vacuoles are observable with the sclerotic scatter technique.

e. Specular Illumination - This is a method of biomicroscopy used in examination of the smooth optical surfaces of the eye. During specular observation the angles of incidence and reflection of the slit lamp beam are equal. The position of the illumination system relative to the eye determines both these angles. The microscope is also set incident with the angle of reflection of the beam (3.13a). During specular reflection the examiner sees a bright light reflected from the optical surface. This reflection is referred to as the zone of specular reflection. Specular reflection is used mainly to observe the corneal and lenticular surfaces, but it may also be used to examine other smooth surfaces such as the conjunctiva.

Procedure in Examination by Specular Reflection

e.1 Position the patient's eyes straight ahead;

e.2 Position the microscope perpendicular to the iris plane;

e.3 Rotate the slit lamp about 45 - 55^{o} to either side of the microscope;

e.4 Produce a corneal parallelepiped beam;

e.5 Determine the position of the catoptric image formed by the cornea of the filament of the bulb;

e.6 Move the parallelepiped beam towards the catoptric image with the joy stick;

e.7 When the parallelepiped beam is coincident with the catoptric image, a bright specular reflection will be seen from both the anterior and posterior corneal surface. Only a small portion of the cornea can be observed at any one time with specular reflection. The small portion seen is determined by the position of the catoptric image. Asking the patient to move the eyes to a determined position (e.g. to lower the catoptric image the patient would look up) the catoptric image can be located at any place on the cornea (Fig. 3.13b).

As the corneal parallelepiped beam is brought closer to the catoptric image of the filament, the stromal layer

of the parallelepiped beam changes in appearance from a dull, whitish-grey to a brighter whitish-grey with many highly luminous spots. This increased brightness of the reflected light has been called "internal specularity" (Walker, 1977). The bright reflection from the anterior corneal surface is whitish and rather uniform in appearance, but the reflection from the corneal endothelium has a dull yellowish-white mosaic pattern. This mosaic pattern is caused by the flat corneal endothelial cells. In fact specular reflection is the only technique with which this cellular pattern can be discerned.

4. KERATOMETRY - OPTICAL PRINCIPLES

The most precise and common way to determine the corneal radius is by means of the ophthalmometer or keratometer. Based upon the doubling principles of Helmholtz (1854) and the imagery of Ramsden (1796), and modified by Javal and Schiotz in 1881, the keratometer has provided the best and most widely used clinical apparatus.

Optical Principles

Luminous mires at the front of the instrument are reflected by the cornea which acts as a convex mirror. An image of the mires is thus formed which is erect, virtual and approximately 4 mm. behind the corneal surface. The distance between the mires and the cornea is such that the

virtual image is focused very close to the focal plane of the cornea. The corneal reflections are measured from the first Punkinje image.

Keratometry employs the relationship which exists between the object and image size of a convex mirror. Thus, the formula:

$$r = \frac{2\,u\,i}{o}$$, where: u = distant of the object
i = the image size
o = object size

Once the radius is known, and assuming the cornea has the index of 1.3375, the formula applicable to a single surface can be used as follows:-

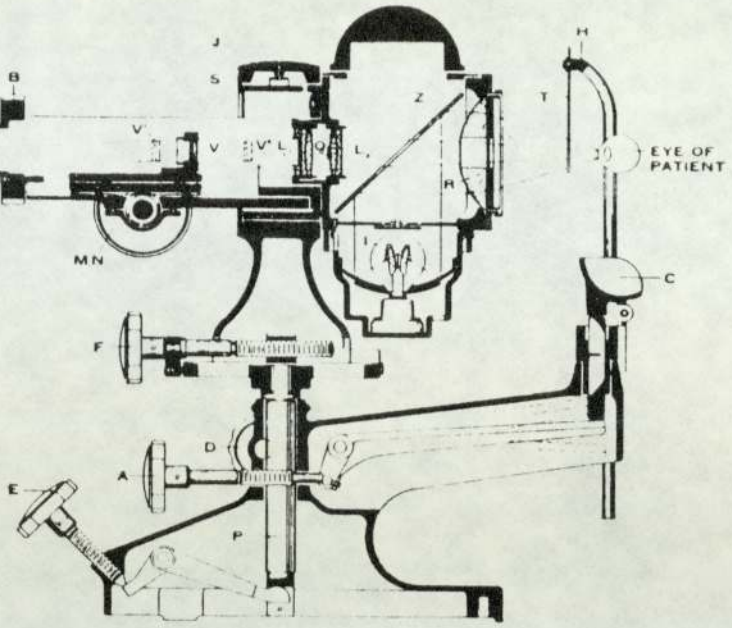
$$D = \frac{1.3375 - 1.0}{r} = \frac{0.3375}{r \text{ (in metres)}} = \frac{337.5}{r \text{ (in mm.)}}$$

Figure 3.14a shows the schematic arrangement of keratometer system.

Use of the Keratometer

The eyepiece of the instrument must be set properly with the observer's accommodation at rest. Wittenberg (1969) stated that fluctuation of observer's accommodation was a possible cause of variability of the results of keratometry (of the order of $\pm$ 0.20D). Zeiss keratometer includes a system so there is no necessity to focus the eyepiece. Each eye is examined singly. The opposite eye is occluded. In keratometry, the mire images formed by the

a.



b.

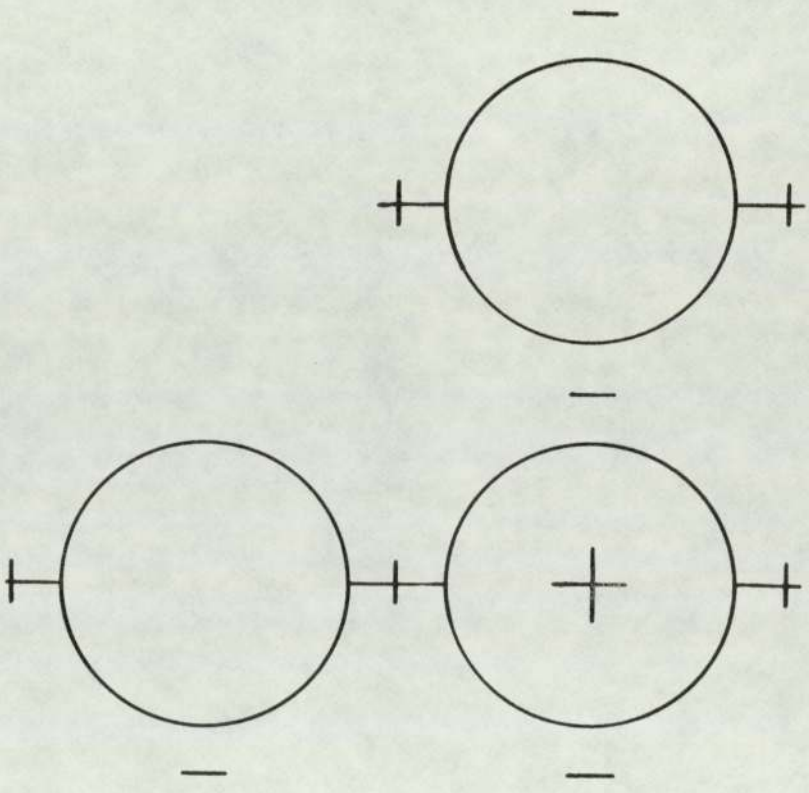


Fig. 3.14 - a. Optical system of Bausch and Lomb keratometer (from Mandell,Contact Lens Practice). b. Appearance of standard mire images after setting.

objective must be precisely focussed, if the radius measurement is to be made accurately. Blurred mire images have a different separation from sharply-focussed ones. The mires should appear at their brightest and sharpest. This is done by singling the lower right-hand circles (Bausch and Lomb keratometer).

The measurement is done by aligning the mires in the principal meridians. This is done by overlapping the plus and minus signs of the keratometer so that only one line appears (Fig. 3.14b). The findings are recorded as the power found in the meridian measured.

B. SOME OCULAR CORRELATIONS OF CORNEAL THICKNESS AND INTRA-OCULAR PRESSURE

Martola and Baum (1968) found no significant difference between the corneal thickness of right and left eyes, or between male and female corneae. They also found no correlation between refractive error and central corneal thickness.

No correlation was found between corneal thickness and age, and between corneal thickness and mean radius of curvature (Lowe, 1969).

It has been established that variations in the anterior corneal radius produce negligible changes in apparent corneal thickness (Maurice and Giardini, 1951;

Ehlers and Hansen, 1971; Arner and Rengstorff, 1972).

