To my wife Pat and children Christopher

and Deborah

SOME ASPECTS OF CORNEAL PHYSIOLOGY IN EXTENDED CONTACT LENS WEAR

by

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IN EXTENDED CONTACT LENS WEAR

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SUMMARY

Changes in the physiology of the human in vivo cornea during periods of contact lens wear are manifest in many different ways. The aim of the experimental work described in this thesis was to examine:-

(1) Apparent in vivo human corneal oxygen uptake rates.

(2) Apparent corneal endothelial cell density.

(1) An existing apparatus for the measurement of in vivo corneal oxygen uptake rates was modified to improve the accuracy and repeatability of measurement. Using this apparatus the following aspects of human in vivo corneal oxygen consumption rates were observed:-

- (a) A wide variation in individual oxygen consumption which conformed to a normal distribution.
- (b) A diurnal study which showed no change in oxygen uptake rate throughout the waking hours.
- (c) A change in oxygen uptake rate induced by changes in the fit of one form of soft contact lens.
- (d) A change in the oxygen demand of a non-contact lens wearing eye, induced by the wearing of a contact lens in the contralateral eye.
- (e) A reduction in oxygen consumption rate during a six month period of extended soft contact lens wear.

(2) An existing photo slit lamp technique for corneal endothelial photography was utilised together with a microfilm projection device to enable cell counts to be carried out. The instrumental variability of the system was determined and no change in apparent endothelial cell densities during a six month period of extended contact lens wear was observed.

It is concluded that the wearing of an extended wear soft contact lens causes a change in apparent corneal oxygen uptake rate, but no change in endothelial cell density.

Key Words: Oxygen, Electrode, Oxygen Debt, Endothelium
Cornea

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CHAPTER 1

ANATOMY AND PHYSIOLOGY OF THE CORNEA

1.1. THE GENERAL STRUCTURE AND FUNCTION OF THE CORNEA

The superficial area of the cornea is 1.3cm^2 (Maurice, 1969). It has a shorter radius of curvature than the remainder of the eye and consequently protrudes from the globe. The annulus so formed between the cornea and surrounding sclera is termed the limbus or sulcus sclerae. When viewed anteriorly, the human cornea is seen to be elliptical. The long meridian lies close to the horizontal and is of average length 11.7mm., whilst the shorter vertical meridian is approximately 10.5mm. (Edelhauser et al. 1979; Hogan, et al. 1971).

The elliptical configuration is due to an anterior extension of the opaque sclera both superiorly and inferiorly. When viewed from the posterior surface the circumference of the cornea appears circular. The central thickness of the human cornea is approximately 0.52mm. increasing to approximately 0.7mm. at the limbus (Maurice and Giardini, 1951(a); Von Bahr, 1948; Mishima and Hedbys 1968; Martola and Baum, 1968). A meridional difference in peripheral corneal thickness has been reported (Hirji and Larke, 1979).

The cornea and sclera form the tough outer protective coat of the eye which encloses the ocular tissues. The cornea is transparent, avascular and does not possess lymph vessels or other channels for bulk fluid flow. Most of the corneal nutrients are received from the

aqueous humour and pre-corneal tear film, and pass across the corneal limiting layers.

Some nutrients enter the peripheral cornea from the peri-corneal blood vessels, but supply only the peripheral cornea (Votockova et al. 1966).

The cornea is composed of five structurally distinct layers. (Figure 1.1).

Epithelium
Bowman's Layer
Stroma
Descemet's Membrane
Endothelium

1.1.1. THE EPITHELIUM

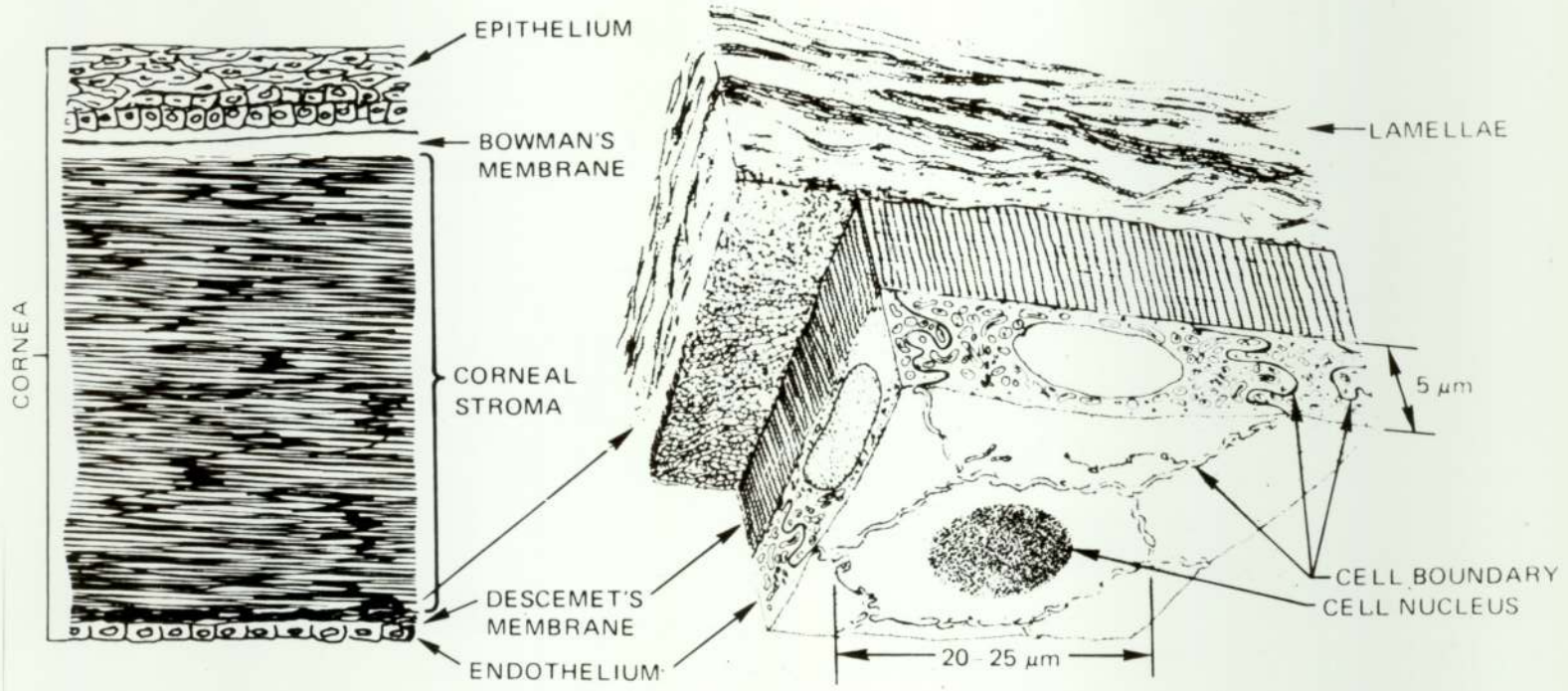
The epithelium occupies about one-tenth of the total corneal thickness. It is composed of five to seven superimposed layers of cells of total thickness between 50u - 90u. (Tripathi and Tripathi, 1972). More recently epithelial thickness has been measured on the in vivo human cornea using a micropachometer. An average value of 62 lu. has been reported (Wilson et al. 1980).

Three groups of cells are identifiable in the epithelium. The innermost layer is formed by a single row of basal cells resting on a basement membrane. The cells are polygonal in shape and measure approximately 18u high, (Hogan et al. 1971; Fine and Yanoff, 1979). The cyto-plasm of the basal cells contains a high concentration

Figure 1.1.

Cross Section of the Cornea

After Knight et al 1978



After Knight, P.M., Link, W.J., Kaufman, H.E. - The Corneal Endothelium, Jan. 1978, Heyer Schulte Medical Optics Centre.

of diffusely distributed tonofilaments, some of which are reported to be actin filaments (Gipson and Anderson, 1977). They are mostly orientated perpendicular to the corneal surface and measure less than 80^{o}A in diameter. Virchow (1910) described intensely staining cells among the basal cells, and their number is reported to increase in some corneal disorders (Tripathi and Garner, 1972). However, Perera (1969) has suggested that the apparently intensely stained cells are fixation artefacts and that the basal cells are of one type only.

Redslob (1935), in a study on embryo chickens and foetal human cornea, demonstrated the presence of a basement membrane under the basal cells of the epithelium. This has been confirmed in the adult human eye (Loewenstein, 1940; Busacca, 1949).

The basement membrane is 300^{o}A to 500^{o}A thick (Tripathi and Tripathi, 1972). It is composed of a lipid layer anteriorly and a reticular fibre meshwork posteriorly. (Teng, 1962). Hemi-desmosomes are scattered along the basal cell membrane, fibrils radiate from them across the space between the basal cell membrane and the basement membrane, attaching the two layers (Pedler, 1962; Jakus, 1961 and Teng, 1962).

The basal cells are characterised by a paucity of cell organelles and these almost disappear as the cells migrate anteriorly to the surface. The mitochondria are filamentous

and small (Kaye and Pappas, 1962; Teng, 1961). The Golgi apparatus however is well developed (Payrau et al. 1967). The chondriosomes normally lie in a perinuclear cap around the basal aspect of the nucleus (Pedler, 1962).

The basal cell layer is a germinal layer. Basal cells mitose and migrate forward to form the wing cell layer, the superficial layer, and are finally desquamated into the tear film. The cell turnover time for the epithelium has been reported to range between 3½ to 7 days (Hanna and O'Brien, 1960; Hanna et al. 1961; Bertalanffy and Lau, 1962), although the rate of mitosis can be inhibited by a number of factors (Friedenwald and Buschke, 1944).

The mid-epithelial zone comprises two to three layers of polygonal-shaped cells whose nuclei lie parallel to the corneal surface. The anterior cell surface is convex and the posterior surface concave. Their lateral extensions are thin and wing-like, giving rise to their name of wing cells. The more superficial the cell's position, the flatter is its appearance. The cytoplasm of the wing cells contains diffusely distributed tonofibrils, a limited number of small thin mitochondria and golgi apparatuses which are more apparent than in the basal cell layer. Wing cells migrate forward to form the outer superficial layers of the epithelium.

This superficial zone consists of two cell layers made up of flat, broad squamous cells, 45u in length and approximately 4u in thickness

(Hogan, et al. 1971). The cytoplasm of the surface cells is characterised by well developed golgi apparatuses. Membrane-bound vesicles are often observed to be fused with the cell membrane and opening into an intercellular space. The superficial cells are eventually sloughed off; they break up and disintegrate on the surface. Occasionally, wing cells can be observed at the anterior surface, which Teng (1962) attributed to the recent loss of some squamous superficial cells. He suggested that the anterior corneal surface presents different appearances dependent upon which stage in the desquamating process the observation is made.

On the outermost cellular surface the cell membrane forms many projections. Pedler (1962) considered these to be the remaining components of desmosomes. Sheldon (1956), Payrau, et al. (1967) and Sugitta (1976) considered them to be microvilli, whilst stereoscan electron microscopy reveals the appearance of microplicae (Blumcke and Morgenroth, 1967).

Pedler (1962) suggested that the microprojections retain the pre-corneal tear film on the cornea by the formation of fluid menisci between adjacent protrusions. Ruskell (1972) was unable to observe microprojections on the apex of monkey cornea which nevertheless maintains a normal tear film. Doubt about this theory of fluid retention was expressed by Maurice and Riley (1968) and Maurice (1969) because of the small size of the projections

compared to the thickness of the tear film, reported by Mishima (1965) as 6.5u.

1.1.2. BOWMAN'S LAYER

This layer was formerly considered to be a separate corneal membrane. It is however not sharply demarcated from the stroma and the term layer rather than membrane is preferred (McTigue, 1967).

Bowman's layer consists of collagen fibrils closely but randomly distributed. The diameter of the fibrils is between 160^{o}A to 240^{o}A (Jakus, 1961), although Kayes and Holmberg (1960) found them to measure 260^{o}A to 360^{o}A and Hogan et al. (1971) 240^{o}A to 270^{o}A. Bowman's layer is 9u to 14u thick, and almost totally acellular (Kayes and Holmberg, 1960; Tripathi and Tripathi, 1972).

1.1.3. THE STROMA

The stroma constitutes nine tenths of the total thickness of the cornea. It consists almost entirely of collagenous lamellae which lie parallel to the corneal surface. Each lamella is made up of collagen fibrils. Jakus (1961) reported their diameter to fall within the range 160^{o}A to 240^{o}A in Bowman's layer, gradually increasing to 275^{o}A to 400^{o}A. The fibrils within each lamella lie parallel to each other (McTigue, 1967) and extend across the whole cornea from limbus to limbus (Virchow, 1910; Smelser

and Ozanics, 1965). Each lamella ranges in width between 9u to 260u and is between 1.5u to 2.5u thick (Salzmann, 1912). The cornea contains about 200 to 250 lamella which are superimposed on each other.

The collagen fibrils are embedded in a ground substance composed primarily of three glycosaminoglycans, keratin sulphate, chondroitin sulphate and chondroitin. Water composes some 78% of stromal volume (Maurice and Riley 1968).

The majority of stromal cells are fixed cells or keratocytes which are modified fibrocytes. They are flattened in the plane of the cornea. Jakus (1961) described them as lying within, rather than between, the collagen lamellae, an observation confirmed by Smelser and Ozanics (1965). Goldman et al. (1968) and McTigue (1965) stated that in man, they can lie within or between lamellae in the anterior stroma, but only between them in the more posterior layers. Maurice (1969) stated that in human cornea, cells make up 2.4% of the total tissue volume. Otori (1967) found similar values for the rabbit. Polymorphonuclear leucocytes are also found within the tissue, but in considerably smaller numbers than the keratocytes.

1.1.4. DESCEMET'S MEMBRANE

Descemet's membrane forms the basement membrane of the corneal endothelium. The thickness at birth of 3u to 4u increases to between 10u and 12u in adult life. Electron

microscopy has revealed that the membrane is formed of a number of very regularly arranged stratified layers. (Jakus, 1956, 1961; Feeney and Garron, 1961; Kaye and Pappas, 1962). The membrane manifests periodic thickenings which bulge posteriorly towards the anterior chamber. These protrusions are termed Hasall-Henle warts. Descemet's membrane is restricted to the posterior surface of the stroma and is thought to be secreted by the endothelial cells throughout life (Hogan et al. 1971; Cotlier, 1975).

1.1.5. THE ENDOTHELIUM

The corneal endothelium comprises a single layer of cells, mainly hexogonal in shape and approximately 5u deep and 18 to 20u wide. Modified desmosomes form its attachment to the overlying Descemet's membrane. The nuclei are large flat and oval, measuring approximately 7u in diameter (Hogan et al. 1971). The cytoplasm possesses the organelles characteristic of cells engaged in active transport and protein synthesis for secretion (Knight et al. 1977). Wolf (1969) demonstrated microvilli on the posterior cell surface and Blumcke and Morgenroth (1967) confirmed the observation using the scanning electron microscope. Each cornea has between 300,000 - 500,000 endothelial cells covering an area of approximately 100mm.^{2} (Svedbergh and Bill, 1972).

The lateral cell membranes, combined with that of the adjacent cell, forms an intercellular border (Jakus,

1961, 1962; Kayes and Holmberg, 1964; Speakman, 1959). The posterior end of the intercellular borders forms a terminal bar (Jakus, 1962; Kayes and Holmberg, 1964; Kaye and Pappas, 1962; Kaye et al. 1962; Donn et al. 1961).

A thin polysaccharide coat on the posterior surface of the endothelium has been described (Kaye and Pappas, 1962). More recently,Schroder and Sperling (1977) and Jacobsen and Sperling (1978) have described the binding of ruthenium-red-osmium tetoxide to a material 60mm. to 150mm. thick on the inner surface of the endothelium. It is believed this layer may be involved in fluid movement across the posterior corneal surface.

In order to maintain its transparency, the cornea must be constantly hypohydrated; an active transport system located in the endothelium has been postulated (Mishima and Kudo, 1967; Dikstein and Maurice, 1972). The endothelium functions as a barrier against an inflow of water and dissolved substances from the aqueous humour into the stroma, and was one of the first transporting tissues on which correlated morphological and physiological studies were made (Donn et al. 1961; Kaye and Pappas, 1962; Kaye et al. 1962). In view of their possible role in fluid and particulate movement across the endothelial layer, the intercellular spaces have been subjected to numerous investigations. Early studies of the Terminal Bar (Junctional Zone) of the rabbit corneal endothelium showed the junction to be continuous in three dimensions

Donn et al. 1961; Kaye and Pappas 1962; Kaye et al. 1962). It was not determined if the junction was a "tight" junction with a zonula occludens (Farquhar and Palade 1963) in which actual fusion of the outer elements of the plasma membrane occurred, or whether the junction was a narrowing of the intercellular space filled with a material of moderate density. Leuenberger (1973) considered that the junctions in rat endothelium should not be considered as "Tight" junctions, but as gap junctions with gaps of approximately 30^{o}A between two apposed cell membranes.

The role of the endothelium in the control of corneal hydration is discussed in 1.5..

1.1.6. PRE-CORNEAL TEAR FILM

In the open eye the tear film is approximately 10u thick (Holly, 1980). It has a complex trilaminar structure. The outer layer consists of a thin non-polar lipid layer derived from the Meibomium glands, which is capable of reducing evaporation from the middle aqueous layer (Iwata et al. 1969). This layer is also believed to protect the tear film from highly polar skin lipids from the surrounding palpebral aperture (Holly, 1973). Nine-tenths of the total tear film thickness is formed by the aqueous layer, derived predominantly from the main and accessory lacrimal glands.

The posterior mucoid layer originates from the conjunctival goblet cells. It coats the anterior surface of the

Figure 1.2

Cross Section of the Pre Corneal Tear Film.

After Holly, 1980.

SUPERFICIAL LIPID LAYER ~0.1μm

consisting mainly of waxy and cholesteryl esters and some polar lipids

AQUEOUS LAYER ~7μm

containing in dissolved form inorganic salts glucose urea and surface active biopolymers proteins and glycoproteins

microvilli

MUCUS LAYER ~0.02-0.05μm

a hydrated layer of mucoproteins rich in sialomucin

corneal epithelial cells enhancing surface wettability and preventing lipid contamination, thus contributing to the stability of the tear film (Holly and Lemp, 1977; Holly et al. 1977). (Figure 1.2).

1.2. SWELLING PRESSURE

The ability of excised cornea to swell when placed in aqueous solutions has long been known (Chevreuil, 1821; Donders, 1857; Schweigger-Seidel, 1869). The greater component in such swelling is the corneal stroma. Payrau et al. (1967) demonstrated that the corneal stroma of most vertebrates including mammals, birds and teleost fishes will imbibe fluid when given free access.

Pieces of whole cat cornea swell up to 5.5 times by weight when placed in water (Kinsey and Cogan, 1942). Human cornea swells to a lesser extent; an upper limit of 2.5 times its initial weight has been demonstrated (Ehlers, 1966).

The swelling pressure developed by the corneal stroma has been described by many authors; Cogan and Kinsey, 1942(b); Dohlman and Anseth, 1957; Pau, 1954; Hedbys and Dohlman, 1963; Hedbys et al. 1963; Fatt, 1968; Fatt and Hedbys, 1970; Klyce et al. 1971. This pressure drops rapidly as the stroma swells and increases as fluid is forced out. The rate of stromal swelling from imbibed fluid across its surfaces can be predicted from the value of the swelling pressure and resistance to the flow of

fluid offered by the tissue (Fatt and Goldstick, 1965).

Swelling takes place uniformly across the thickness of the stroma, except possibly in the anterior irregular layers (Kikkawa and Hirayama, 1970).

The two sites for stromal expansion are the collagen fibrils and the ground substance surrounding them. Aurell and Holmgreen (1953) suggested the former, although much evidence now suggests that expansion occurs in the ground substance. Heringa et al. (1940) and Woodin (1954) demonstrated a reduction in the water-binding capacity of the stroma following extraction of stromal mucoids. Cetylpyridinium chloride precipitation of stromal acid glycosaminoglycans similarly reduces the swelling pressure as does digestion by hyaluronidase (Hedbys, 1961; Green et al. 1976). Francois et al. (1954) found no difference in the diameter of collagen fibrils from normal and swollen corneae and Maurice (1957) in refraction studies provided further evidence to suggest swelling occurs in the interfibrillar substance. No change in fibril hydration was observed with changing corneal hydration.

1.2.1. WATER BINDING

The cation content of rabbit whole cornea has been studied by many authors (Harris and Nordquist, 1955; Harris et al. 1956; Langham and Taylor, 1956; Harris, 1960; Hara, 1966; Davson, 1949). Stromal interstitial fluid is hypertonic to both aqueous humour and plasma (Otori, 1967). Mishima

and Hedbys (1967) calculated that the stroma should be hypertonic to the aqueous as a result of normal evaporation from the eye. The binding of stromal extracellular fluid has been discussed by several authors. Davson (1949) suggested that the stromal cellular fluid is in Donnan equilibrium with the aqueous humour. However, this requires the chloride ion concentration in the stroma to be less than that in the aqueous humour. Otori (1967) demonstrated stromal and aqueous chloride ion concentration to be almost equal. These results were in good agreement with previous investigators (Kinsey, 1951; Langham and Taylor, 1956; Bito and Davson, 1964; Kinsey, 1967). Otori (1967) favoured a stronger binding force and Friedman and Green (1971) suggested the Donnan contribution to the swelling pressure to depend on the molecular organisation of the stromal ground substance.

The corneal stroma is rich in acid glycosaminoglycans and protein (Laurent and Anseth, 1961; Anseth and Laurent 1961; Maurice, 1962). It is suggested that cations bind to the negative charges on the glycosaminoglycans giving rise to the cation excess found in the stromal extracellular fluid. Interfibrillar swelling possibly develops as a result of mutual repulsion of these negative charges. The cation excess must be maintained, as changes in the ion concentration of the stroma will affect stromal swelling and hydration (Otori, 1967; Hodson, 1971(a)); and hydration and thickness of the stroma show a linear relationship to one another over a given range (Hedbys and Mishima, 1966).

An increase in hydration is accompanied by an increase in stromal thickness and a situation is eventually reached when light passing through the stroma is scattered (Maurice, 1957).

1.3. CORNEAL TRANSPARENCY

Prior to the advent of the electron microscope it was suggested that the transparency of the cornea resulted from a uniformity of its refractive index. However, the measured refractive index of corneal collagen is 1.47 whilst that of the ground substance is 1.34 (Maurice, 1957). Maurice (1957) proposed that the stromal fibrils are arranged such that they form a series of diffraction gratings. This would promote the directional constructive interference along the path of the incident light. Scattered light in other directions would be eliminated by destructive interference. This theory requires the separation of stromal collagen fibrils to be less than the wavelength of light.

Goldman et al. (1968) described "lakes" in the stroma of dimensions exceeding $1\frac{1}{2}$ wavelengths which occurred both within and between lamellae. It was suggested that these lakes represented the inhomogeneity in refractive index which gives rise to the opaqueness of swollen cornea. The lattice theory of corneal transparency has been questioned. Smith (1969) described a model which is essentially based on the uniform refractive index theory.

However, the lattice theory is widely accepted as the most reasonable explanation of stromal transparency at the present time, and subsequent theoretical studies have confirmed the contention that transparency depends on an ordered arrangement of collagen fibrils (Cox et al. 1970; Benedik, 1971; Farrell et al. 1973). The reduction of transparency following an increase in corneal hydration can be explained in terms of the lattice theory. The excess fluid may alter the regularity of the collagen fibrillar spacing, thus affecting their function as diffraction gratings and increasing the amount of scattered light.

1.4. CORNEAL PERMEABILITY

Intactness of epithelium and endothelium is essential for the maintenance of normal corneal hydration; rapid swelling of the stroma occurs when these limiting membranes are damaged (Maurice and Giardini, 1951(b)).

In understanding the role of these limiting layers in the maintenance of normal hydration, it is important to know their permeability characteristics.

The permeability of the cornea may enter into the normal physiology of the tissue in three aspects.

a) The supply of metabolites to its composite layers.
b) The barrier the limiting layers oppose to

the achievement of a physico-chemical equilibrium between the stroma and fluids external to the cornea.

c) The mechanisms for maintaining stromal hydration.

The water permeability of the epithelium has been studied (Stanley and Winston-Salem, 1972; Stanley et al. 1966; Mishima and Hedbys 1967; Green and Green, 1969; Donn et al. 1963). The endothelium is also permeable to water, (Stanley and Winston Salem, 1972; Rhee et al, 1971; Stanley et al.1966; Mishima and Hedbys, 1967; Green and Green, 1969; Mishima, 1975) and has a permeability valuer greater than that of the epithelium (Green and Green, 1969).

The permeability of Descemet's membrane has been described by Fatt, (1969).

The concept of membrane permeability to water involves two possible mechanisms of water movement.

1. The diffusional exchange of water molecules (Cogan and Kinsey 1942(a); Donn et al. 1963).

2. The net volume flow of water driven by osmotic or hydrostatic pressure (Cogan and Kinsey, 1942(a), 1942(b).

Von Bahr (1956; 1962) suggested that both corneal limiting

layers act as semi-permeable membranes. A net volume flow of water through them could be created by an osmotic pressure gradient.

The epithelium offers high resistance to the movement of all ionic and other fat insoluble substances (Maurice, 1953; Cogan et al. 1944). It is probable that these substances penetrate the epithelium by the intercellular spaces. The outer cellular layer should offer the greatest resistance to movement since the flattened cell shape will increase the path distance from one cell face to the next and reduce the total length of the cell borders on the surface (Maurice, 1969). Substances which are soluble in both fat, as well as in water, penetrate the epithelium rapidly (Swan and White, 1942). Such substances are soluble in the cell membranes and pass directly across the cell layer.

The endothelium is far more permeable to ions than the epithelium (Maurice, 1951, 1955, 1961). The obstruction offered by the endothelium depends upon the solute particle size and is little affected by the charge or temperature (Sery et al. 1962; Maurice and Watson, 1965; Sery and Nagy, 1967; Mishima and Trenberth, 1968). The movement of sugars across the endothelium is an exception. It is a rapid energy-dependent movement (Hale and Maurice, 1969), for which a special transport mechanism has been postulated (Czaky, 1965).

The barrier formed by the posterior corneal surface is almost entirely due to the endothelium. Descemet's membrane provides a negligible resistance (Maurice, 1961) and large molecules can pass it freely (Donn et al. 1963).

The observation that the endothelium offers obstruction dependent on particle size, which is independent of charge and temperature suggests that the greater part of the penetration is through inert pores which could correspond to the intercellular spaces (Maurice, 1953, 1961). Hodson (1968) places doubt on the intercellular spaces as being the only pathway of solute movement due to the presence of zonular occludens. It is suggested that some fluid flow takes place through the cells.

1.5. CONTROL OF HYDRATION AND THICKNESS

The first quantitative investigation of corneal swelling following the removal of its limiting layers was that of Leber (1873, 1903). He concluded that the ability of the cornea not to swell in the normal living eye was due to a high degree of impermeability of its limiting layers and thus the stroma was denied access to fluids. Cogan and Kinsey (1942(b)) attributed the deturgesced state of the normal stroma to be due to osmotic forces, and suggested the stromal tendency to imbibe fluid was balanced by the osmotic withdrawal of fluid into the supposedly hypertonic aqueous humour and tears. They appreciated that only impermeability to salts was necessary; that is, the limiting

layers should act as perfectly semipermeable membranes. Bock and Maumenee (1953) demonstrated that deturgescence of the corneal stroma was not solely dependent on osmotic forces. Salts would penetrate the endothelium if bathed in a hypertonic aqueous, allowing the stroma to swell. Such salts would not be free to leave the tissue fluid (Davson, 1949; Maurice, 1951). A hypertonic tear film could maintain thickness by continually withdrawing fluid from the stroma (Green and Downs, 1973).

Such a hypothesis assumes the tears to be hypertonic and implies that tears which are isotonic or hypotonic would cause swelling. However, the cornea maintains normal thickness when bathed in solution isotonic to blood and shows only limited swelling when hypotonic solutions are used (Von Bahr, 1956; Mishima and Maurice, 1961), although more recently a marked increase in thickness has been reported (Kempster and Larke 1978). Such an osmotic mechanism clearly is not employed to regulate corneal hydration.

From the properties of the stroma and the limiting membrane it is to be expected that the cornea should imbibe fluid continuously even when the cellular layers are intact. The in vitro cornea swells at low temperatures although it can maintain its thickness under similar conditions at body temperature. This suggests that thickness is controlled by the active removal of fluid that leaks into the stroma. The demonstration of such an active process requires evidence that fluid is transported out of the stroma, and that this is linked to the metabolic activity of the cornea.

It is insufficient to demonstrate that the tissue swells as the rate of swelling increases when corneal metabolism is interfered with, for this could result from a decrease in the passive resistance of the limiting layers to water movement. The cornea in vitro will swell when metabolism is inhibited by refrigeration. It will return to its normal thickness when subsequently incubated at physiological temperature. This phenomenon known as the temperature reversal effect, has been demonstrated by several authors (Davson, 1955; Harris and Nordquist, 1955; Langham and Taylor, 1956; Donn et al.1959(a); Mishima and Kudo, 1967; Dikstein and Maurice, 1972; Kikkawa, 1969).

It is suggested that an active process located in the cornea is instrumental in maintaining corneal deturgescence and therefore transparency. This view was originally postulated by Davson (1949). Dehydration does not take place in the presence of metabolic inhibitors (Philpot, 1955; Langham and Taylor, 1956; Brown and Hedbys, 1965), or in the absence of oxygen (Harris and Nordquist, 1955), which suggests that it is due to cellular activity.

The exact location, within the cornea, of a site for such active transport has been extensively investigated. Four possible sites have been postulated.

1. Limbus
2. Stroma
3. Epithelium
4. Endothelium

Since temperature reversal can occur in excised cornea (Donn et al.1959(a); Mishima and Kudo, 1967) the limbus cannot be considered as a possible site of the active process. Maurice (1969) states that if the site were stromal, contraction internally of the stromal tissue would be required to force fluid out. Dohlman and Anseth (1957) could not demonstrate a change in the swelling pressure of freshly excised stroma incubated in a physiological medium at $4^{o}C$ or $37^{o}C$, and the presence of a full imbibition pressure in isolated stroma can be established within several minutes of its excision from the body (Hara and Maurice, 1969).

By exclusion, the active pump resides in either the corneal epithelium or endothelium.

The first membrane to be implicated was the epithelium. A transcorneal potential has been demonstrated (Donn et al. 1959(a),(b);Ehlers and Ehlers, 1968; Friedman and Kupfer, 1960; Green, 1965,1966(a); Kikkawa,1966(a); Maurice, 1967(a); Modrell and Potts, 1959; Fee and Edelhauser, 1970). This was shown by Donn et al. 1959(a)) to be the result of an active transport of sodium ions inwards across the corneal epithelium. Damage to the corneal epithelium causes an almost total depletion of the trans corneal potential (Friedman and Kupfer, 1960; Potts and Modrell, 1957; Donn et al. 1959(a). It is suggested that such a potential is generated in the most superficial layers of the epithelium (Maurice, 1967). Green, 1966(b), 1968,1969(a),(b), 1970) and Friedman and Green,(1971) have suggested a mechanism of corneal hydration based on an epithelial sodium pump

whereby the concentration of sodium in the stroma is increased. As a consequence, the amount of sodium bound by the stromal glycosaminoglycans is also increased and the attraction of the polyanion for water decreased, possibly as a result of a conformational change.

However, Mishima and Kudo (1967) demonstrated that the cornea can maintain constant normal thickness for a considerable length of time in the absence of the epithelium. They further demonstrated that the epithelium was not necessary for temperature reversal of the thickness to take place. Riley (1971) and Dikstein and Maurice (1972) have further demonstrated that active transport across the epithelium is not essential for a deturgesced cornea. Active transport of chloride ions out of the cornea into the tears has been demonstrated (Van der Hayden et al. 1975; Kylce, 1975; Zadunaisky, 1966; Ploth and Hogben, 1967). Green (1965) claims that there is also a chloride component in the epithelial transport mechanism of the rabbit, although this opinion was questioned (Maurice and Riley, 1970).

Klyce (1977) has shown that the chloride ion flux can thin a swollen cornea, when the endothelial surface is sealed, by transporting water out of the cornea. The amount of water moved is small compared to that moved by the endothelium. The exact nature of the active secretion by the epithelium is at present still uncertain. Evidence supporting the location of an active transport system in the endothelium has been put forward (Mishima,

and Kudo, 1967; Trenberth and Mishima, 1968; Maurice, 1972; Dikstein and Maurice, 1972). The nature of the observed fluid movement remained obscure. Initial attempts to detect any electrical activity associated with a pump were unsuccessful, (Potts and Modrell, 1957; Donn et al. 1959(b);Friedman and Kupfer, 1960); and a potential difference across the endothelium had not been consistently detected (Kikkawa,1966(a),(b);Green, 1967; Hodson, 1971(b).