Grieten and Weekers (1962) confirmed that the corneal radius of eyes with closed angle glaucoma was steeper than in normal eyes, and found the mean corneal radius was steeper in glaucomatous eyes than in normal eyes. However, Tomlinson and Leighton (1972) found that the vertical corneal radius was significantly flatter in a low-tension glaucoma sample than in either the open-angle glaucoma sample or in the normal controls. The horizontal corneal radius showed similar trends, but did not reach significance.

Tomlinson and Leighton (1972) found no significant correlation between corneal thickness and intra-ocular pressure. Ehlers and Riise (1967) studied 45 patients, and concluded that when no difference in pressure was present, no difference in corneal thickness occurred and when pressure decreased, so corneal thickness increased.

Ehlers (1970) studied 8 patients with unilateral unoperated glaucoma and clinically-normal corneae. No statistically significant difference in thickness between the glaucomatous and normal corneae was found.

Tornquist (1957) showed that in eyes with acute glaucoma the radius of curvature of the cornea was 4% steeper on average than in normal eyes.

Mandell and Polse (1969) studied 12 patients with

mild to very advance keratoconus. The mean value for the central thickness for keratoconic patients was 0.377 mm. (0.13 to 0.505 SD $\pm$ 0.11), whereas the mean for the normals was 0.506 mm. (0.43 to 0.56 SD $\pm$ 0.04).

CHAPTER IV

A. METHOD

1. TECHNIQUES OF VISUALIZING THE HUMAN CORNEAL ENDOTHELIUM - LITERATURE REVIEW.

2. GENERAL METHOD.

CHAPTER IV

A. METHOD

1. TECHNIQUES OF VISUALIZING THE HUMAN CORNEAL ENDOTHELIUM IN VIVO - LITERATURE REVIEW.

Vogt (1920) was the first who developed a technique for observing the endothelium by specular illumination with the slit lamp. However, the magnification was not sufficient for a detailed study of the cellular morphology. In 1968 Maurice introduced a new specular microscope suitable for photographing the endothelium in situ at high magnification. This microscope consisted essentially of a light source which is passed through a slit aperture and condensing lens. The slit beam is directed through an objetive lens of the microscope and onto the cornea. Modifications of the instrument were made by Laing et al (1975), so that it became possible to examine and photograph the corneal endothelium at high magnification in vivo. Further changes were introduced by Bourne and Kaufman (1976a)and then by Sherrard (1977). The optical principles of the clinical specular microscope have been well explained by Laing et al (1979b). The main disadvantage of this technique is that it requires applanation of the cornea with a dipping cone lens and necessitates using topical anaesthetic.

Bron and Brown (1974) seems to be the first to photograph the human corneal endothelium in vivo at high magnification, requiring no physical contact with the eye. They used the macrophotography unit constructed by Brown (1970). The apparatus consisted of a macrophotographic objetive fitted to a reflex camera by means of an extension tube. The whole apparatus was mounted upon a photo-slit lamp (Zeiss) which provided illumination for viewing and synchronized flash for exposure. A more successful attempt has been reported by Holm (1978). The technique consisted in a specially fitted microscope supplied with an objetive with a long working distance. The camera is mounted horizontally on a bracket attached to a photo-slit lamp. This first non-contact specular microscope was made by Preisler Instrument, Sweden after suggestion by Dr. Olle Holm. This apparatus with slight modifications was used by Olsen (1979b). Photographs obtained with a modified slit lamp have also been reported by Laule et al (1978). The slit lamp was modified with the addition of a beam splitter, a 2X negative lens and a camera arm for stereo photography. The endothelial photographs were projected onto glossy white paper, giving a total magnification of 200X. Holden and Zantos (1979) also used a standard photo-slit lamp to obtain photographs of the corneal endothelium, rephotographing the original slide in order to obtain a sufficiently large

magnification to distinguish individual features of the cellular structure.

McCarey (1979) introduced some modifications to the macrophotography unit described by Brown in 1970, so it was possible to increase magnification and resolution of the endothelial cell image.

Koester et al (1980) used both contact and non-contact specular microscopy to photograph the corneal endothelium. Through the use of a scanning mirror system, the field of view had been expanded to 0.9mm. in diameter.

Barr and Schoessler (1980) used the Holden and Zantos (1979) technique. Through modifications of the fixation system of the Nikon slit lamp, they concluded that similar central areas of the endothelium could be photographed at different times by different observers.

2. GENERAL METHOD

a. SUBJECTS

A group of (40) healthy subjects (20 males and 20 females) were studied. The subjects were divided into two groups. One group, consisting of twenty (20) un-

complicated hard contact lens (PMMA) wearers. The other group, consisting of twenty (20) normal subjects who had not been adapted to contact lens wear. All eyes were normal by slit lamp examination and had no history of ocular abnormalities. The age of the subjects varied between 16 and 38 (24 mean $\pm$ 4.75 SD). Most of the subjects ranged between 19 and 30 years of age (85%). This condition was prefered to avoid as much as possible changes in cell density due to age (Bourne and Kaufman, 1976; Laule et al, 1978; Olsen, 1979b; Lopes Cardozo, 1979).

b. ENDOTHELIAL PHOTOGRAPHS

The system used in this study to obtain endothelial photographs has been described before (Belisario et al, 1980)*. It consisted of a reflex camera body which was fitted by an extension tube (85mm. long) to one of the eyepieces of a Zeiss Jena Photo-slit lamp (Fig. 4.1).

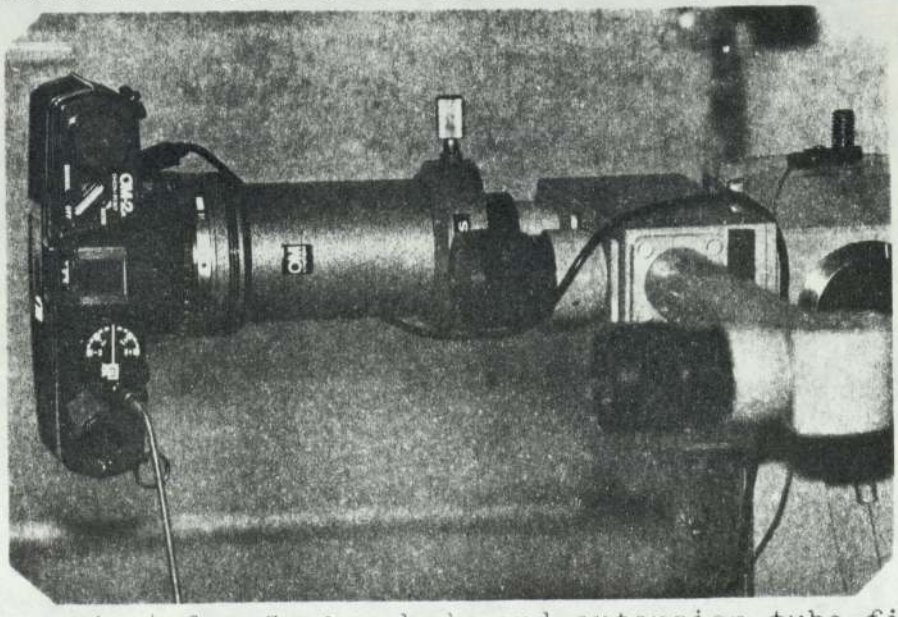


Fig.4.1 - Camera body and extension tube fitted to the slit lamp and flash system.

The endothelium was photographed in the area of specular reflection (about mid point between limbus and corneal apex). The angle of observation was kept at about 50 degrees, thus avoiding distorted dimensions on the photomicrographs (Olsen, 1979_a).

Specular reflection was obtained as explained in chapter III. It was necessary to use maximun viewing illumination and 40X magnification to focus the endothelium critically. A small fixation point of approximately o.75 mm. diameter was used so that eye movements and micro-nystagmus of gaze were reduced as far as possible.

Photographs were taken using high flash intensity with Kodak Ektachrome film of both 50 ASA and 64 ASA. The former material is intended for tungsten illumination and results in some colour discrepancy (Figs. 4.2; 4.3; 4.4 and 4.5).

c. ENDOTHELIAL CELL DENSITY

Cell density was determined from the photographs obtained. The estimation was made by counting the cells in a photographic field of known area. The photo-slit lamp was used to photograph a surface of a graticule with calibration lines etched on it at 10 micron marks. This

*Publication included in appendix 1.

calibrated photograph was then projected onto a screen at a final magnification of 540X; that is, the 10 micron marks were 5.4mm. apart. The endothelial photographs were projected on the same screen at the same magnification and position so that the cells could be counted and an estimation of cells per square millimeter made. The area counted in each photograph was approximately 0.1 $mm.^2$, and the readings were expressed as cells per square millimetre.