Maurice (1951, 1969) suggested that such a potential need only be in the order of 1mV if it was directly associated with an active transport of the sodium ion. A potential of this magnitude has been demonstrated; aqueous side negative (Fischbarg,1972(a),(b);Barfort and Maurice, 1972, 1974; Fischbarg, 1973; Fischbarg and Lim, 1973; Hodson, 1974). This potential is eliminated when the endothelial cells are removed (Fischbarg, 1973; Hodson, 1974). Sodium and bicarbonate ions must be present for the pump to be operational (Hodson,1971(b);Dikstein and Maurice, 1972). Clearly the electrical potential is generated in the endothelial cells and, in its sodium and bicarbonate requirements the spontaneous potential correlates closely with the endothelial pumping activity (Hodson, 1974; Fischbarg and Lim, 1974).

Several hypotheses have been put forward to explain the nature of the endothelial pump. If the electrical potential is produced by an electrogenic ion pump, it cannot be a sodium ion pump commonly found in many biological membranes as it has the wrong polarity, that is, of the wrong orientation for an electrogenic sodium pump

(Hodson et al. 1977; Hodson and Miller, 1976). In an attempt to explain the pumps requirement for a cation and an anion Hodson (1974) proposed that the endothelium pumped salt into the aqueous humour. The electrical potential is created by an electro-osmotic effect caused by the reabsorption of translocated salt back across the endothelium into the corneal stroma.

This hypothesis required that the trans-endothelial electrical potential should be reduced to zero in the absence of the stroma. It has been demonstrated that this is untrue (Hodson et al, 1977). Fischbarg and Lim, (1974) proposedthat the pump removed sodium ions from the cornea and allowed protons into it. No quantitative foundation is described for this model, which according to Hodson and Miller (1976) cannot readily be tested.

Hodson et al. (1977) concluded that the endothelial pump acts primarily by pumping an anion into the aqueous humour. Experiments to identify such an anion have eliminated chloride, phosphate, sulphate (Hodson, 1971(b), 1974) and any anion metabolised from glucose by the endothelial cells themselves (Riley et al. 1977).

Bicarbonate and hydroxyl ions remain as substrates for an anion pump. Since bicarbonate is the only anion found to be essential to the maintenance of corneal hydration the concept of a bicarbonate pump was postulated. Hodson and Miller (1976) supported this view and Riley (1977) reported

the existence in the endothelium of an anion-sensitive ATPase which is most active in the presence of bicarbonate. Hull et al. (1977) have confirmed the presence of a net bicarbonate flux across the endothelium into the lens-side. Mayes and Hodson (1978) have demonstrated that the active endothelial bicarbonate ion flux can be coupled to and generate flows of water, comparable in size to trans endothelial fluid flows previously observed in corneal physiology (Davson, 1955; Mishima and Kudo, 1967; Dikstein and Maurice, 1972; Fischbarg and Lim, 1974). Mayes and Hodson (1979) have shown that the endothelial bicarbonate pump operates both in vivo and in vitro to reduce the stromal bicarbonate ion level.

Hodson (1977) suggested that the endothelial cells pump bicarbonate ions alone into the aqueous humour. Bicarbonate ions originating in the stroma provide two thirds of the substrate. The remaining third is provided by the intracellular conversion of exogenous carbon dioxide to bicarbonate. Metabolic carbon dioxide does not significantly contribute, as a result of its low concentration. The relative contribution of bicarbonate and carbon dioxide to net bicarbonate movement has been investigated (Kelly and Green, 1980). No tenable evidence for the participation of carbon-dioxide exists.

Albeit that the existence of a bicarbonate ion pump located in the corneal endothelium has been determined, a detailed model of its action has still to be formulated. It is unlikely that it is the only ion-pump active in the endothelium (Hodson and Miller, 1976). There is considerable

evidence for a sodium pump in these cells (Hodson, 1971(b), Dikstein and Maurice, 1972; Fischbarg and Lim, 1974). The membrane potentials, lens side negative, are inconsistent with the active extrusion of an anion alone.

It is possible that an active sodium pump exists, but its activity must be symmetrical across the endothelium and not polar. Sodium transport takes place exclusively across the lateral margins of the endothelial cells (Kaye et al. 1965; Kaye and Tice, 1966). It is likely that this would lead to the required symmetry and the apparent absence of any trans-endothelial electrical activity associated with the active transport of sodium.

1.6. METABOLISM AND NUTRITION

The cornea is composed of living reproducing cells. Some are involved in active transport processes and others with synthetic processes. They have significant energy requirements (Langham, 1955; Langham and Taylor, 1956; Harris and Nordquist, 1955; Davson, 1955; Mishima and Kudo, 1967; Dikstein and Maurice, 1972; Schwartz et al. 1954). Corneal cells require predominantly oxygen, glucose and amino acids.

The avascular nature of the cornea implies that nutrients enter at the periphery from the limbal capillaries or across the corneal limiting membranes via the aqueous humour and tears.

Limbal blood vessels play only a minor role in corneal nutrition. If the potential supply of nutrients from the limbal region is interfered with, either by making a deep pericorneal incision (Gruber, 1894; Leber, 1903; Gunderson, 1939), or by destroying the peripheral vascular supply (Votockova, 1962; Votockova et al. 1966), no impairment of corneal function occurs.

Maurice (1960) observed the movement of fluorescein and haemoglobin in the plane of the cornea following its introduction to the peripheral stroma. No central drift of the coloured spots, which was consistent with a fluid flow, could be observed. This is in accordance with the high resistance to water offered by the stroma. Under the hydrostatic pressure differences available, there would not be any appreciable current of liquid allowed to flow (Hedbys and Mishima, 1962). The absence of stromal channels for fluid flow has been confirmed due to the lack of spreading of high molecular weight haemoglobin (Maurice, 1957). However, Wakui and Suguira (1965 (a), (b), (c), described the movement of dyes through the cornea in channels associated with nerve trunks and Collins (1966) described the development of a lymph system beneath the epithelium in pathological corneae.

However, such routes could not represent a significant source of nutrition for the cornea (Maurice, 1969; Maurice and Riley, 1970). Tissue fluid in the normal stroma is considered to be stagnant. Any solute movement should follow the laws of diffusion. Diffusion of serum albumen

in the rabbit (Maurice and Watson, 1965), and of fluorescein in man (Maurice, 1967(b)) have been described. Molecules entering the cornea via the limbal blood supply are almost entirely lost to the anterior chamber before they reach the centre of the cornea. Clearly the vascular system supplies only a very limited peripheral region of the cornea with metabolites.

The relative importance of tears and aqueous humour as a source of metabolites has been investigated.

1.6.1. AMINO ACID SUPPLY

The high turnover rate of epithelial cells infers a considerable demand of amino acids for protein synthesis. Balik (1958) found a high concentration of amino acids in the tears, however, Riley (1977) stated that absorption of amino acids occurred across the endothelium.

1.6.2. GLUCOSE SUPPLY

Glucose supply is important in tissues, both as an energy source and as a building block that is incorporated into a wide range of cell products necessary for maintenance of cell integrity and function (Roberts and Parlebas, 1965). Bock and Maumenee (1953) implanted thin water-impermeable polyethylene sheets in the stroma. After 48 hours the epithelium and stroma anterior to the disc had degenerated whilst those tissues posterior to the disc

remained normal. Similar results were obtained by Knowles (1961). The aqueous was thus implicated as the primary metabolic source. However, Pollack (1962) described the implantation of water impermeable polypropylene sheets between the stromal lamellae in cats. They were tolerated for more than one year without pathological changes. Choyce (1965) demonstrated that 8mm. acrylic discs could be tolerated in human cornea indefinitely. The possibility of a glucose supply from the tears is not therefore eliminated. However, the tear glucose concentration is relatively small in man (Giardini and Roberts, 1950) and would not significantly contribute to corneal metabolism (Maurice, 1969). The glucose concentration is more than ten times greater in the aqueous (Reim et al. 1967). Many values of aqueous glucose concentration have been reported (Bruun-Laursen and Lorentzen, 1974, 1975; Pohjola, 1966; De Beradinis, 1965; Reddy and Kinsey, 1960; Reim et al. 1972; Kinsey, 1953; Alaerts, 1955; Walker, 1933).

Further evidence supporting the aqueous humour as the main source of glucose supply is provided by the increased hydration and loss of the temperature reversal effect when glucose is removed from the bathing solutions (Mishima and Kudo, 1967).

The exchange of glucose across the endothelium is more rapid than would be expected on the basis of its molecular size, and maintains the glucose level of the cornea at a similar level to that found in the aqueous humour and blood (Reim and Lichte, 1965; Hale and Maurice, 1969). An

active movement of glucose from the aqueous humour into the stroma has been suggested (Hale and Maurice, 1969). Maurice (1969) suggested that the molecular size of glucose was similar to that of the sodium ion and thus the permeability for the two substances across the endothelium would be similar. This permeability value (Maurice, 1951) would permit glucose to enter from the aqueous humour in excess of the glucose consumption of the whole cornea under aerobic conditions (Langham, 1954). The biological half life of glucose is approximately 8 minutes. If the external glucose supply was cut off, the glucose in the cornea would be almost completely exhausted within 30 minutes (Fatt, 1978).

An alternative source of metabolic energy is derived from glycogen. Glycogen deposits are stored solely in the epithelium (Calmettes et al, 1956), although recent histochemical evidence for glycogen in the endothelium has been described (Malinin and Bernstein, 1979). Estimates of corneal glycogen concentration have been carried out (Kamei, 1959; Herman and Hickman, 1948(a),(b), Reim and Lichte, 1965). Herman and Hickman (1948(a)) found a glycogen consumption rate of 25ug/hr for excised tissue at $32^{o}C$ in air, after endogenous glucose was exhausted. Under such conditions the supply should last for 40 hours (Maurice, 1969).

Smelser and Ozanics (1953) demonstrated a glycogen depletion in 2 hours as a result of contact lens wear.

The glucose consumed by the cornea is incompletely oxidised. It has been estimated that 84% of the glucose is metabolised no further than lactate in the rabbit (Maurice, 1969). The epithelium and stroma have equal rates of lactic acid formation for equivalent tissue weights (Maurice and Riley, 1968).

Lactic acid produced by glycolysis is removed into the aqueous humour; only a small fraction is lost via the epithelium to the tears (Reim et al. 1972; Ruben and Carruthers, 1972; Riley, 1972). However, Fatt, (1978) stated that diffusion of lactic acid posteriorly to the aqueous humour is unlikely as the aqueous concentration of lactate is high, and thus lactic acid is lost to the tears.

1.6.3. OXYGEN SUPPLY

As already stated, the limbus does not significantly contribute to the supply of corneal metabolites. The cornea respires only across its surface, an adequate supply of oxygen is necessary to maintain normal corneal hydration (Fischer, 1930; Duane, 1949 ; De Roetth, 1950; Langham and Taylor, 1956; Langham, 1952, 1954; Dikstein and Maurice, 1972; Freeman, 1972; Mishima and Kudo, 1967).

Langham (1952) demonstrated a fall in lactic acid production when the atmospheric oxygen tension was increased; the converse is also true (Langham, 1951, 1952). This implicates the atmosphere as being the major supply of

corneal oxygen. It further demonstrates that the cornea is not respiring at its maximum rate under normal atmospheric conditions.

Barr et al. (1976) also conclude that the three principle corneal layers receive oxygen from the air under normal open eye conditions in rabbits.

The cornea lends itself to a simple mathematical model when oxygen diffusion through it is being considered. The model is that of a plane sheet, infinite in two dimensions, to which the standard diffusion equation based on Fick's Law can be applied. The oxygen consumption rate and oxygen permeability for each layer must be known.

An oxygen tension profile was calculated (Fatt and Beiber, 1968) using data derived from previous experiments (Takahashi and Fatt, 1965; Maurice, 1962; Takahashi et al. 1967). This profile has been modified (Fatt et al. 1974) following the work of Freeman (1972) and Freeman and Fatt (1972).

The oxygen tension in the aqueous humour has been investigated extensively (de Haan, 1922; Heald and Langham, 1956; Dreckhahn and Lorenzen, 1958; Neuman, 1958; Jacobi, 1966, 1968; Wegener and Moller, 1971; Kwan et al. 1972; Barr and Silver, 1973). The reported range of values recorded has been large, 10 to 80mm.Hg.. A value of 55mm. Hg. is normally accepted (Maurice, 1969; Fatt, 1978; Fatt et al. 1974).

In the closed eye situation, the aqueous oxygen tension is the same as in the open eye. However, the oxygen tension at the anterior corneal surface is reduced. Oxygen must therefore be derived from an alternative source. This is provided from the capillary bed in the palpebral conjunctiva. Fatt and Beiber (1968) and Kwan and Fatt (1970) have demonstrated that the capillary bed creates an oxygen tension at the corneal surface of about 55mm Hg. Since no signs of corneal distress are present following periods of eye closure it is concluded that this oxygen tension allows normal corneal metabolism to occur.

The consumption of oxygen by the cornea is further discussed in Chapter 2.

1.7. PATHWAYS OF METABOLISM

Carbohydrate metabolism predominates in the cornea, as indicated by a respiratory quotient of unity. (De Roetth, 1950). Glucose is the primary mono-saccharide in this process. The major pathways involved in glucose metabolism are first, the Embden-Meyerhof pathway followed by the Krebs tricarboxylic or citric acid cycle and, second, the direct oxidation of glucose by the hexose monophosphate shunt.

The principal pathway for the oxidation of glucose is the Embden-Meyerhof pathway. In addition to both aerobic and anerobic glycolysis, complete operation of the citric

acid cycle has been confirmed in the epithelium (Kuhlman and Resnik, 1959).

Glucose is also degraded in the epithelium by the hexose monophosphate shunt. Glucose-6-phosphate is oxidised by a two-step process to yield ribulose-s-phosphate (Kinoshita and Masurat, 1954; Kinoshita et al. 1955).

It is estimated that 65% of the glucose is metabolised by the Embden-Meyerhof pathway (Kinoshita and Masurat, 1959; Kinoshita, 1962). This agrees with the work of Katz and Wood (1959) who state that 35% of glucose is utilised via the hexose monophosphate shunt.

CHAPTER 2

THE HISTORY, DEVELOPMENT AND PHYSIOLOGICAL IMPLICATIONS OF CONTACT LENSES

2.1. THE HISTORY AND DEVELOPMENT OF CONTACT LENSES

Probably the earliest reference to contact lenses is that attributable to Leonardo da Vinci, whose contribution lay in determining the basic optical principles of neutralising the refractive power of the cornea in water. His work has been well reviewed (Ferrero, 1952; Hoffstetter and Graham, 1953; Gasson, 1976). Enoch (1956) described the subsequent work in this area of, Descartes (1637) and Duke-Elder (1970) discussed the further contribution of De la Hire (1685).

Young (1801) provided the stimulus which led to the first optical correction of astigmatism by Airy (1827), and Herschel (1845) linked the earlier theoretical considerations with the emerging clinical use of contact lenses.

Such clinical development was continued with the work of Fick (1888), who utilised contact lenses to increase visual acuity in keratoconus. At the same time, Kalt in Paris was using contact lenses to treat the advancement of keratoconus and thus originating the technique of orthokeratology. Muller (1889) described the correction of high myopia using contact lenses. Thus, by 1890 the foundations had been laid for the correction of refractive errors, remoulding the corneal shape, and neutralising corneal irregularity. Over the 1920-1930 period the trial fitting set emerged. Heine (1929) developed a large

afocal lens set based on the initial four lens set developed by Stock.

It gradually emerged that contact lens comfort could be significantly improved by modifications, both in lens design and subsequent fitting procedures.

Advances were made both in impression techniques and in fitting philosophies such that all day tolerance of contact lenses was reached by the late 1940's. In 1957 the corneal lens was described and eventually became established as the most widely used form of contact lens (Bier, 1957).

2.2. THE HISTORY AND DEVELOPMENT OF POLYMERS FOR CONTACT LENS APPLICATION

The origin of polymers for contact lens application can be traced back to the introduction of polymethylmethacrylate (P.M.M.A.) in the 1930's. (Feinbloom, 1936, 1937, 1938). The original lenses were composed of a ground glass corneal portion with a moulded plastic scleral band. Subsequently, the first moulded all plastic scleral contact lenses were produced. The term "polymer" encompasses a wide range of materials, manifesting differing physical and chemical properties. They may be naturally occurring or synthetic. Currently available contact lenses are all manufactured from synthetic materials, and may be classified into three groups.

a) Thermoplastic polymers (e.g. P.M.M.A.)

b) Synthetic Elastomers (e.g. Polydimethyl Siloxane)

c) Hydrogels

The varying physical properties of these contact lens materials require different fitting criteria to be adopted. Thermoplastic polymers, in general, have a low oxygen permeability. They are fitted, as a result, to allow a tear movement between the contact lenses and the anterior corneal surface. This provides the oxygen required for normal corneal metabolic function to take place. Elastomeric contact lenses are also fitted in a similar manner, albeit they are significantly more permeable to oxygen than the thermoplastic lenses. They suffer however, from the hydrophobic nature of their surfaces (Yasuda, 1967; Becker, 1966; Hill and Cuklanz, 1967). Attempts to modify the surface chemistry in order to make the lenses hydrophilic have been made (Rizzut, 1974; McVannell et al. 1967; Mizutani and Miwa, 1977).

P.M.M.A. has remained the most widely used contact lens material, approached only in popularity by the hydrogels following their inception in the 1950's (Lim and Wichterle, 1956). Lim and Wichterle (1956) proposed the use of covalently cross-linked glycol methacrylates for surgical prosthesis. The application of synthetic hydrogels for contact lens usage was subsequently outlined (Wichterle and Lim, 1960). It is worthy of note that Mann (1938)

described the work of Galeskowskey (1886), who suggested the application of a gelatine disc impregnated with both cocaine and sublimate of mercury directly onto the cornea following cataract extraction. This procedure provided antiseptic cover and relieved post operative pain and thus was the earliest reference to a soft hydrophilic contact lens appliance and to the use of such a lens as a dispenser of ophthalmic medication. Herschel (1845), however, had previously described the application of a tube, containing animal jelly to the cornea, in order to correct an irregular corneal surface.

The original hydrogel contact lenses were produced from infrequently cross-linked poly 2 - hydroxylethyl methacrylate (P.H.E.M.A.). A variety of cross-linked polymers yielded transparent hydrogels which retained significant qualities of a polar solvent under equilibrium conditions. Such synthetic hydrogels consist of numerous sub-units termed monomers which when joined together form a polymer. Identical monomers join together forming a homopolymer and different monomers form a co-polymer (Refojo, 1978). Such polymer chains can be joined together by a cross-linking agent. Cross-linked polymers will swell but they cannot be permanently deformed by heat or pressure, and thus resemble thermoplastic polymers. Hydrogels used for contact lens manufacture can be described as cross-linked hydrophilic polymers. They swell in water forming an elastic gel-like material. P.H.E.M.A. is fabricated by lightly cross-linking H.E.M.A. with ethyleneglycol dimethacrylate (E.D.M.A.). H.E.M.A. can also be copolymerised

with other hydrophilic monomers. The one most commonly encountered in contact lens application being vinyl pyrollidone.

Because of their hydrophilicity and softness hydrogels are suitable in many ways for contact lens application. There are however inherent problems associated with the use of hydrogel lenses regarding their tensile strength and in the correction of astigmatism. Improvements in design and fitting of the methacrylate lens have retained its popularity as a contact lens material. It does however have a number of deficiencies, in particular its rigidity and virtual impermeability to oxygen. The elastomeric hydrophobic materials require surface treatment, as already stated, to render them suitable for contact lens application, and difficulties during lens manufacture have been encountered (Bitonte and Keates, 1972).

It would be an advantage to develop a material showing improvements in respect of these drawbacks encountered with existing lens materials. Ng et al. (1976) have reviewed some proposed materials. More recently a new generation of contact lens materials termed 'Gas Permeable' materials have emerged; using the concept of a flexible thermoplastic polymer of high oxygen permeability. They have attempted to combine the optical properties of the methacrylate lens with the greater flexibility and oxygen permeability of the hydrogel material.

2.3. PHYSIOLOGICAL CONSIDERATIONS OF CONTACT LENS WEAR

OXYGEN SUPPLY

When a contact lens is placed on the cornea a new environment for the cornea is created. As has been already discussed, the supply of glucose and other metabolites derived from the anterior chamber will remain essentially unaffected, as does the cabon dioxide efflux rate (Fatt, et al. 1969). However, due to the avascular nature of the cornea its oxygen requirements are obtained from the atmosphere (Fatt and Beiber, 1968). The direct access of dissolved atmospheric oxygen is affected by the presence of a contact lens (Fatt et al. 1969). The physiological embarrassment so produced may cause an increase in anaerobic glycolosis, increase in lactic acid concentration, depletion of glycogen storage levels and loss of corneal deturgescence with a corresponding increase in corneal thickness (Smelser, 1952; Riley, 1969; Polse and Mandell , 1970; Uniake et al. 1972).

Clearly a contact lens which has the minimal affect on corneal physiology is to be favoured. Most oxygen reaching the anterior corneal surface does so as a result of permeating the hydrogel contact lens material. The measurement of oxygen permeability of hydrogel materials has been studied (Holly and Refojo, 1972; Refojo, 1973; Yasuda et al. 1966). Carter (1972) has described a tear

pump underneath a hydrogel contact lens following the observation of red blood cells moving beneath a soft lens which originate from a drop of blood placed in the tear pool beyond the edge of the lens. The relative importance of such a tear pump mechanism in contributing to corneal oxygenation is not at this time fully appreciated.

Albeit that the avascular cornea was suspected of deriving all or part of its oxygen required for respiration directly from the air, no quantitative invivo measurements were reported until those of Hill and Fatt 1963(a),(b). They used a polarographic oxygen sensor to monitor the depletion of oxygen from the air-saturated solution in a small chamber that was part of a scleral contact lens. Haberich (1966) used a direct volumetric estimation of oxygen in a gas-filled chamber attached to a contact lens. Both methods were slow and time consuming and the procedure was simplified (Hill and Fatt, 1964(a),(b). The chamber on the scleral contact lens was replaced by a thin polyethylene membrane covering the oxygen sensor, which acted as an oxygen reservoir. This procedure is more rigid although it is somewhat less precise than the "chamber" method (Fatt, 1978). As such, it is only useful for determining the relative oxygen flux, for example, when the effect of a drug or contact lens on oxygen uptake rate is being monitored. An exact mathematical analysis of data derived in this way is not available because the multi-layered cornea and sensor membrane form too complicated a system. An empirical procedure has been used (Jauregui and Fatt, 1971). This relates the oxygen uptake by the cornea (Q),

the membrane thickens (l), the solubility of oxygen in the membrane (K), the time taken to reduce the oxygen tension within the initial reservoir by a factor of ten (T), and the oxygen tension of the limited reservoir (P).

$$Q = \frac{Pkl}{t_1} \qquad \text{(Jauregui and Fatt, 1971)} \qquad (1)$$

Fatt et al. (1969) showed that the oxygen flux into the anterior surface of a multi-layered cornea would be expressed as

$$Q = a\,p^{B} \qquad (2)$$

where a and B are empirical constants.

(a = 0.24×10^{-6} m l O_2/cm^2 x sec x (mm Hg $\frac{1}{2}$)

(B = 0.5

(P = Oxygen Tension at the epithelial contact lens interface.

Using these results Fatt and St. Helen (1971) derived an expression for oxygen flux through a contact lens.

(Dk = permeability of lens material)

(l = lens thickness)

(Pa is the oxygen tension at the anterior surface of the contact lens in the open eye).

$$Q = \underset{\text{Contact Lens}}{\frac{(Dk)}{L}} \underset{\text{Contact Lens}}{(Pa - P)} \qquad (3)$$

For the closed eye Pa is the oxygen tension of the palpebral conjunctiva 55mm Hg (Fatt and Beiber, 1968; Kwan and Fatt, 1970). The contact lens and cornea are joined at a mathematical plane. Therefore

Q cornea = Q contact lens (Equation 2 = Equation 3)

$$\therefore \quad a\,P^{B} = \frac{(Dk)}{l}_{\text{contact lens}} \quad (Pa - P) \qquad (4)$$

Given the properties of a soft contact lens $\frac{(Dk)}{l}$ and the state of the eye, open or closed, the oxygen tension at the cornea - contact lens interface can be calculated.

Utilising the oxygen consumption data of Freeman (1972) and Dk values of Freeman and Fatt (1972), the oxygen flux into the cornea, together with the oxygen tension under a contact lens as a function of its oxygen transmissibility, has been re-examined (Fatt et al. 1974) (Figure 2:1(a)(b).

This differs from the earlier work of Fatt et al. (1969) in several respects.

1. The relationship is linear with respect to P
   thus equation (2) becomes $Q = aP + B$ (5)
   (a = constant = 0.66×10^{-6})
   (B = constant - 0.50×10^{-6})

2. An oxygen consumption rate for epithelium and endothelium was used that was independent of oxygen tension down to zero.

Figure 2.1.(a)

Oxygen flux into a cornea as a function of oxygen tension at the anterior epithelial surface

After Fatt, 1978.

———————— Fatt and St. Helen, (1971)

---------- Fatt et al. (1974).

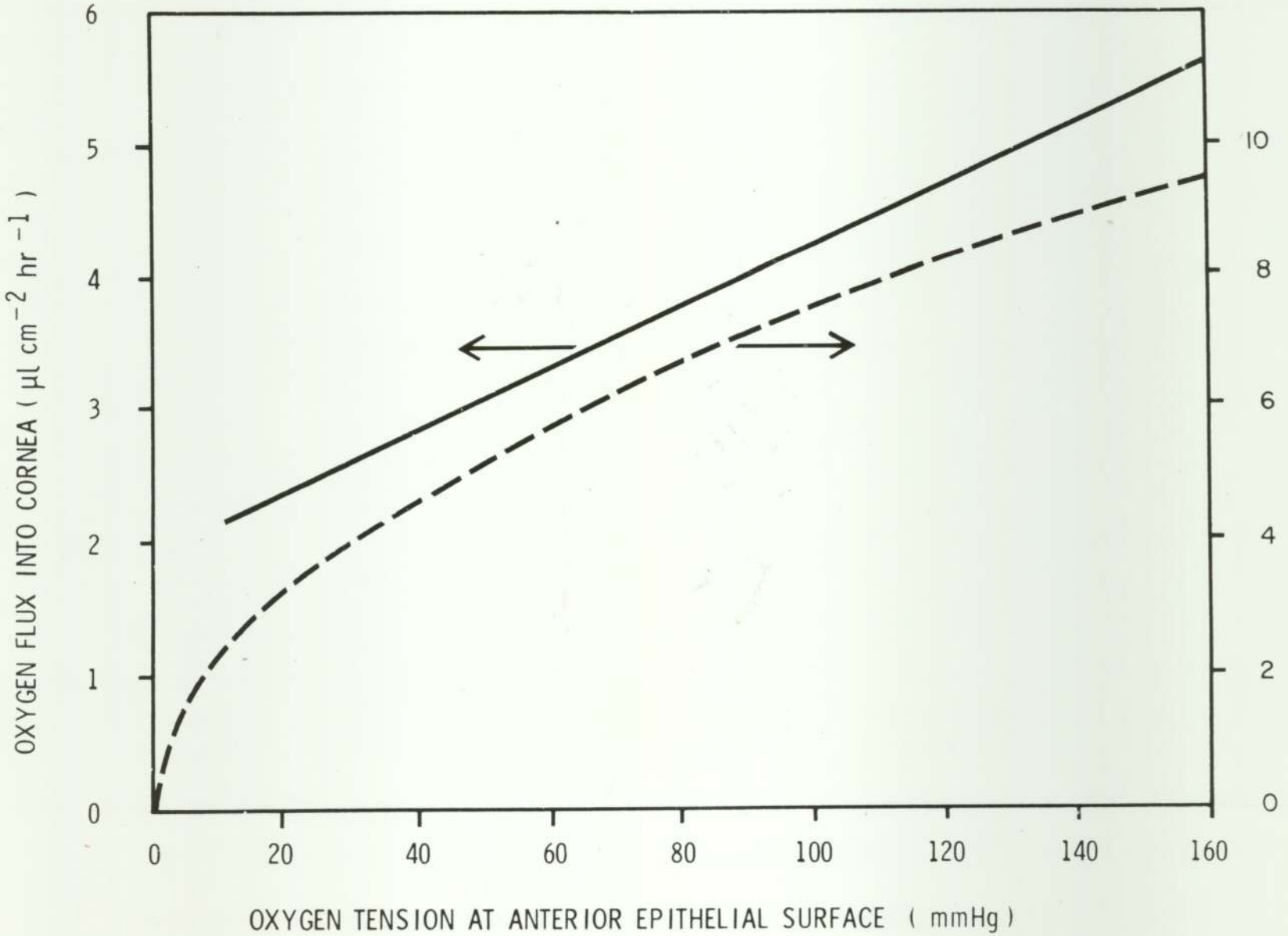

OXYGEN FLUX INTO CORNEA (µl cm^{-2} hr^{-1})
OXYGEN TENSION AT ANTERIOR EPITHELIAL SURFACE (mmHg)
0
1
2
3
4
5
6
0
2
4
6
8
10
0
20
40
60
80
100
120
140
160

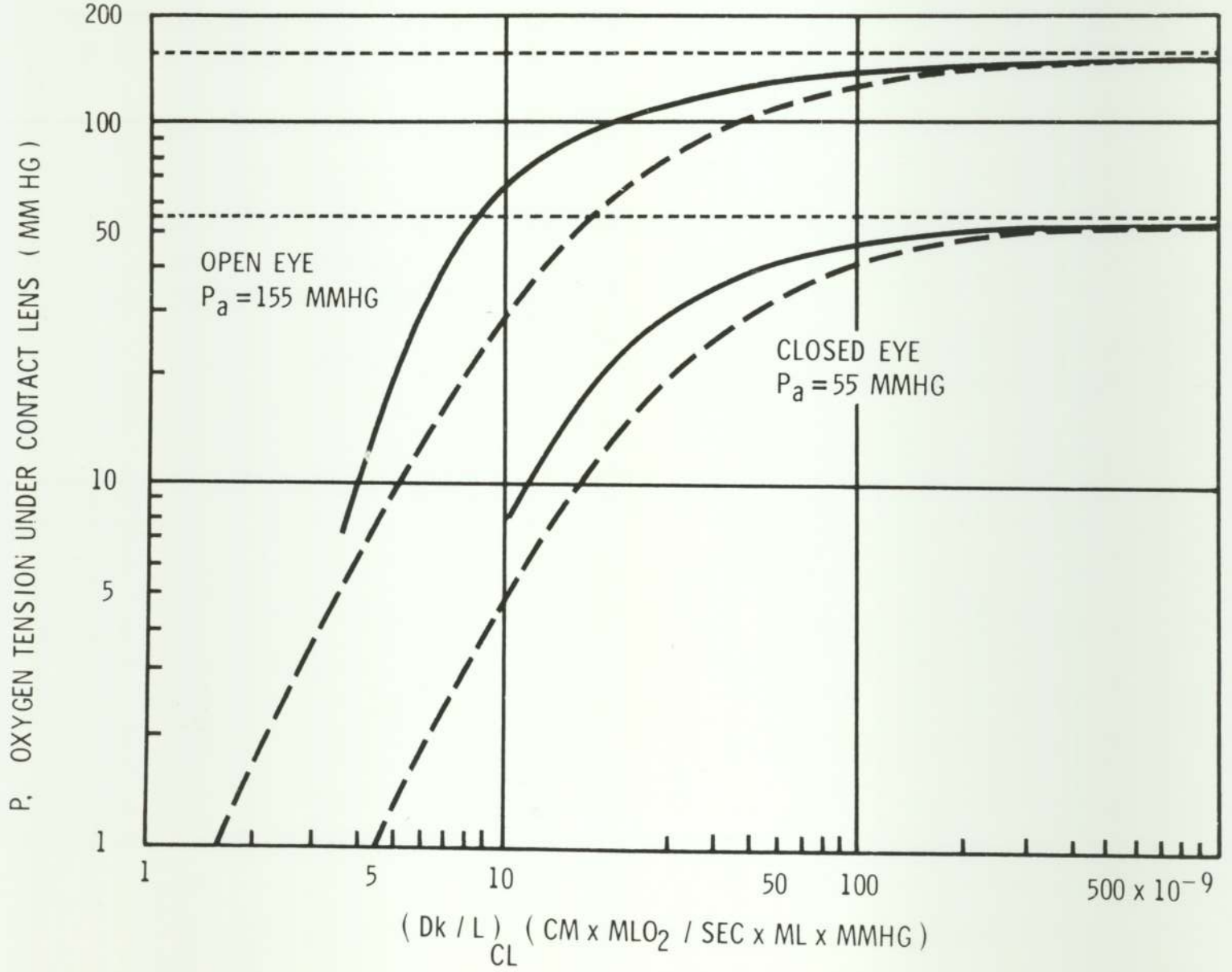
Figure 2.1.(b)

Oxygen tension under a contact lens as a function of the oxygen transmissibility of the lens.