Endothelial cell density was estimated from the mean counting between two or more photographs for each eye. No further enlargment of the original negatives was needed.

d. CORNEAL RADIUS

Central corneal radii in both principal meridians were taken using a Bausch and Lomb keratometer. The mean value between these two principal meridians for each subject was taken.

e. CORNEAL THICKNESS

The central corneal thickness was measured in every case using a modified Haag-streit pachometer (Mishima and Hedbys, 1968). Description of the apparatus

and the method employed have already been outlined in chapter III. Three consecutive readings were taken on each subject, and the mean value was derived.

f. INTRA-OCULAR PRESSURE

Intra-ocular pressure on each subject was derived using a Goldmann tonometer. Description of the apparatus and measurement principles have been previously described in chapter III.

The cornea was anaesthetized with two drops of benoxinate (oxybuprocaine) hydrochloride o.4%, and fluorescein was instilled to stain the tear fluid. The slit lamp diaphragm was opened fully to allow the maximun light onto the prism. The angle between microscope and illumination was set at about 60 degrees. The pressure dial was turned to 1 gram (10 mm.Hg.) to put tension on the spring coil and prevent prism vibration. By adjusting the tension when the full area of the 3.06 mm. was applanated, the two semi-circles appeared so located that their inner edges just touch.

Small vertical or horizontal adjustments were often needed to centre the pattern. The tonometer face was cleaned between subjects, using ether.

g. DATA ANALYSIS

Differences in endothelial cell densities between control and contact lens wearers group; and between males and females, were analysed using the student-t test.

Differences in intra-ocular pressure and corneal thickness between control and contact lens wearers group were also analysed using the student-t test.

Correlations of cell densities with time wearing contact lenses; mean central corneal radius; intra-ocular pressure and corneal thickness were calculated using the test of significance for Pearson r. Scatter diagrams were also plotted for each correlation showing regression lines for both control and contact lens wearers groups.

Changes in mean cell density with time of wearing contact lens were plotted. The standard error of the mean for each group of subjects was represented.

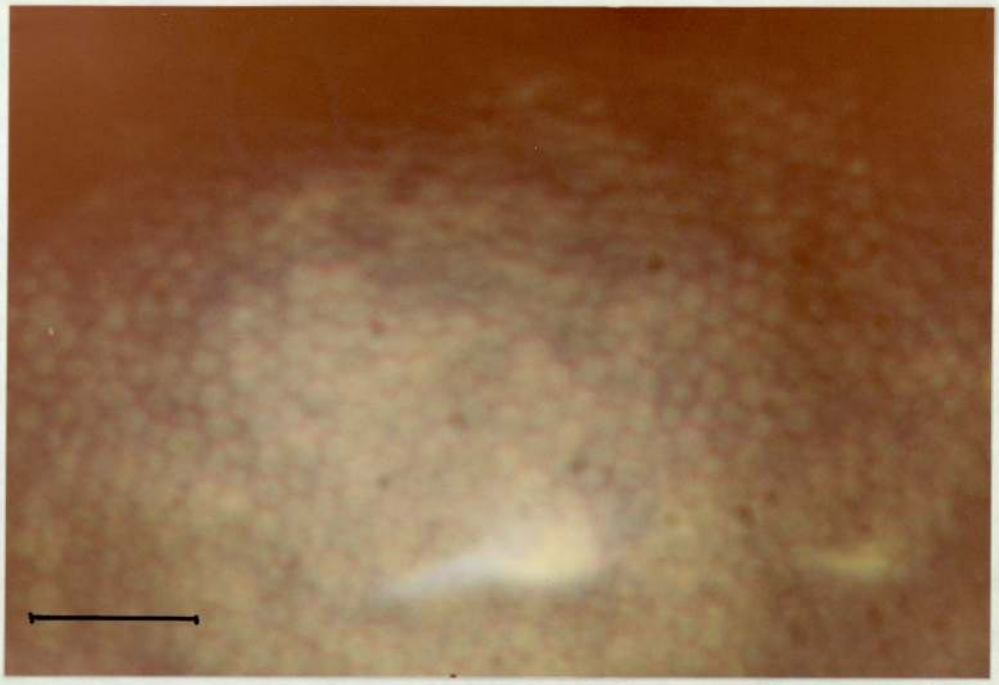


Fig. 4.2 - Corneal endothelium of a 16 year-old boy using 64 ASA film. Bar gauge= 100 microns.

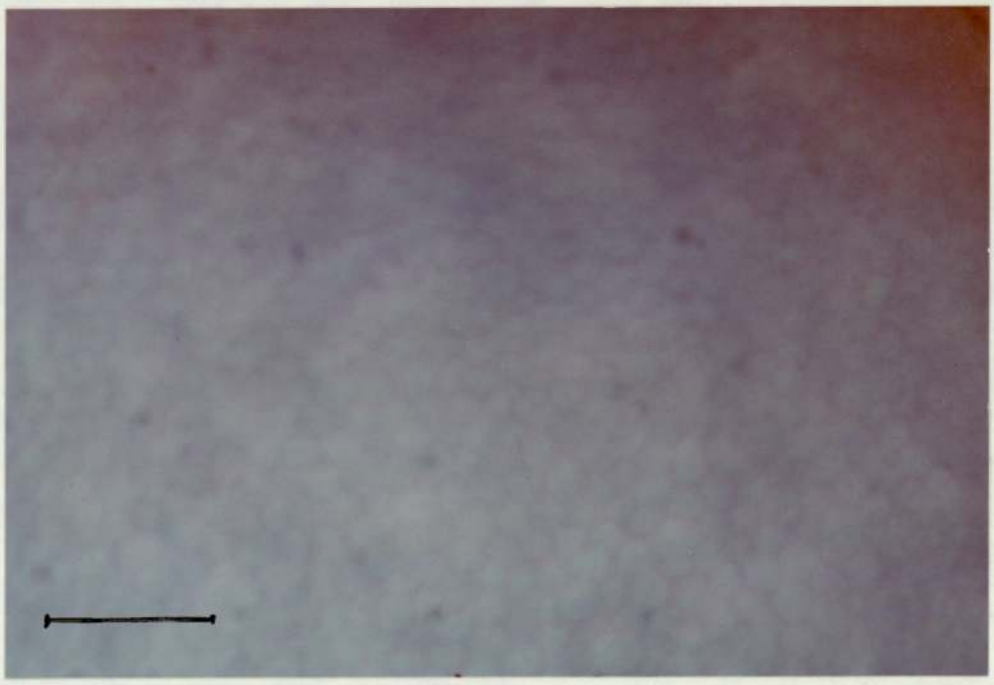


Fig. 4.3 - Corneal endothelium of a 24 year-old woman using 50 ASA film (artificial light). Bar gauge = 100 microns.

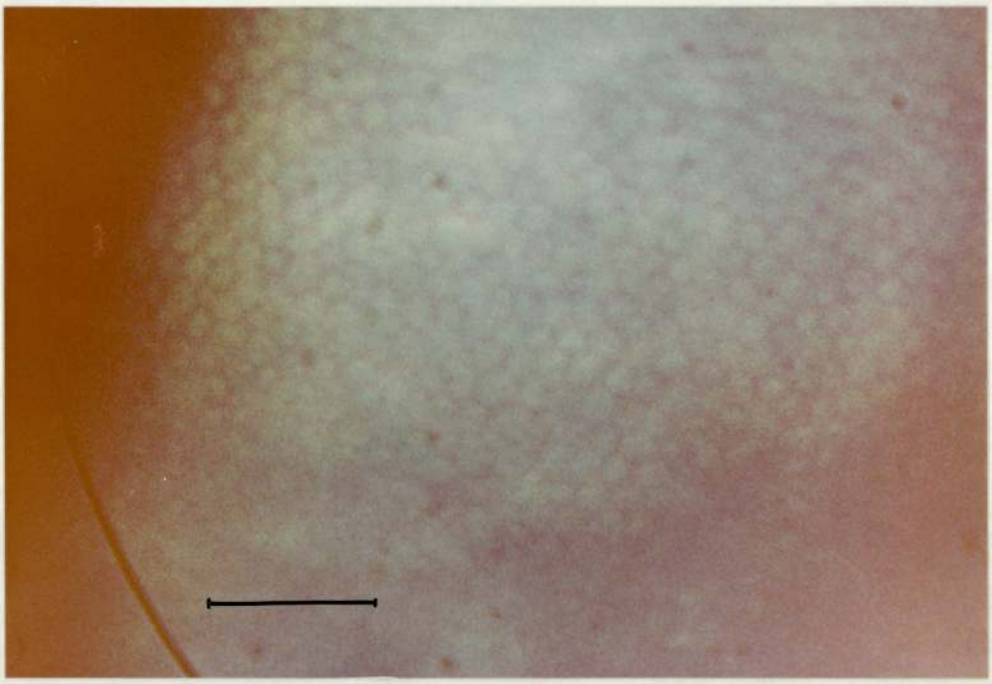


Fig. 4.4 - Corneal endothelium of a contact lens wearer of 20 years of age using 50 ASA film (artificial light). Bar gauge= 100 microns.