After Fatt, 1978

_____ Fatt and St. Helen, 1971.

---------- Fatt et al. 1974.


200
100
50
10
5
1
P. OXYGEN TENSION UNDER CONTACT LENS (MM HG)
OPEN EYE
P_a = 155 MMHG
CLOSED EYE
P_a = 55 MMHG
1
5
10
50
100
500 x 10^{-9}
$(Dk/L)_{CL}$ (CM x MLO_2 / SEC x ML x MMHG)

3. The calculated flux is lower for all values of oxygen tension.

Therefore, equation (4) is re-written as

$$\frac{Dk}{l} = \frac{0.50 \times 10^{-6} + 0.66 \times 10^{-6} p}{Pa - P}$$

2.4. PHYSIOLOGICAL CONSIDERATIONS OF EXTENDED SOFT CONTACT LENS WEAR

For a normal cornea in the open eye situation the oxygen tension at the anterior corneal surface is 155mm Hg. and that of the aqueous humour 55mm Hg. In the closed eye situation no signs of physiological distress are manifest and thus it is presumed that the cornea is receiving an adequate supply of oxygen, although a diurnal change in corneal thickness has been reported (Hirji and Larke, 1978; Mandell and Fatt, 1965; Gerstmann, 1972). The minimum oxygen tension, below which the cornea enters respiratory distress, has been examined. If such a value is known a contact lens can be produced to ensure an oxygen tension value to be available in excess of this amount. Such a critical oxygen tension was found to range between 11-19mm. Hg. (Polse and Mandell, 1970). Below this critical oxygen value the cornea hydrates and swells. By observing the level of succinic dehydrogenase in the corneal epithelium while exposing the epithelial surface to reduced oxygen tensions for a specific period of time, a similar oxygen tension requirement of 19mm Hg. was obtained (Rengstorff, et al. 1974). Recently, this value has been re-examined

and a higher figure of 30mm Hg. suggested (Mandell and Farrell, 1980).

A value of 15mm Hg. has been assumed as a basis for calculations. From Figure 2:1b the transmissibility of the contact lens necessary to maintain such an oxygen tension in the closed or open eye situation can be predicted, and the oxygen permeability can be calculated for a given contact lens thickness.

(a) From the data of Fatt and St. Helen (1971)

Corneal flux = $Q = aP^B$ ..... Eq.2. P = 15mm Hg.

$\therefore Q = 0.24 \times 10^{-6} \times 15^{\frac{1}{2}} = 0.9295 \times 10^{-6} cm^{-3} cm^{-2} sec^{-1}$

$= 0.9295 \times 3600 \ 1 \ cm^{-3} \ W^{1}$

$= 3.344$

(b) From the revised data of Fatt et al. (1974)

Corneal flux = $Q = 0.66 \times 10^{-6} \times 15 \times 0.50 \times 10^{-6}$

$= 5.99 \times 10^{-7} \ cm^{3} cm^{-2} sec^{-1}$

$= 2.1564$

These values of oxygen consumption agree with the experimental values reported (Hill and Fatt, 1963(a), Jauregui and Fatt, 1972).

If the epithelial oxygen consumption rate under a tight fitting contact lens is equal to the influx of oxygen through the hydrogel contact lens, the transmissibility of the lens material required to maintain the critical oxygen tension during periods of sleep can be determined.

(a) From the data of Fatt and St. Helen (1971) - $Dk = \frac{ap^B}{Pa-P}$ 4

p = 15m Hg. Pa = 55m Hg. l = 0.025 cms.

$$\frac{Dk}{l} = \frac{0.24 \times 10^{-6} \times 15^{\frac{1}{2}}}{55-15} = \frac{0.929 \times 10^{-7}}{40} = 2.3225 \times 10^{-9}$$

$$\therefore \text{ Permeability} = Dk = 2.3225 \times 10^{-9} = 58.06 \times 10^{-11}$$

$$cm^3 cm^2 sec^{-1} mm\ Hg^{-1}$$

(b) According to Fatt et al. 1974.

$$\frac{Dk}{l} = \frac{0.50 \times 10^{-6} + 0.66 \times 10^{-6} \times 15}{40}$$

$$= 1.497 \times 10^{-8}$$

$$\therefore \text{ Permeability} = Dk = 1.497 \times 10^{-8} \times 0.025 = 37.43 \times 10^{-11}$$

$$cm^3 cm^2 sec^{-1} cm^{-3} mmHg^{-1}$$

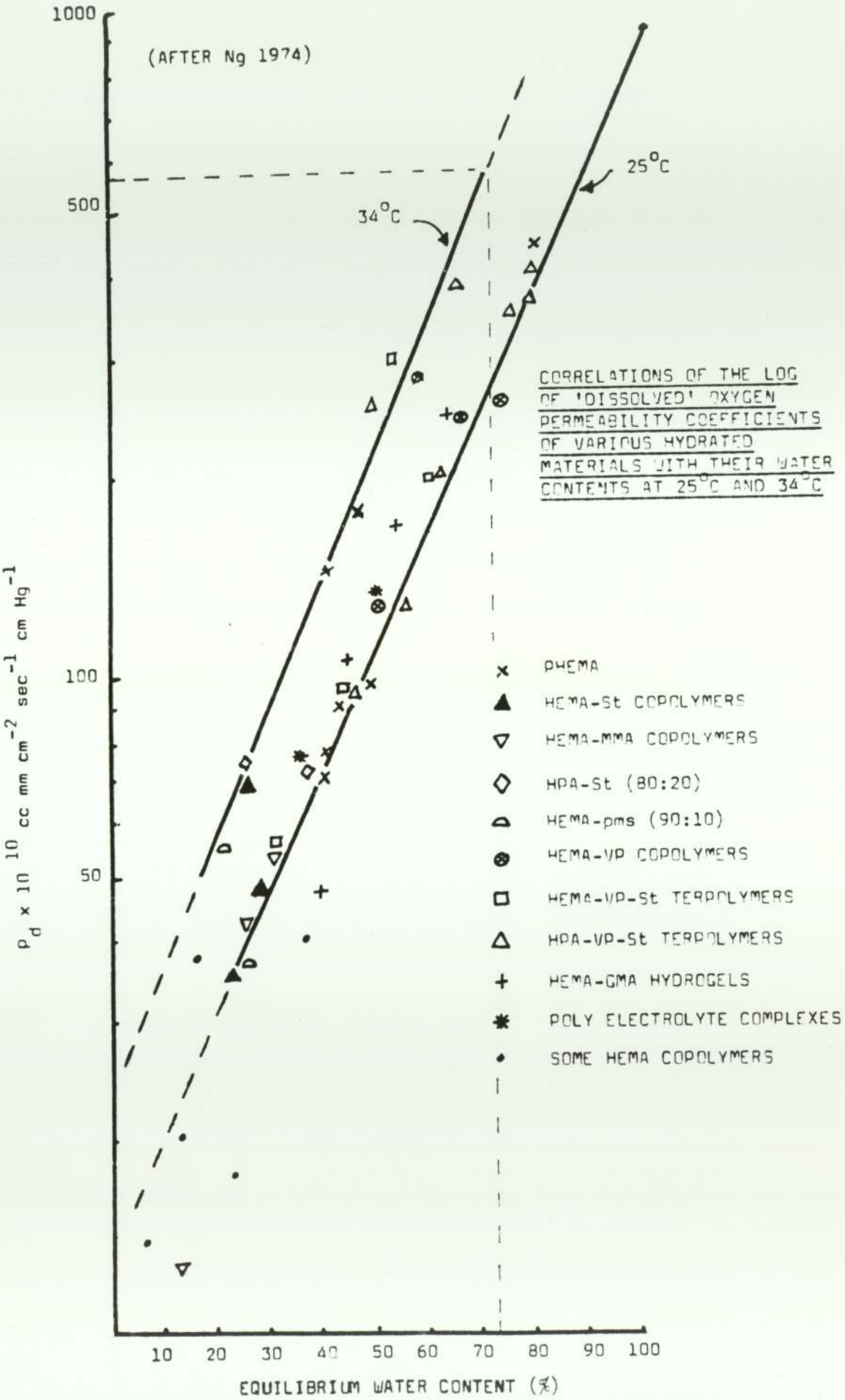
Therefore to ensure a minimum critical oxygen tension of 15mm Hg. at the anterior corneal surface during periods of sleep a hydrogel contact lens 2.5mm thick must have an oxygen permeability of 58×10^{-11} (Fatt and St. Helen, 1971). 37×10^{-11} (Fatt et al. 1974).

The data of Fatt et al. (1974) is probably the most accurate available at this time (Fatt, 1978). Therefore, a contact lens of thickness 0.025 cms. would have to be made from a hydrogel material exhibiting a dissolved oxygen permeability in excess of $37 \times 10^{-11} cm^3 cm^2 sec^{-1} cm^{-3} Hg^{-1}$ in order to ensure that the minimum oxygen tension at the contact lens corneal interface was 15mm Hg. From the work of Ng (1974) and Ng and Tighe (1976) this value

Figure 2.2

Correlations of the log of 'dissolved' oxygen permeability coefficients of various hydrated materials with their water contents at 25°C and 34°C.

After Ng, 1974


(AFTER Ng 1974)
1000
500
100
50
34°C
25°C
$P_d \times 10^{10}$ cc mm cm^{-2} sec^{-1} cm Hg^{-1}
10 20 30 40 50 60 70 80 90 100
EQUILIBRIUM WATER CONTENT (%)
CORRELATIONS OF THE LOG OF 'DISSOLVED' OXYGEN PERMEABILITY COEFFICIENTS OF VARIOUS HYDRATED MATERIALS WITH THEIR WATER CONTENTS AT 25°C AND 34°C
PHEMA
HEMA-St COPOLYMERS
HEMA-MMA COPOLYMERS
HPA-St (80:20)
HEMA-pms (90:10)
HEMA-VP COPOLYMERS
HEMA-VP-St TERPOLYMERS
HPA-VP-St TERPOLYMERS
HEMA-GMA HYDROGELS
POLY ELECTROLYTE COMPLEXES
SOME HEMA COPOLYMERS

requires a hydrogel material of at least 63% equilibrium water content at 34^{o} (Figure 2:2).

(Dk units are 100x smaller than Pd units).

A contact lens manifesting these characteristics of suitability for extended wear is Sauflon p.w.$^{T.m.}$

The equilibrium water content of Sauflon p.w.$^{T.m.}$ is summarised in Table 1a and the physical properties in Table 1b.

TABLE 1a

EQUILIBRIUM WATER CONTENT OF SAUFLON p.w.$^{T.m.}$

(Larke, 1981)

Lens in Container:	Equilibrium Water Content
18^{o}C. pH 6.5 Toxicity 0.9% NaCl	82.3%
Lens on Eye : Eye Open	
34^{o}C. pH 7.4 Toxicity 1% NaCl	78.5%
Lens on Eye : Eye Closed	
37^{o}C. pH 6.8 Toxicity 0.9% NaCl	77.96%

NaCl = Sodium Chloride.

TABLE 1b

PHYSICAL PROPERTIES OF SAUFLON p.w.$^{T.m.}$

Refractive Index Dry	1.525	Cordrey, 1977
" " Wet	1.360	"
Linear Expansion Factor	1.800	"
Tensile Strength kg/cm^2	1.000	"
Water Content Normal Saline 20°C	80%	"
Dissolved oxygen permeability $cm^2 cm^3 sec^{-1} cm^{-3} mmHg^{-1}$	48.6	Fatt and Morris, 1977.

Sauflon lenses have a sufficiently high water content to meet the theoretical demand of ensuring an oxygen tension of 15mm Hg. at the anterior corneal surface during periods of sleep. It is therefore a theoretically potential extended wear contact lens even up to a thickness of 0.025cm. Lathe cut Sauflon p.w.$^{T.m.}$ lenses have a single base curve with a minimum hydrated front optic diameter of 8mm and a hydrated edge thickness of 0.5mm. Their overall diameter is 13.00mm. and hydrated centre thickness does not exceed 0.025 cms. (Figure 2:3).

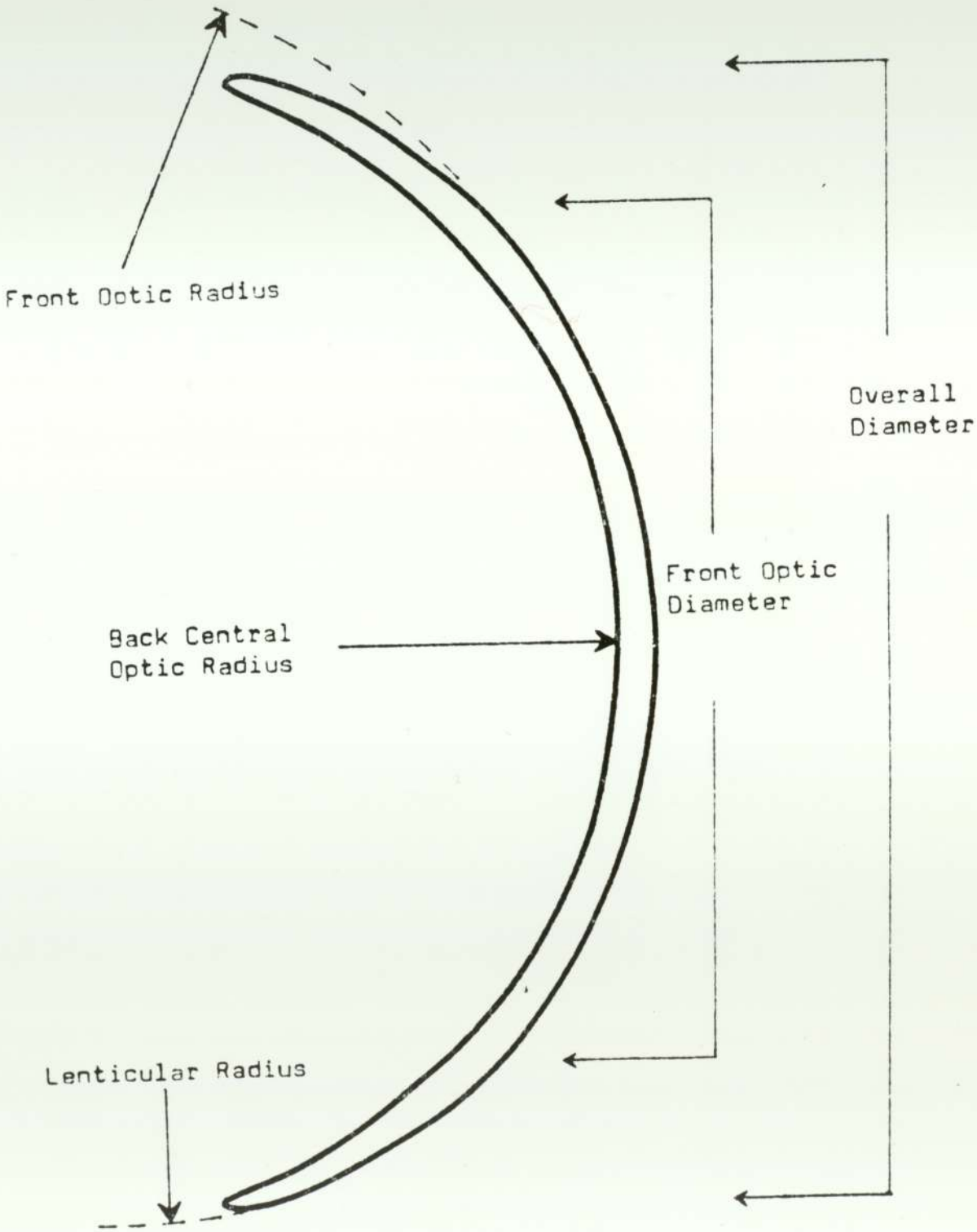
An increase in oxygen transmissibility can be effected by increasing the oxygen permeability of the hydrogel material for a given contact lens thickness. However, the contact lens thickness can be reduced for materials of lower oxygen permeability thus creating the same effect. This later technique forms the basis of the development of ultra

Figure 2.3

A schematic Sauflon 85 Contact Lens profile.