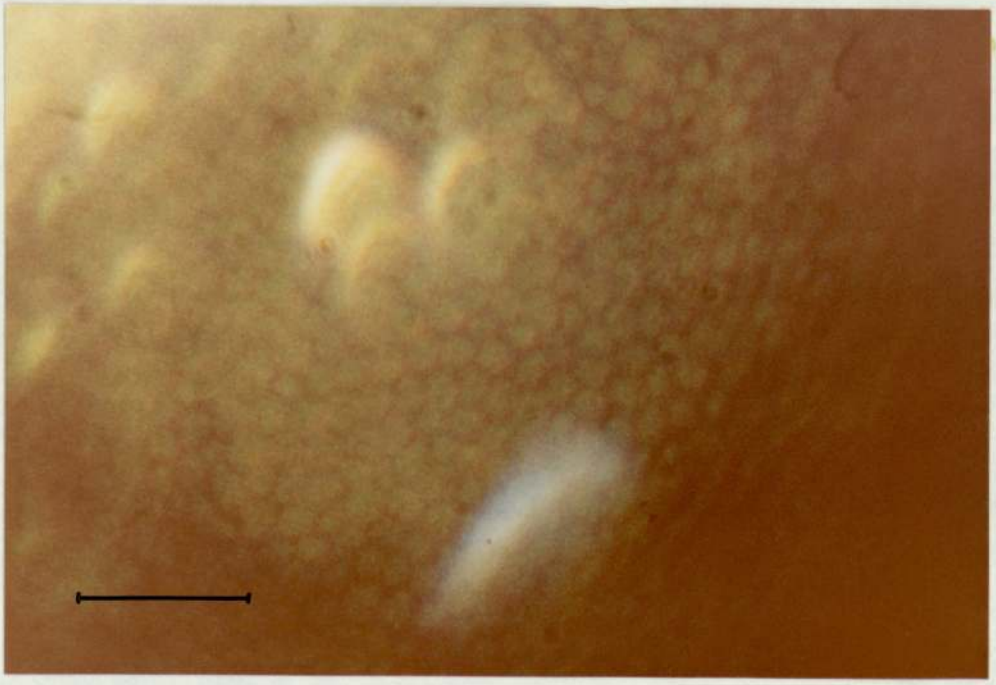


Fig. 4.5 - Corneal endothelium of a contact lens wearer of 20 years of age using 64 ASA film. Bar gauge= 100 microns.

CHAPTER V

1. RESULTS

1. RESULTS

Tables 1 and 2 summarize the results of both control and contact lens subjects. Each subject has been assigned a number which corresponds on the individual case record.

Figure 5.1 and 5.2 shows the frequency distribution of age and endothelial cell densities ($mm.^2$) among the 40 subjects examined. Endothelial cell density distribution ranged from 2452 to 2992 (mid point intervals), representing 75% of the total sample population.

Mean endothelial cell counts in the contact lens wearers group was 2756 $\pm$ 375 (SD) and 2856 $\pm$ 279 (SD) in the control group. The difference between these mean values represented 3.5%. A statistically significant difference appeared at P= 0.20 (1.55>t.2, student's t-test).

Correlations between the results from males and females were made taking 3 groups: considering all subjects together as one group, and with control and contact lens wearers groups separately. There was no significant difference in endothelial cell density (P>0.2, student's t-test).

No significant differences in intra-ocular pressure, corneal thickness or central corneal radius were found between control and contact lens wearers group ($P>0.2$, student's t-test).

A significant negative correlation was found between endothelial cell densities and time of wearing contact lenses ($r=-0.45$, $P<0.05$), see figure 5.3. When the subject of 108 months (9years) and 204 months (17 years) were excluded from the sample, the significance level appeared at $P<0.1$ ($r=-0.36$). Figure 5.4 shows a graphical representation of the changes occurred to the mean cell counts at different periods of wearing contact lenses.

A highly significant positive correlation was found between central corneal radius and cell density among the control group ($r=0.62$, $P<0.002$). Among the contact lens wearers group a positive correlation appeared at $P=0.2$ ($r=0.24$, $P<0.2$), see figure 5.5.

There was no correlation of cell density with corneal thickness either with the control or with the contact lens wearers group ($r=-0.20$, $P>0.25$; $r=-0.014$, $P>0.25$), see figure 5.6.

A slight significant negative correlation was found between intra-ocular pressure and cell density (Fig. 5.7). This significance level was found to be at $P<0.25$ for the control group, and $P=0.2$ for the contact lens wearers group (Pearson $r=-0.19$ and -0.20 respectively).

Table 1

CASE No.	AGE (years)	SEX	MEAN CORNEAL RADIUS (mm.)	CORNEAL THICKNESS (mm.)	IOP(mmHg.)	CELL DENSITY (mm^2.)
1	24	F	7.54	.58	14	2800
2	23	M	8.01	.54	10	2900
3	16	M	7.36	.54	16	3400
4	23	M	7.30	.52	12	3100
5	25	F	8.18	.54	13	2800
6	29	M	8.23	.56	17	2700
7	22	F	8.08	.52	14	2900
8	26	M	8.30	.52	15	2600
9	35	M	7.80	.56	18	2500
10	27	M	7.94	.56	14	2500
11	22	F	7.73	.56	18	2700
12	26	F	7.94	.52	14	2800
13	27	M	7.94	.53	10	2600
14	27	F	8.23	.55	17	2650
15	19	M	7.73	.48	14	3000
16	20	M	7.89	.51	11	3200
17	18	F	7.34	.53	13	3500
18	25	F	7.58	.51	14	2750
19	29	M	7.73	.56	16	2875
20	26	F	7.96	.50	12	2400

Table 2

CASE No.	AGE (yrs)	SEX	MEAN CORNEAL RADIUS (mm.)	CORNEAL THICKNESS (mm.)	IOP(mmHg.)	TIME USING CONTACT LENSES	CELL DENSITY (mm^2.)
1	22	F	7.46	.50	14	3yrs	3000
2	20	F	7.61	.54	14	2yrs	2550
3	21	M	8.23	.48	17	4½yrs	2500
4	20	M	8.44	.58	12	1½yrs	2700
5	24	M	8.13	.52	14	4½yrs	2400
6	25	M	7.99	.60	16	6mths	2600
7	20	F	7.58	.56	15	3½yrs	2600
8	24	M	8.06	.54	14	3yrs	3400
9	32	M	7.76	.54	13	3yrs	3050
10	20	M	7.76	.58	15	1½yrs	2900
11	22	F	7.82	.54	12	1½yrs	2700
12	35	F	7.58	.58	16	17yrs	2250
13	38	F	7.56	.50	16	2yrs	2600
14	21	F	7.71	.54	13	10mths	3600
15	20	M	7.42	.48	16	9mths	3300
16	24	F	8.10	.48	12	3yrs	2400
17	20	F	8.65	.54	14	3½yrs	2350
18	21	M	7.96	.50	15	9yrs	2500
19	21	F	7.91	.49	18	2yrs	2600
20	25	F	8.33	.56	14	3½yrs	3120