A SCHEMATIC SAUFLON T.M. 85

CONTACT LENS PROFILE



thin and hyper-thin contact lenses.

2.5. CORNEAL OXYGEN SUPPLY AND OXYGEN TENSION PROFILES

Corneal hypoxia induces swelling and loss of corneal transparency. Hypoxia of longer duration causes necrosis. Such observations were made following:- (i) ligation of the cilial arteries and the arteria carotis interna, (ii) reduction of the oxygen tension at the anterior corneal surface, and (iii) following the prolonged wearing of ill fitted contact lenses. (Heald and Langham, 1956; Langham 1955; Smelser and Chen, 1955; Weekers, 1940; Zander, 1975). An insufficient endothelial oxygen supply can directly influence vision, as impairment of normal endothelial function may reduce corneal transparency (Dikstein and Maurice, 1972; Maurice, 1972).

Corneal oxygen supply has been investigated (Fatt and Beiber, 1968; Fatt et al. 1974; Takaheshi and Fatt, 1965). Barr and Silver (1973) and Barr and Roetman (1974) maintain that under open eye conditions the whole cornea receives oxygen from the air. This conclusion is based on oxygen tension measurements in the anterior chamber and investigation of corneal oxygen diffusion. Grote and Zander (1976) re-examined the situation having determined both the oxygen diffusion constant of the cornea and the oxygen solubility coefficient of the aqueous humour, values which previously were unknown or not exactly

determined and which contributed significantly to the conclusions drawn.

Their work produced an oxygen tension profile for the cornea in the open eye condition, which corresponded to that of Kwan et al. (1972) and supported the view that the corneal endothelium is primarily supplied with oxygen by the aqueous humour in both open and closed eye conditions. (Figure 2.4). Fatt et al. (1974).

Grote and Zander (1976) further discussed the contribution of both diffusion and convection in allowing sufficient oxygen to reach the posterior corneal surface, although Bert and Fatt (1969), have demonstrated the effect of convection on the distribution of oxygen to be small in the mammalian cornea.

2.6. CONTACT LENS EFFECTS ON THE CORNEAL ENDOTHELIUM

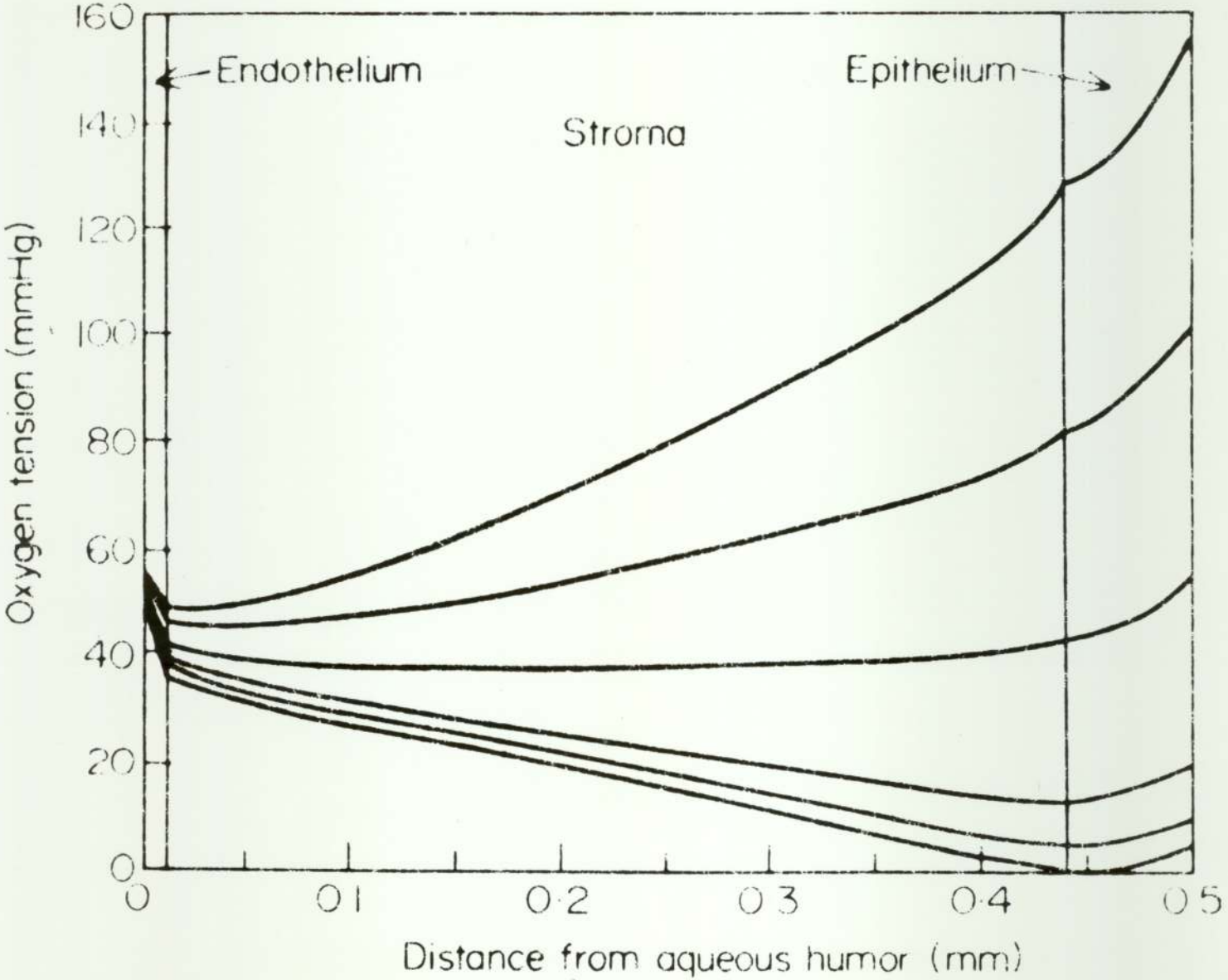
A contact lens placed on the corneal surface causes a reduction in the partial pressure of oxygen at the contact lens/corneal interface. The physiological implications and physical contact lens characteristics needed to minimise this effect have been discussed (see Section 2.4.).

Zantos and Holden (1977) and Holden and Zantos (1978) described the appearance of endothelial blebs following the insertion of soft contact lenses in unadapted patients. Such blebs were reversible on cessation of lens wear and

Figure 2.4

Oxygen tension profiles in the cornea for various oxygen tensions at the anterior surface. The upper curve represents the open eye; the third curve from the top is for the closed eye.

After Fatt et al. 1974.


160
140
120
100
80
60
40
20
0
Endothelium
Epithelium
Stroma
Oxygen tension (mmHg)
0
0·1
0·2
0·3
0·4
0·5
Distance from aqueous humor (mm)

may be related to the relative anterior anoxia created by the contact lens (Holden and Zantos, 1980), thus supporting the view that the corneal endothelium receives its oxygen from the anterior corneal surface. This is however contrary to the oxygen profile data of Fatt (1978). Such blebs have subsequently been described following periods of hard contact lens wear. Barr and Schoessler (1980).

2.7. EXTENDED WEAR CONTACT LENSES

Following the introduction of hydrogel contact lenses, the concept of extended wear became more feasible. Such a type of lens partially removes the difficulties of insertion and removal associated with daily wear lenses, and the complications arising from the frequent use of antiseptic solutions may be reduced or largely avoided. Such advantages are of benefit particularly to aphakics, and the use of extended wear lenses for therapeutic applications has been reported: Waltman and Kaufman, 1970; Gasset and Kaufman, 1970; Leibowitz and Rosenthal, 1971; Podos et al. 1972; Hillman, 1974; Gould, 1974; Boyd, 1979).

De Carle (1972) reported extended wear using 68% equilibrium water content hydrogel contact lenses on non-pathological eyes. Further reports followed (Gasson, 1976; Zantos and Holden, 1977; Burnett-Hodd, 1977; Binder and Woodwood, 1980).

Binder and Worthen (1977) concluded that the 'continuous wear concept' was a viable option for cosmetic lens wear. However, the use of such lenses is not without some clinical difficulties. Josephson (1979) described the appearance of corneal microcysts after long term use of extended wear lenses and this finding has been substantiated (Humphreys et al. 1980; McMonnies, 1981).

CHAPTER 3

OXYGEN UPTAKE OF THE HUMAN IN VIVO CORNEA

3. OXYGEN UPTAKE OF THE HUMAN IN VIVO CORNEA

3.1 INTRODUCTION

The corneal endothelium derives its oxygen from the aqueous humour, whilst the corneal epithelium is dependent on receiving oxygen from the atmosphere (Fatt, 1978). The epithelium is the primary consumer of oxygen in the cornea due to its large number of cells. The corneal consumption rate of oxygen has been examined. (Bessey and Wolbeck, 1939; Fischer, 1940; Lee and Hart, 1944; Robbie et al. 1947; De Roetth, 1950, 1951; Langham, 1952, 1954, 1955). However, all of these studies used excised corneal tissue. The assessment of in vivo oxygen consumption was not made until the development of eletrochemical methods of gas analysis. Hill and Fatt (1963 (a)) first reported the use of a polarographic oxygen sensor in determining apparent corneal oxygen consumption. Their early sensors were supported in a scleral contact lens whilst subsequent work differed in that contact between sensor and cornea was direct, (Hill and Fatt, 1963 (b); Hill and Fatt, 1964 (a); Farris et al. 1965; Jauregui and Fatt, 1971, 1972). More recently measurement of oxygen flux into the cornea has been measured by pressing an oxygen sensor onto a soft contact lens being worn on the eye (Fatt, 1978). The purpose of the experiments now reported is to examine aspects of apparent human in vivo oxygen consumption rate in relation to soft contact lens wear.

3.1.1 THEORY OF ELECTRODE OPERATION

Gaseous hydrogen and oxygen are released at the surface of wires submersed in water when connected to the positive and negative poles of a direct current electrical source. This phenomenon was demonstrated in 1800 by Nicholson (Glassstone, 1946). Danneel (1897) demonstrated the reverse reaction to be possible and further showed that the current flow during this electrolytic reaction was proportional to the concentration of gaseous dissolved oxygen. An electro-chemical half-cell reaction may be given as:-

$$O_2 + H_2O + 4\bar{e} = 4\ O\bar{H}$$

(Koltoff and Jordan, 1952; Lingane, 1961).

The term oxygen sensor is applied to a combination of cathode and reference anode. Although polarographic oxygen sensors differ in detail according to their application, they all depend on the chemical reduction of oxygen at an electrical-conducting surface by the electron pressure imposed on that surface by an externally applied potential.

The anode is an oxygen sensor, commonly made of silver. The reaction taking place at its surface is given as: $Ag \rightarrow Ag + e^-$. The electrolyte usually contains chloride ions and the silver ions are deposited as the salt.

$$Ag^{+} + Cl \rightarrow AgCl \qquad \text{(Fatt 1976).}$$

The cathode is made of a noble metal, often platinum, and acts as an electron donor. The reduction of dissolved oxygen to hydroxyl ions at or near the cathode surface gives rise to an electric current if a reference electrode is also in the solution.

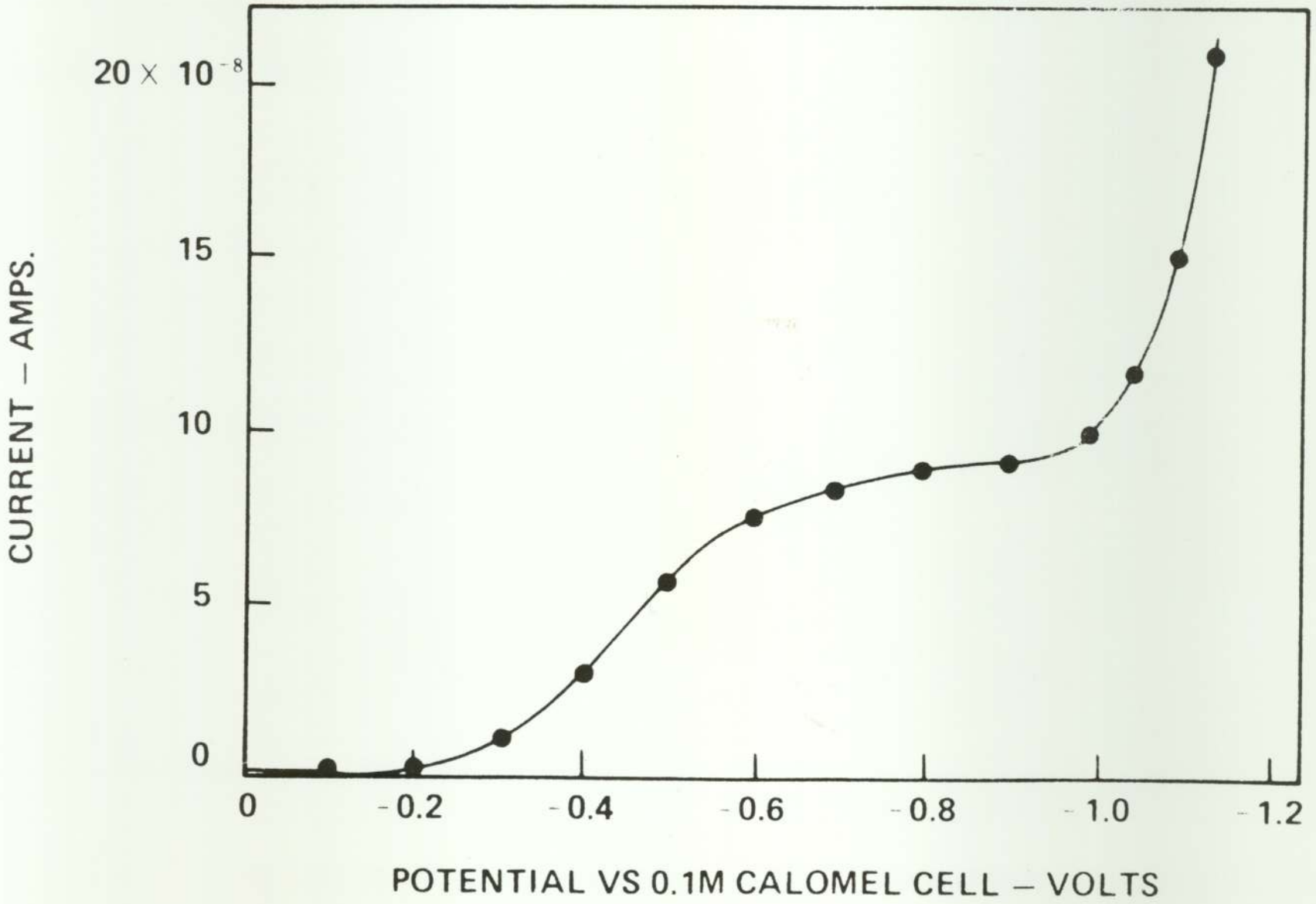
In theory, if there is no potential difference between the reference anode and oxygen cathode there will be no current flow. However, impurities in the solution often cause a small current to become established. Figure 3.1 shows a plot of current flow against applied voltage. Below -0.2V there is little oxygen reduction. Above this value there is an increase in current, proportional to the applied potential difference; there is adequate oxygen available at the cathode and current is only limited by the applied potential. As the potential difference increased to -0.6V, a plateau is reached when increasing the potential difference further does not produce a significant increase in current. Above -1.00V other electrode reactions occur and a steep increase in current output results.

Oxygen diffusion to the cathode surface is maximal at a potential above -0.6V but below -1.00V. Within this range the electrode is said to be "polarised" as it adopts the externally applied potential with little or no change in current flow (Koltoff and Lingane, 1952). Difficulty

Figure 3.1

Steady state current observed when the voltage applied is varied from 0 - 1.1 V with respect to a calomel cell

After Davies, 1962. Redrawn from Fatt, 1976.


20 × 10⁻⁸
15
10
5
0
CURRENT – AMPS.
0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
POTENTIAL VS 0.1M CALOMEL CELL – VOLTS

arises in isolating the sensor components (Anode, Cathode, and Electrolyte) from the test solution. Clark (1956, 1959), placed anode and cathode side by side, and together with the electrolyte, covered them all with a thin oxygen permeable membrane to isolate them from all other components. A membrane of low oxygen diffusion rate used in this way results in covered sensors producing a lower current output than a bare sensor, for a given oxygen tension.

Membrane covered sensors are less sensitive to flow or stirring effects. Stirring will increase the sensor current output above that obtained in a stagnant liquid.

An increase in membrane thickness causes an increase in response time and decrease in sensor current (Fatt, 1976; Stuck et al.1971). To eliminate the effects of both flow and stirring, the membrane needs to be of thickness equivalent to six electrode diameters and have a diffusion co-efficient/oxygen solubility product equal to the test solution. Such a membrane is not feasible, and the design of an oxygen sensor requires a compromise between cathode size, membrane thickness and the gaseous diffusion characteristics of the membrane material.

3.1.2 APPARATUS

The oxygen sensors used in the present study were made by Mr J Treherne from the Medical Physics Department at University College Hospital, London, under the direction

of Dr D Parker and designed in conjunction with Dr C G Wigham. The sensor of the Clark type, consists of a 25u platinum wire cathode embedded in a water resistant epoxy resin.

Surrounding the resin is a ring of silver 2.8 mm thick, which forms the anode. A 12u teflon membrane, D606 (Radiometer V.A. Howe Limited) is held in place over the end of the eletrode by a stainless steel ring that fits firmly over the delrin (ICI) casing. Figure 3.2. shows the electrode assembly.

The sensor was connected to a high impedance amplifier which has controls for range selection, zero adjust and calibration. A constant voltage supply of -0.8V was connected to the amplifier and provided the polarising voltage for the sensor. A variable resistance adjusted amplifier output to match the chart recorded input voltage requirement. The chart recorder is a Vitatron Model UR40 (Vitatron U.K. Limited).

The practical arrangement of the apparatus is shown in Figure 3.3.

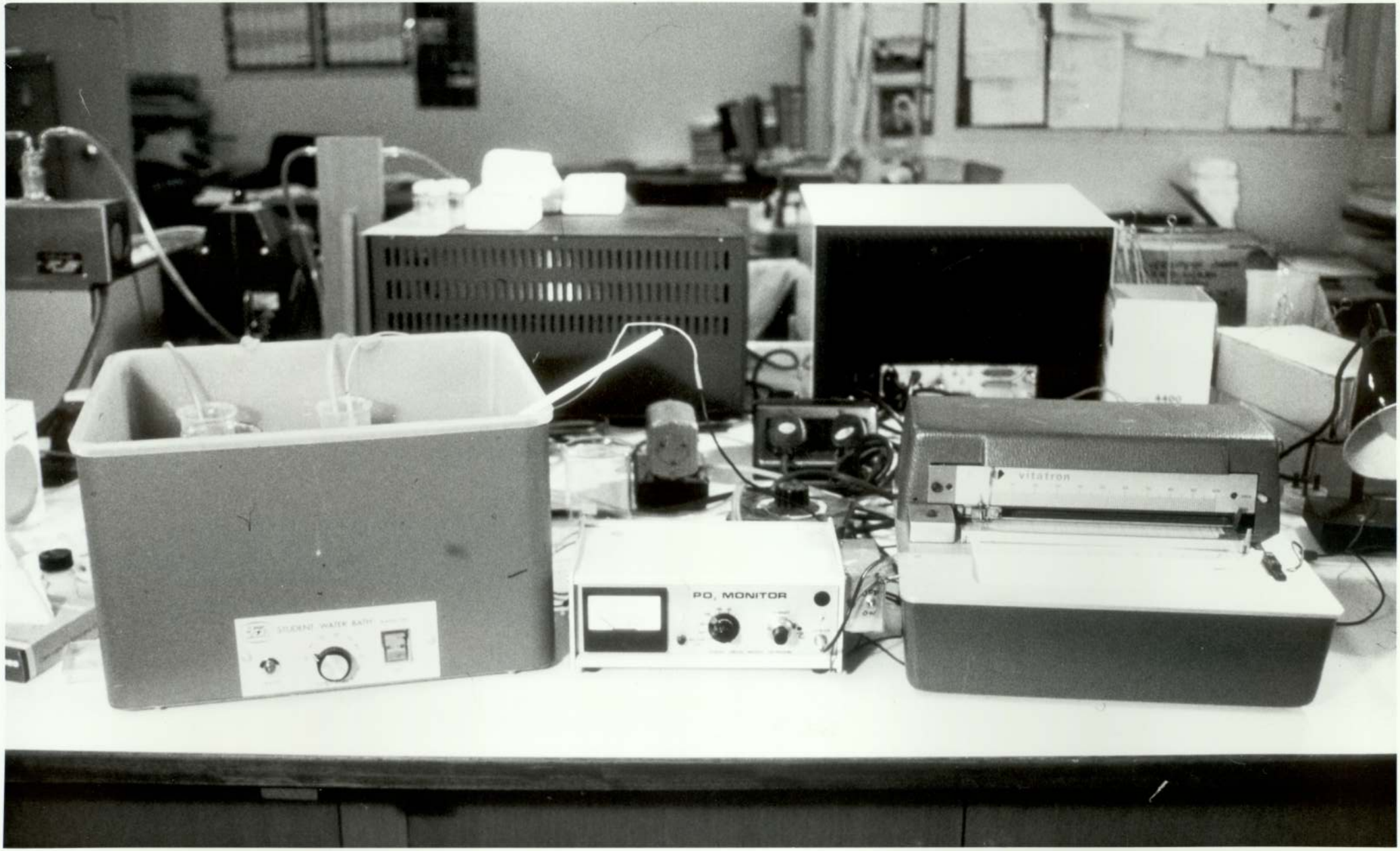
The senor was mounted in a hydrogel sleeve (Sauflon 70) which enhances its stability on the cornea and facilitates ease of handling. The design of the sleeve is as described by Wigham (1978).

Figure 3.2

Oxygen Sensor and Hydrogel Sleeve

Figure 3.3.

Laboratory Arrangements of
Oxygen Sensor Peripheral Apparatus.


PO, MONITOR
vitatron

3.1.3 METHOD

The electrode was assembled afresh every day, using a new membrane, clean sleeve and fresh electrolyte. The sensor was filled with electrolyte as described in 3.1.5. The whole sensor was washed in 0.9% sodium chloride to remove traces of electrolyte from the outside and the hydrogel sleeve was slid into position. The sensor was calibrated by placing it in nitrogen saturated 0.9% sodium chloride solution maintained at 34^{o}C in a water bath. The amplifier was set to read zero using the set zero gain control. The sensor was transferred to an air saturated 0.9% sodium chloride solution also at 34^{o}C and the amplifier set to read 155 mm Hg using the calibration control. The chart recorder was adjusted to give a zero reading for zero amplifier output. The potential divider was adjusted such that 100% scale reading represented an amplifier output corresponding to 155 mm Hg. The response time was estimated from the time taken for an 80% response in moving from a nitrogen saturated solution to an air saturated solution and fell within a two second period.

The properties of this electrode system have previously been described (Wigham, 1978). Following calibration the oxygen sensor assembly was maintained at 34^{o}C in nitrogen saturated sodium chloride until required for use. The assembled sensor was used for up to three hours, after which time it was completely re-assembled and re-calibrated.

3.1.4 SOURCES OF INSTRUMENTAL ERROR

Values of apparent corneal oxygen consumption vary with different sensors. As stated previously (2.3) such values of corneal oxygen consumption obtained are relative and not absolute values. The main source of such variation is due to membrane tension, the volume and thickness of the electrolyte and the sensor response time (Fatt, 1979). The layer of electrolyte trapped between the membrane and electrode surface is considered as part of the oxygen reservoir with the tear film between the membrane and the cornea. The thickness of both layers is unknown and likely to be variable with each assembly of the sensor. The small volume of the oxygen reservoir allows rapid depletion of the available oxygen such that steady state uptake of oxygen cannot be assumed. Such a system is therefore only of value where relative information is required, and the term apparent oxygen uptake has been adopted in this work. (Larke et al. 1981).

3.1.5 STEPS TAKEN TO IMPROVE ACCURACY OF SENSOR OUTPUT

(I) METHODS OF ELECTROLYTE INSTILLATION

The method of instilling the electrolyte described by Wigham (1978) required an excess of fluid to be present. The excess fluid escaped when the sensor cap and electrode assembly were screwed together. However, the flush

fitting thread resisted such fluid escape and the volume of electrolyte and the pressure on the membranes may vary considerably at each assembly.

To overcome this problem, the sensor cap and electrode assembly were joined together before instilling the electrolyte. One drop of electrolyte was placed on the electrode tip from a 1 ml syringe (Gillette Industries), with a No. 18 needle. This gave greater control over electrolyte volume. The membrane was then carefully placed onto the electrolyte/electrode tip and secured with the stainless steel retaining ring. Care was taken to ensure the absence of air bubbles which was confirmed visually using a binocular microscope.

(II) RESPONSE TIME

The response time was estimated from the time taken for an 80% response in moving from an air saturated solution to a nitrogen saturated solution and vice versa. The initial response time of five seconds corresponds to that of Wigham (1978) and is faster than others reported, (Fatt, 1979; Efron and Carney, 1979), The time of the contact between the electrode and the cornea is less; consequently, the response time is of greater significance as an error source, (Fatt, 1979). It was found that by careful electrode assembly to ensure no oxygen bubbles

remained in the electrolyte layer, the response time could be reduced to a maximum of two seconds. This response time was checked prior to, during and after all experimental work.

(III) CALCULATION OF RESULTS

The graphical method of calculation described by Wigham (1978) was dependent on the visual assessment of a best-fit line through the data points. This linear regression is carried out by the method of least squares and subsequently the whole mathematical procedure has been transferred to a computer programme. (Appendix I). This improved the accuracy of the regression line fit and allowed the results to be obtained with greater speed.

(IV) ELECTROLYTE SOLUTION

A small quantity of electrolyte (Potassium Hydroxide) came into contact with one subject's cornea, producing a mild alkaline burn. Consequently, an alternative electrolyte was sought. Potassium Chloride (0.1M) (Parker, 1978) and sodium chloride (0.9%) (Fatt, 1979) were suggested. Sensor response time was compared using all three electrolyte solutions. No statistically significant difference was obtained. (Appendix 2). Owing to the availability and non-irritant nature of sodium chloride 0.9% it was adopted as the electrolyte for future experimental work.

3.1.6 PATIENT SELECTION

All subjects used in experiments detailed in this chapter were volunteers who fell within a previously determined acceptance profile (Appendix 3). Prior to the commencement of any experimental procedure each subject was issued with a written description of the experiments in which he was to participate. The subject was then requested to sign a consent form and to complete personal history details. (Appendix 4, 5 and 6).

EXPERIMENTAL PROCEDURES

3.2 EXPERIMENT 1

TO DETERMINE INSTRUMENTAL PRECISION AND EFFECTS OF REPEATED OXYGEN MEASUREMENT OF APPARENT CORNEAL OXYGEN CONSUMPTION RATE.

3.2.1 INTRODUCTION

After improving the accuracy of the measurement of oxygen consumption rate, (3.1.5), it was necessary to assess the instrumental variability of the electrode assembly. It was further decided to ascertain if apparent corneal oxygen consumption rate was affected by the technique of repeated measurement. This information was required to assist in the experimental design of subsequent work. To achieve this ten repeated measurements of apparent corneal uptake rates were taken on three male subjects at five minute intervals.

3.2.2 METHOD

Each subject was placed in the supine position and instructed to fixate a distant object overhead. Objective cover testing was carried out to eliminate patients with a strabismus or high phoria such that there was no movement of the eye induced by the placement of the oxygen electrode. The subject was told to keep his eyes as still as possible once the sensor was in position. The sensor was mounted in a hydrogel sleeve and held in contact with the unanaesthetised cornea for approximately 30 seconds. This procedure was repeated 10 times allowing a five minute interval between successive readings. Following termination of the experimental procedure, all subjects were examined with the aid of a slit lamp and fluorescein. The practical arrangement is shown in Figure 3.4.

3.2.3 RESULTS

The results of the successive measurements on the same subjects are shown in Table 1. One way analysis of variance revealed no statistically significant differences between the groups. (Table 2). The mean value of apparent corneal oxygen uptake and coefficient of variance are shown in Table 3.

Figure 3.4.

Oxygen Sensor being placed on
Unanaesthetised Cornea

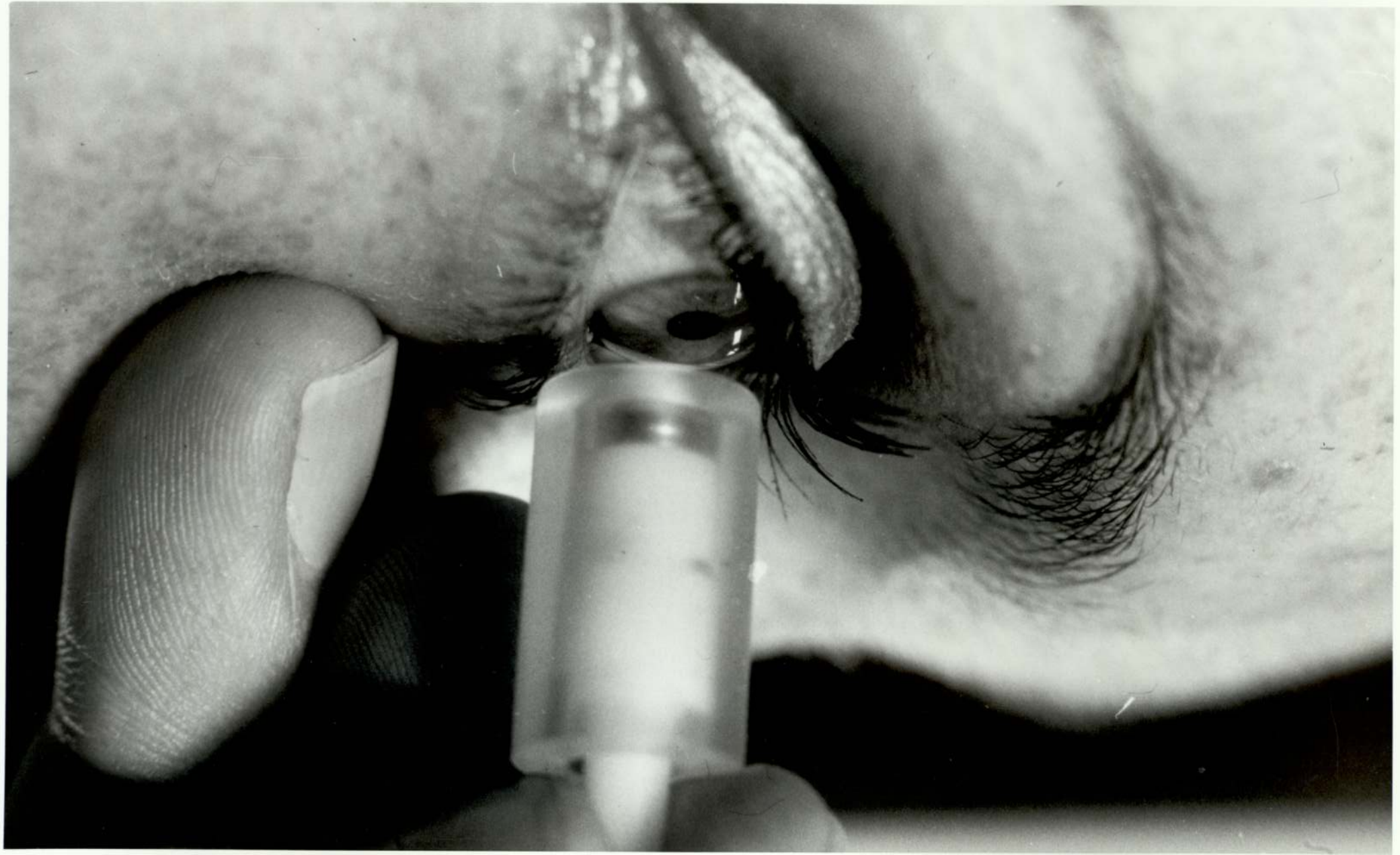


TABLE 1

Values for calculated apparent corneal oxygen consumption rate in microlitres per hour per square centimetre of corneal surface, ($ulO_2cm^{-2}Hr^{-1}$) measured on three subjects ten times at five minute intervals between each reading.