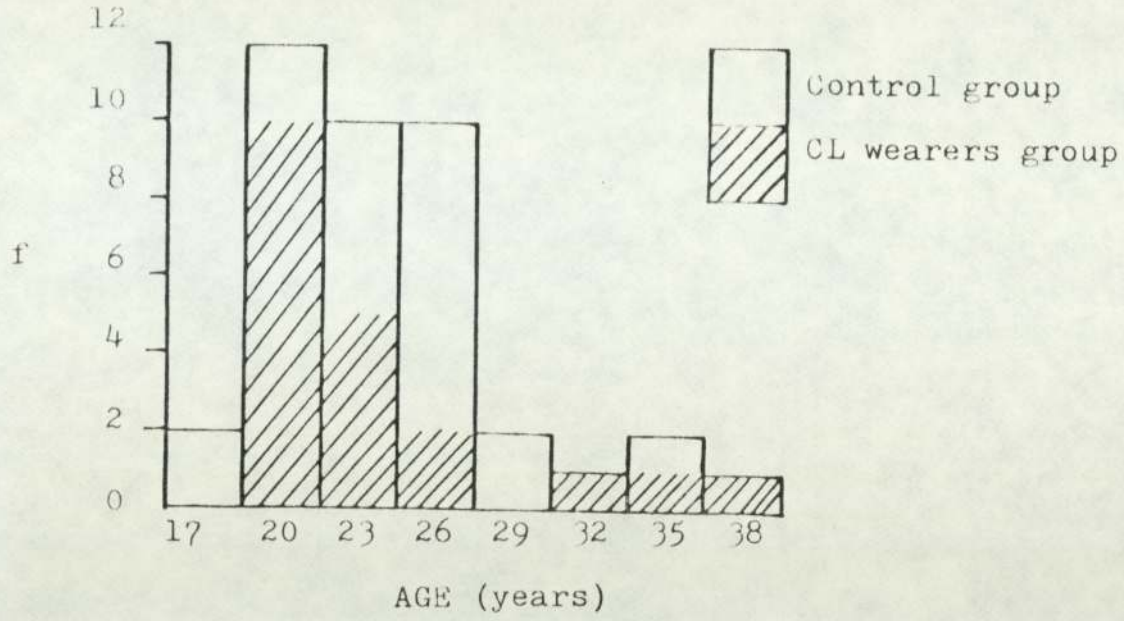


Fig. 5.1 - Frequency distribution of age (mid point class intervals) among the 40 subjects studied.

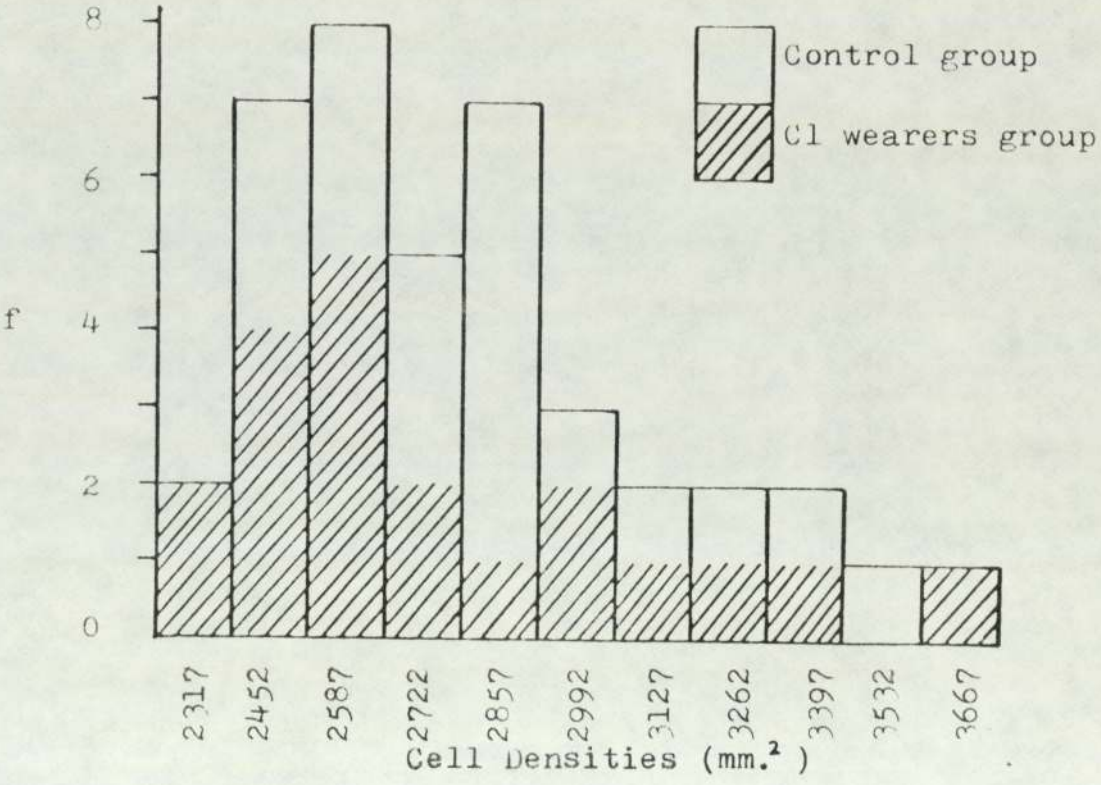


Fig. 5.2 - Frequency distribution of cell densities (mid point class intervals) among the 40 subjects studied. Class intervals on abcissa represent a range of 135.

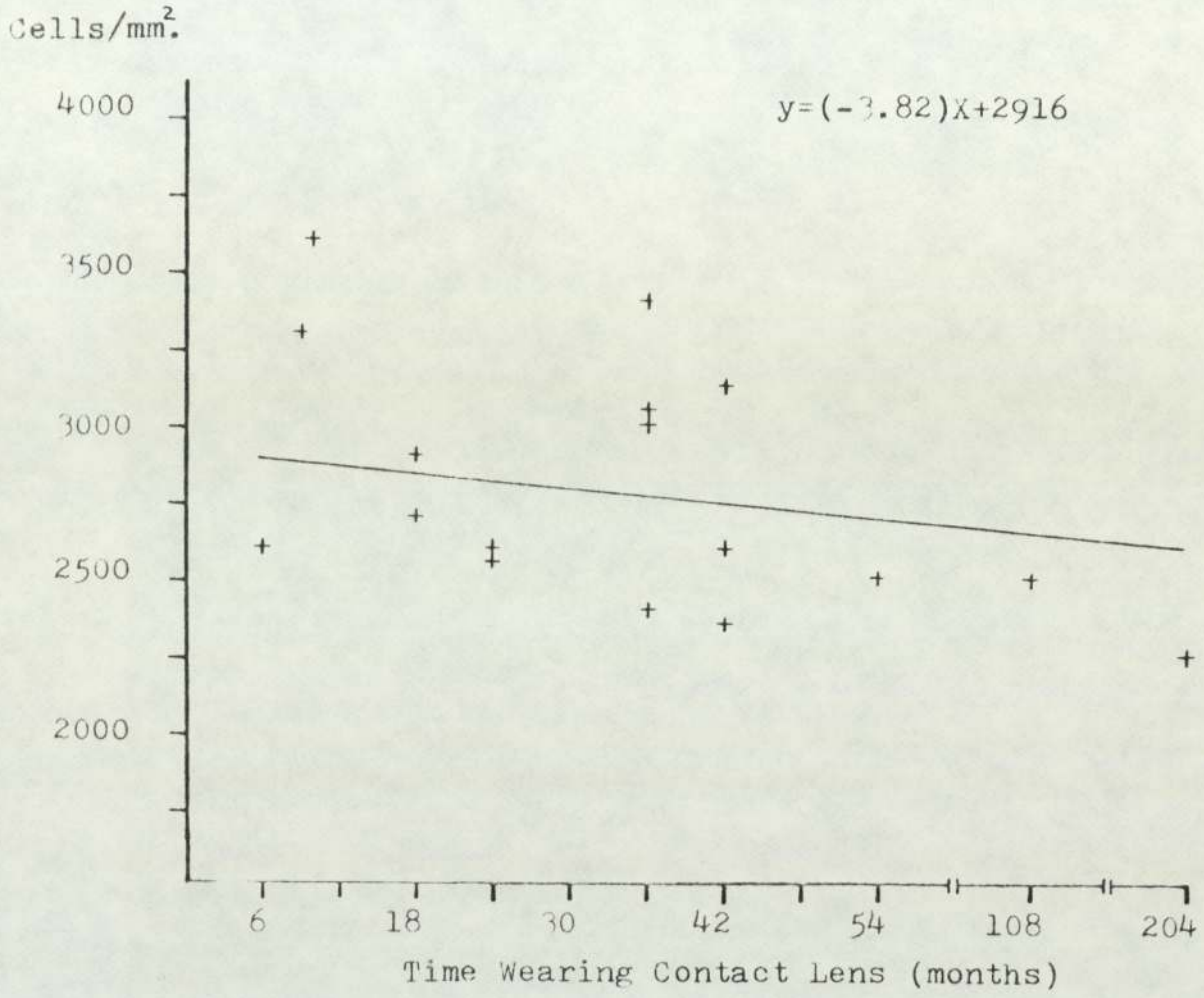


Fig. 5.3 - Endothelial cell density vs. time of wearing contact lens. A significant correlation at $P < 0.005$ level appeared ($r=-0.45$). Excluding subjects of 108 and 204 months the significance was lower ($r=-0.36$, $P < 0.1$).

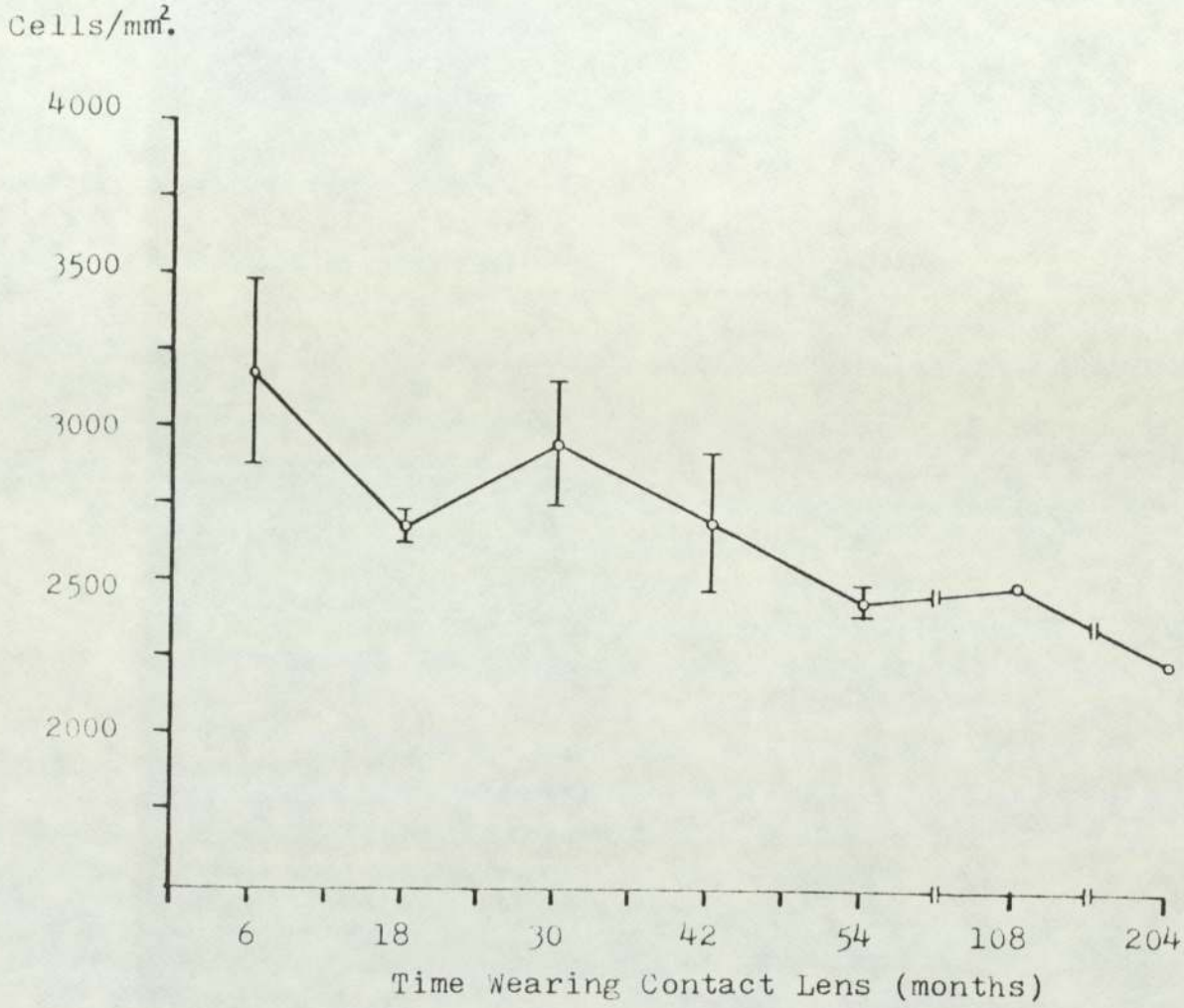


Fig. 5.4 - Changes in cell density with time of wearing contact lens (mid point intervals). Data point represent mean cell density for different groups of subjects (from 6 to 54 months N= 3; 6; 4; 3 and 2 respectively) ± the standard error of the mean.

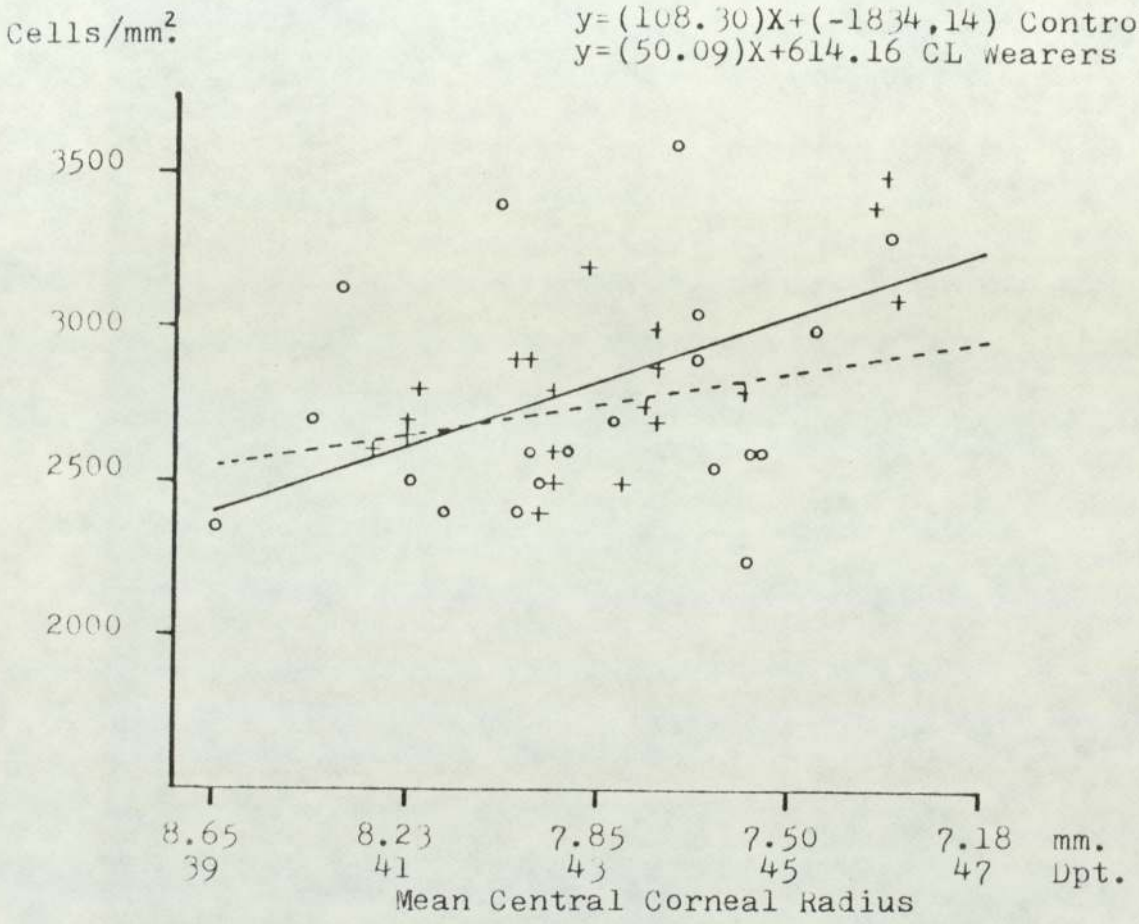


Fig. 5.5 - Cell density vs. mean central corneal radius for both control (cross) and contact lens wearers group (circle). Solid line represents linnear regression for the control group and dotted line for the contact lens wearers group. A significant correlation at $P < 0.002$ level appeared for the control group and only at $P < 0.2$ for the contact lens wearers group.

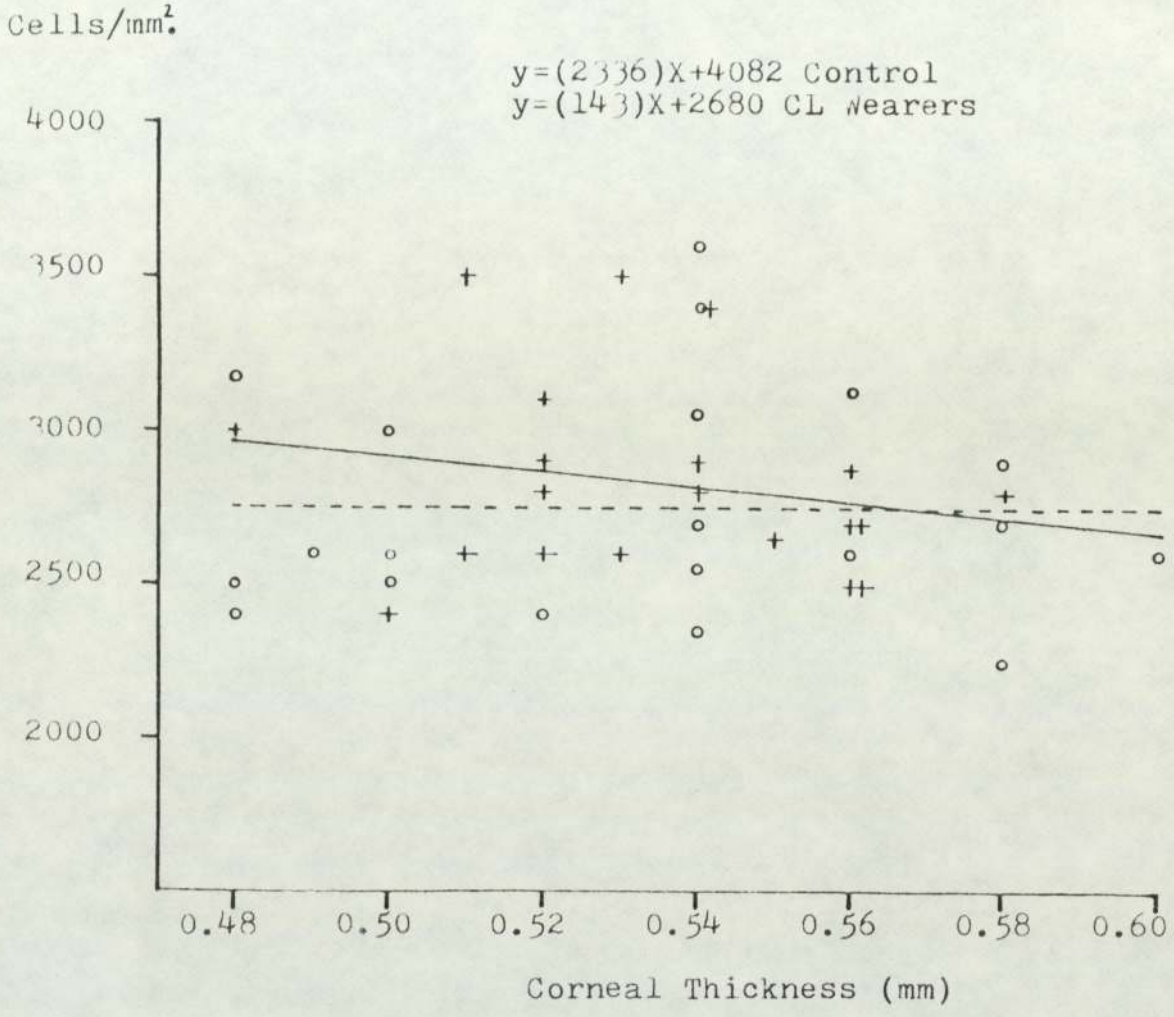


Fig. 5.6 - Cell density vs. central corneal thickness. No correlation was demonstrated for both control (cross) and contact lens wearers group (circle). Solid line represent linnear regression for control group and dotted line for the contact lens wearers group.

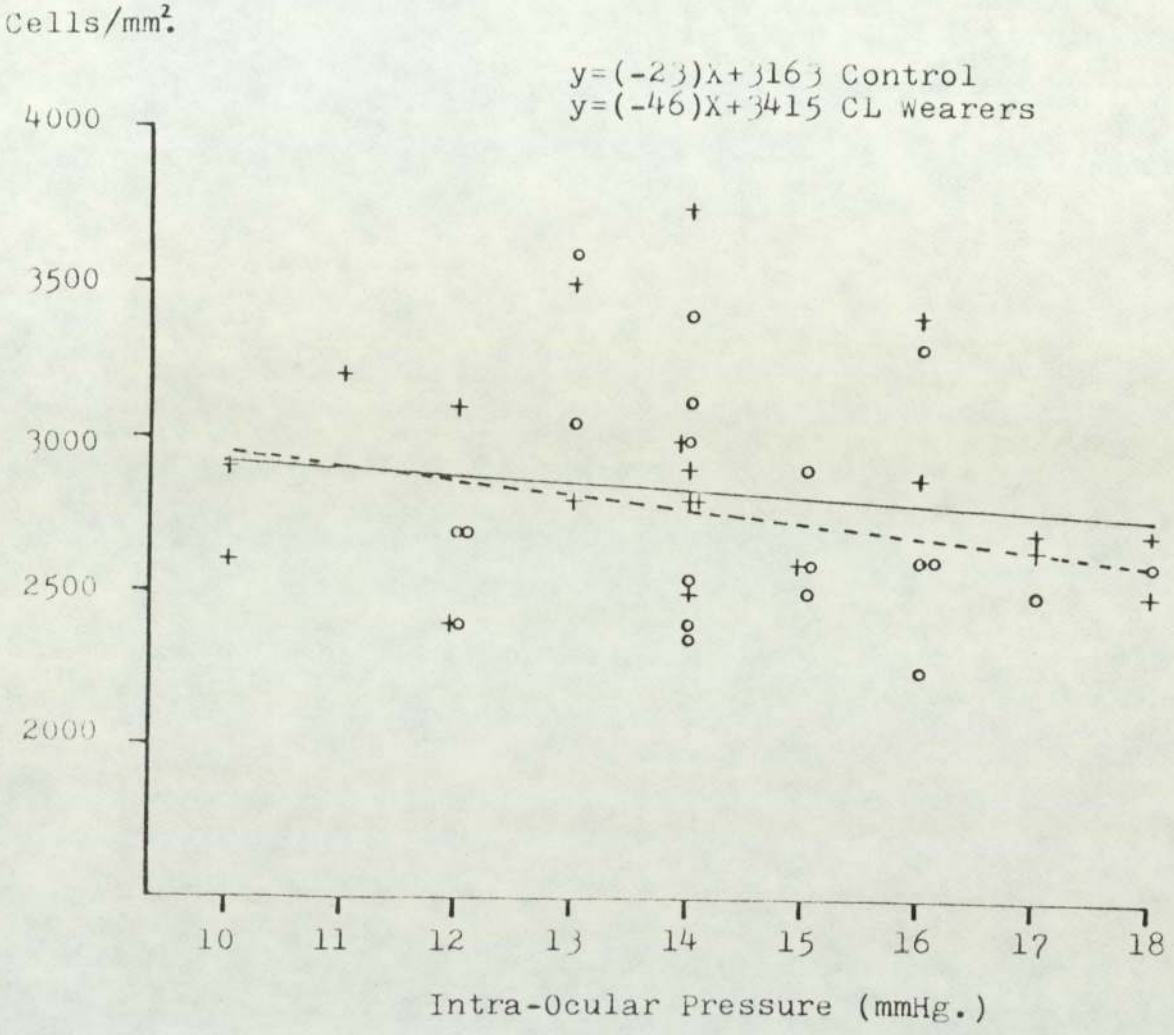


Fig. 5.7 - Cell density vs. intra-ocular pressure. A slight significant correlation was observed at $P < 0.25$ level for the control group (cross) and at $P=0.20$ for the contact lens wearers (circle). Solid line represent linnear regression for the control group and dotted line for the contact lens wearers group.

CHAPTER VI

1. DISCUSSION

1. DISCUSSION

Endothelial response to contact lenses has been described by various authors. Zantos and Holden (1978) noted that transient endothelial changes occurred soon after wearing soft contact lenses. These changes consisted of increased separation between cells and circumscribed black zones (endothelial "blebs") obscuring or displacing the mosaic. Removal of the lenses resulted in a rapid return of endothelial mosaic to a normal appearance. McMonnies and Zantos (1979) found endothelial bedewing in patients who were chronically intolerant to contact lens wear. They noted that recovery of the eye may take several days. Barr and Schoessler (1980) monitored the time course of the endothelial bleb response to hard contact lenses. They noted that the maximum mosaic changes occurred approximately 30 minutes after placing the contact lens, and that the mosaic gradually return to normal in about 4 hours.

In this study a significant negative correlation was observed between cell density and time of wearing contact lenses ($P<0.05$). However, there is some uncertainty arising over the two additional data (108 and 204 months) as to whether they might be discrepant results. This led to speculation that further work, in the

nature of a more extensive and protracted longitudinal study would be necessary for assessing the true rate of decrease in endothelial cell density.

According to the findings a significant difference of 3.5% ($P<0.2$) between the control and contact lens wearers group appeared. This difference suggested that contact lens wearers tend to have a smaller number of endothelial cells than the non-wearers. However, it is believed that if this result is to be considered as conclusively significant, then further work with a larger sample population must be carried out in anticipation of finding a greater level of significance.

An increase in endothelial cell population occurred with mean corneal radius. The highly significant positive correlation ($P<0.002$) for the control group suggested that subjects with small central corneal radii (high dioptric power) would have greater endothelial cell densities. For the contact lens wearers group, this correlation was only slightly significant ($P<0.2$). This low level of significance could be due to the changes in curvature occurring in the cornea due to the contact lens. Changes in corneal curvature through contact lens wear have been noted by previous authors such as Rengstorff (1969); and Brungardt & Potter (1972).

We documented a gradual decrease in endothelial cells with increments in intra-ocular pressure for both control and contact lens wearers groups. Due to the low levels of significance, it would lead one to suggest that more investigations should be made with other samples before considering this finding as conclusively significant.

The effect of intra-ocular pressure on the endothelial cell density has been reported in the past. Irvine and Irvine (1956), noted that in two cases which presented a considerably lower density of cells than the average, had been affected with glaucoma. Setala (1979) noted in patients affected with acute glaucoma, that a rise in intra-ocular pressure lasting at least three days caused a clear decrease in the endothelial cell density.

We found a lack of significant correlation between endothelial cell density and corneal thickness. This is in agreement with the results of Bourne and Kaufman (1976), who did not find any correlation in 40 normal eyes.

No correlation in cell densities between males and females was found. This has been also observed by

Olsen (1979b); Lopes Cardozo (1979); and by Laule et al (1978).

In correlating intra-ocular pressure, corneal thickness and central corneal radius between control and contact lens wearers groups, again, there was no significance.

The photographing technique used in this project, proved to be an efficient way in studying the human corneal endothelium in vivo.

APPENDIX 1

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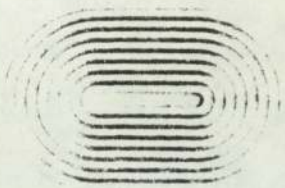


PUBLISHED FORTNIGHTLY

A method of observing and photographing human corneal endothelial cells *in vivo*

J.B. Bellisario-Reyes, A.J. Kempster, A.G. Sabell

Since the introduction of a new specular microscope by Maurice (1968) there has been a major change in the approach to the investigation of the human corneal endothelium *in vivo*. Different techniques have been developed which require no physical contact between the eye and the instrument. A modified slit lamp technique which has been used for observing, photographing, and estimating cell density of the corneal endothelium is described

The importance of the corneal endothelium for the maintenance of normal corneal physiology is well established. Interruption of this cell structure can result in irreversible oedema, leading to loss of corneal transparency.

Vogt[1] in 1920 was the first person to use the slit lamp biomicroscope to observe the corneal endothelium by specular illumination. However, the magnification was insufficient for a detailed study of the cellular morphology. In 1968, Maurice[2] introduced a new specular microscope suitable for photographing the endothelium *in situ* at high magnification. This apparatus consisted essentially of a light source, slit aperture and condensing lens, the slit beam being directed through the objective lens of the microscope onto the cornea by methods long established for metallurgical and petrological microscopy. Modifications of the microscope were made by Laing *et al*[3], Bourne and Kaufman[4], and then by Sherrard[5].

The optical principles of the clinical specular microscope have been well explained elsewhere[6]. The main disadvantage of this technique is that it requires applanation of the cornea with a dipping cone lens and necessitates using a topical anaesthetic in every case.

Bron and Brown[7] in 1974 photographed the human corneal endothelium *in vivo* at high magnification requiring no physical contact with the eye. They used the macrophotography unit constructed by Brown[8] in 1970. The apparatus consisted of a macrophotographic objective fitted to a reflex camera by means of an extension tube, the whole apparatus being mounted on to the eyepiece of a Zeiss photoslit lamp, which provided illumination for viewing, and synchronised the flash. A more successful attempt has been reported by Holm[9] in 1978. The equipment consisted of a specially fitted microscope supplied with an objective of long working distance. The camera was mounted horizontally onto a bracket attached to a photoslit lamp. This first production non-contact specular microscope was made by the Preisler Instrument Company, Sweden, to the instructions of Dr Olle Holm. A similar apparatus with slight modifications was used by Olsen[10]. Laule *et al*[11] used a standard photoslit lamp with the additions for stereophotography. Their endothelial photographs were projected onto glossy white paper, giving a total magnification of 200x. Holden and Zantos[12] also used a standard photoslit lamp to obtain photographs of the corneal endothelium, rephotographing the original slide in order to obtain a sufficiently large magnification to distinguish individual features of the cellular structure.

Materials and methods

A Carl Zeiss (Jena) photoslit lamp with a very simple and cheap modification has been utilised to photograph the corneal endothelium. This basically consists of a reflex camera body which is fitted by an extension tube (85 mm long) to one of the eyepieces of the photoslit lamp (Figures 1A and 1B).

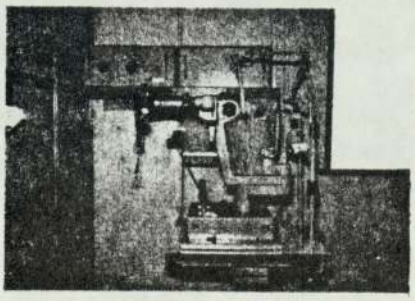


Figure 1A. The Zeiss Jena photoslit lamp with reflex camera attached

Figure 1B. The camera body and extension tube (Olympus L adaptor) fitted to the slit lamp and flash system

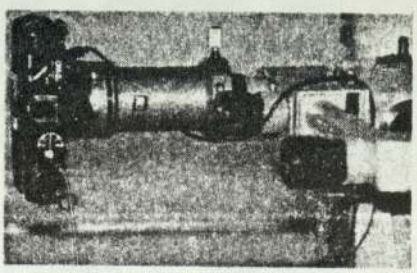
It is necessary to focus the eyepiece so that a clear image can be seen in the viewfinder of the camera. To obtain the specular reflection, a slit beam of medium width should be set at approximately 60 degrees to the microscope camera axis. It is necessary to use maximum viewing illumination and 40x magnification to focus the endothelium critically. By varying the subject's direction of gaze, the specular image can be viewed at different points on the cornea. A small fixation point of approximately 0.75 mm is necessary so that some eye movements and micronystagmus of gaze are reduced.

Photographs can be taken using medium flash intensity and good results have been obtained with Kodak Ektachrome film of both 50 and 64 ASA. The former material is intended for tungsten illumination and results in some colour discrepancy.

The estimation of cell density was made by counting the cells in a photographic field of known area. The surface of a graticule with calibration lines etched on it at 10 micron intervals was photographed using the modified photoslit lamp. This calibrated photograph was then projected onto a screen at a final magnification of 540x; that is, the 10 micron marks were 5.4 mm apart. The endothelial photographs were projected on the same screen at the same magnification and position so that the cells could be counted and an estimation of cells per square millimeter made.

Results

The method described here permitted direct microscopic photography and observation



of the corneal endothelial cell layer (Figures 2A and 2B). This particular method allowed approximately a hundred cells to be recorded on each photograph, and enabled the structural cell formation and density to be seen in good detail.

Discussion

Sufficient magnification and resolution of the endothelial cells is obtained from the original slide, thus avoiding the necessity for further rephotographing and subsequent loss of detail. No special equipment is required other than a standard camera back and extension tube, which are very readily obtainable.

The use of a camera lens is unnecessary, thus reducing the light loss and enabling a slower film to be used in order to improve resolution. This method of observation is particularly easy when a photograph is not required, because the magnification obtained is sufficient to enable individual cell structure to be examined.

Figure 2A. Normal endothelium of a 24-year-old woman using Ektachrome film 50 ASA (artificial light). Bar gauge = 100 microns

Figure 2B. Normal endothelium of a 16-year-old boy using 64 ASA film. Bar gauge = 100 microns

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