Repeated Measurement No.	1	2	3	4	5	6	7	8	9	10
Subject 1.	5.8	6.1	5.7	6.9	6.6	7.3	7.5	7.5	7.3	5.3
Subject 2.	7.3	7.3	7.6	7.8	7.5	7.3	7.1	7.2	7.7	7.2
Subject 3.	6.2	7.1	6.7	6.4	8.6	7.1	7.1	6.9	6.0	7.1

TABLE 2

ONE WAY ANALYSIS OF VARIANCE

(From the Data in Table 1.).

	SS	dF	MS	F	
Treatments	3.43	9	0.38	0.69	not significant
Error	11.03	20	0.55		
TOTAL	14.46	29			

TABLE 3

CO-EFFICIENT OF VARIANCE

(From the Data in Table 1)

	Mean Value of Corneal Oxygen Consumption Rate	Standard Deviation	Co-efficient Of Variance
Subject 1	6.6	0.821	12.5%
Subject 2	7.4	0.235	3.2%
Subject 3	6.9	0.717	10.4%

Average Coefficient of Variance

= $\pm$ 8.7%

3.2.4 DISCUSSION AND CONCLUSIONS

The absence of a trend in the data for repeated measurement on the same eye suggests that the degree of trauma to the corneal epithelium, as the electrode is successively applied, does not result in a detectable change in apparent corneal oxygen uptake rate. This is consistent with the slit lamp appearance of the corneae following the experiment, when only superficial epithelial damage was observed. Damage to the superficial epithelium would be unlikely to significantly affect the corneal oxygen requirement, which would be greatest in the cells of highest metabolic activity in the basal layers. This finding permitted the successive repeated measurement of oxygen consumption rate to be carried out in the future experimental work. The minimum time interval that could elapse between successive readings without altering the oxygen consumption rate was not determined. A time interval of five minutes as used in the experiment was found to be adequate for the practical work detailed in this thesis.

The coefficient of variance was $\pm$ 8.70%. Although this is greater than that inherent in some other forms of ocular measurement, for example, pachometry $\pm$ 2.5% (Larke, 1981; Hirji and Larke, 1978), it is similar to that previously reported for oxygen sensors used to measure the oxygen permeability characteristics of hydrogel materials for contact lens application (Fatt and Morris, 1977), and for

other oxygen sensors (Fatt, 1979), and thus was deemed to be acceptable for this and future experiments.

3.3 EXPERIMENT 2

DISTRIBUTION OF HUMAN IN VIVO OXYGEN UPTAKE RATE IN A POPULATION SAMPLE OF 88 SUBJECTS

3.3.1 INTRODUCTION

This experiment was designed to examine the distribution of human in vivo apparent oxygen consumption rate in a population sample using the oxygen sensor described previously (3.1.2). This information was required to assist both in the experimental design and proposed statistical analysis of the longer term effect of extended soft contact lens wear on apparent corneal oxygen consumption rate (Chapter 4). Using the modified electrode and method of assembly the distribution of corneal oxygen uptake rate in a population of normal eyes, previously determined by Wigham (1978), was re-examined.

3.3.2 METHOD

The apparent corneal oxygen consumption rate was measured on eighty-eight volunteer subjects, both male and female. The experimental procedures and patient selection were as described for Experiment 1, (3.1.6). Measurements were carried out at random times throughout the day and on different days. Male and female volunteers were used in this and subsequent work to provide the required number of suitable subjects for each experiment.

It was not possible to examine the potential effect of the menstrual cycle on oxygen uptake of the cornea due to the potentially long time of observation and complications due to the numerous types and frequent use of oral contraceptives.

3.3.3 RESULTS

The raw values and distribution of a population sample are listed in Table 4 and shown graphically in Figure 3.5. D'Agastino's test for normality (Zar, 1974) revealed no statistically significant departure from a 'normal' distribution for a probability $p < 0.01$. (Table 5).

The mean value of apparent corneal oxygen uptake rate was 6.5 ul O_2 $cm^{-2}hr^{-1}$, which is identical to that previously found by Wigham (1978).

3.3.4 DISCUSSION AND CONCLUSIONS

The mean value obtained for corneal oxygen uptake rate is in good agreement with previously determined values using a similar sensor (Wigham, 1978). This is, however, higher than the values previously reported (Hill and Fatt, 1963(a)(b); Jauregui and Fatt, 1971, 1972; Farris et al. 1965). As previously stated, the values obtained are relative and not absolute. This is due to both variations in sensor design and the mathematical

TABLE 4

RAW VALUES OF APPARENT HUMAN CORNEAL OXYGEN UPTAKE RATES ON 88 VOLUNTEERS

($ulO_2\ cm^{-1}hr^{-1}$)

Experiment 2

5.583	7.675
5.158	5.637
5.191	5.674
7.720	6.370
4.794	8.250
2.638	7.147
5.038	5.777
5.080	8.926
6.190	9.690
6.521	9.440
7.940	8.406
7.288	7.056
6.507	7.689
6.230	7.563
6.850	7.347
4.542	5.538
5.456	7.475
5.339	8.166
6.490	9.107
6.630	3.636
7.670	4.222
5.638	4.780
4.703	7.180
5.778	9,287
8.185	6.272
6.332	4.149
8.814	7.056
6.294	
9.900	5.480
5.770	5.504
6.550	8.580
5.690	5.440
7.620	4.616
8.203	5.747
6.710	5.920
7.461	6.432
6.194	8.243
7.125	6.438
7.525	6.903
6.326	5.538
9.612	5.180
7.104	6.020
6.041	5.001
6.037	4.860
	5.470

Mean = 6.5 ulo2 $cm^{-2}hr \cdot 1$ Standard Deviation = 1.461

Figure 3.5.

Population distribution of apparent corneal oxygen consumpton rate in 88 volunteer subjects

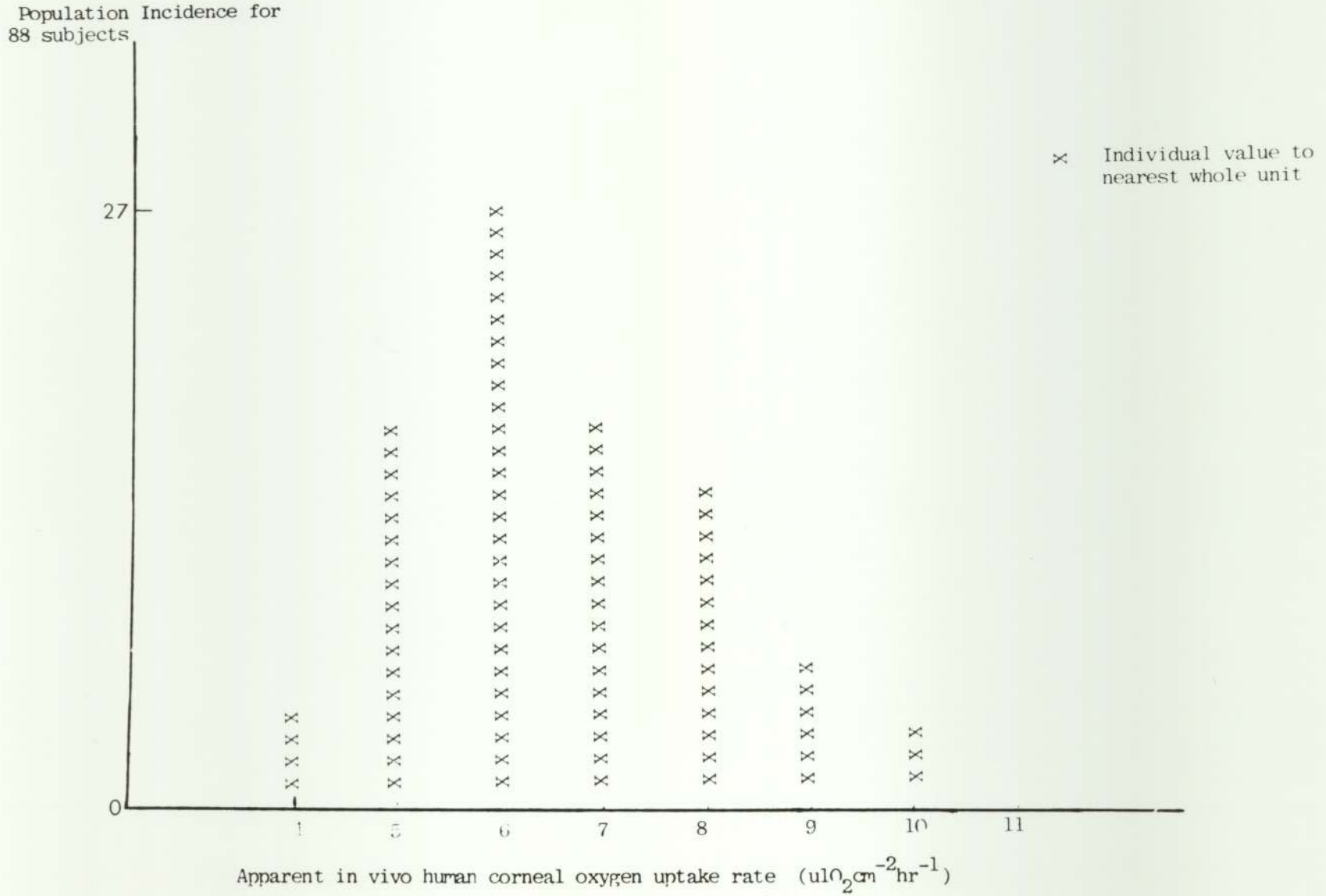

Population Incidence for
88 subjects
27
0
x Individual value to
nearest whole unit
1
5
6
7
8
9
10
11
Apparent in vivo human corneal oxygen uptake rate (ulO_2 cm^{-2} hr^{-1})

TABLE 5

D'AGASTINOS TEST FOR NORMALITY

POPULATION SAMPLE DISTRIBUTION OF APPARENT CORNEAL OXYGEN CONSUMPTION RATE MEASURED ON 88 VOLUNTEER SUBJECTS.

Grouped Oxygen Uptake Rate ulo2cm^{-2}hr^{-1}	O_2 Uptake Rate $\bar{x}i$	Observed Frequency fi	$Efi\bar{x}i$	$Efi\bar{x}i^{-2}$
< 3.5		1))4		
3.5 - 4.5	4	3)	16	64
4.5 - 5.5	5	17	85	425
5.5 - 6.5	6	27	162	972
6.5 - 7.5	7	17	119	833
7.5 - 8.5	8	14	112	896
8.5 - 9.5	9	6	54	486
9.5 - 10.5	10	3	30	300
> 10.5				
		Efi = 88	Efixi = 578	$Efixi^{-2}$ = 3976

$$SS = 3976 - \frac{(578)^2}{88} = 179.59091$$

T = 3062 D = 0.27678

Since D is neither $\leq$ 0.2707 nor $\geq$ 0.2870 ($p < 0.01$) the null hypothesis (Ho) of population normality cannot be rejected.

assumptions made in calculating the oxygen consumption rate. The variation between the results obtained in the present study and those previously reported in the literature, may be explained by these factors. The similarity between the present results and those of Wigham (1978) would be expected due to the similar nature of the sensor and mode of calculation in each case. The distribution of apparent epithelial oxygen uptake rates is somewhat greater than for whole body respiration (Bell et al. 1976). Such a wide divergence of uptake rates may partly explain the generally observed differences in the manner to which patients respond to contact lens wear. The difference between the highest and the lowest values for respiration rate recorded in the present study is approximately three fold. Allowing for a margin of error of $\pm$ 8% on these values, this still represents a considerable difference between individuals. One explanation may be given in terms of differing numbers of epithelial cell layers in the cornea (Hill, 1977). In rabbits, as few as three and as many as seven layers of cells have been observed. A similar circumstance in man would provide a satisfactory interpretation of the observed results. A wide variation in individual subject response to contact lens wear has been observed with regard to corneal swelling and oedema (Sarver et al. 1981; Barr and Schoessler, 1981; Tomlinson et al. 1981). Large individual variations in some aspects of corneal physiology do occur and this may provide

part of the explanation for the success or failure of contact lens wearing subjects. The wide variation in individual corneal oxygen utilisation confirmed in this study suggests that a knowledge of the pre-fitting corneal oxygen requirement may be clinically useful in determining the oxygen transmissibility or fit characteristics of the contact lens of first choice.

3.4 EXPERIMENT 3

THE EXAMINATION OF POSSIBLE DIURNAL (WAKING HOUR) VARIATION OF HUMAN IN VIVO APPARENT CORNEAL OXYGEN CONSUMPTION RATE

3.4.1 INTRODUCTION

No significant change in corneal oxygen consumption rates during the waking hours was demonstrated (Wigham 1978). However, a statistically significant diurnal change in corneal thickness has been observed both with and without contact lens wear (Hirji, 1978; Fatt and Chaston, 1981). Following the modifications to the oxygen sensor, the diurnal oxygen uptake rate was re-examined during the waking-hours (9.00 - 21.00 hours), as the occurrence of any such change would affect the design of future experimental work.

3.4.2 METHOD

Patient selection and instruction were the same as described for previous experiments. Apparent corneal oxygen uptake rates were measured on the right eye of five volunteer male

caucasian subjects at three hourly intervals between 9.00 and 21.00 hours. As a result of previous work (Wigham, 1978), each successive measurement was separated by a 48 hour period to minimise the influence of possible induced epithelial damage. Only subsequently was it found that a significantly shorter time interval could elapse between successive measurements without affecting the results, (3.2). The measurement times were allocated using a Latin Square design.

3.4.3 RESULTS

A Latin Square illustrating the results is shown in Table 6 and 6(A). A three-way analysis of variance was performed on the data. (Table 7). No statistically significant trend in the data was obtained due to either diurnal variation or variation between patients. However, a statistically significant difference ($p < 0.05$) on different days was found.

3.4.4. DISCUSSION AND CONCLUSIONS

No diurnal variation in apparent corneal oxygen consumption rate was detected. The significant difference that exists between results taken on different days may be attributed to (a) differing electrode responses on different days; (b) two unusually high readings on Day 3 and (c) variations in indivudual patients' oxygen requirements on different occasions.

TABLE 6 Latin Square showing calculated values for apparent corneal oxygen consumption rate for five subjects measured between 9.00 and 21.00 hours. ($ul O_2\ cm^{-2} hr^{-1}$).

Time of Day (Hours)	Period Between Measurements (Hours)					
	0	24	48	72	96	YZ
9.00	6.412 A	6.412 E	9.203 D	6.301 C	6.901 B	35.229
12.00	6.484 B	7.111 A	10.769 E	7.619 D	8.672 C	40.655
15.00	6.001 C	7.114 B	6.958 A	7.991 E	7.201 D	35.295
18.00	5.449 D	6.632 C	7.777 B	6.314 A	7.191 E	34.363
21.00	7.001 E	5.421 D	6.664 C	5.982 B	7.884 A	32.952
Y1	32.347	32.720	41.371	34.207	37.849	178.494

Subjects indicated by A, B, C, D, E.

Pt's	A	B	C	D	E	
Yk	34.679	34.288	34.270	35.893	39.364	178.494

TABLE 6(A) Results from Table 6 tabulated to show individual subject values.

Time of Day (Hours)	SUBJECT				
	A	B	C	D	E
9.00	6.412	6.901	6.301	9.203	6.412
12.00	7.111	6.484	8.672	7.619	10.769
15.00	6.958	7.114	6.001	7.201	7.991
18.00	6.314	7.777	6.632	5.449	7.191
21.00	7.884	5.982	6.664	5.421	7.001

Subjects indicated by A, B, C, D and E.

TABLE 7

3 WAY ANALYSIS OF VARIANCE FOR DATA FROM TABLE 6

Total SS	=	1305.64
Mean SS	=	1274.40
(Diurnal SS) Rows	=	6.859
(Order SS) Columns	=	11.830
(Patients) SS	=	3.685

SV	df	SS	MS	F	
Total	25	1305.98			
Mean	1	1274.40			
Due to Diurnal	4	6.859	1.714	2.234	not significant
Due to Order	4	11.830	2.957	3.855	significant (p 0.05)
Due to Patients	4	3.685	0.921	1.200	not significant
Error	12	9.206	0.767		

From tables:

F = (4,12) = 5.41. F. (4,12) = 3.26

0.01 0.05

Due to the improved method of electrode assembly calibration and measurment of response time, it is unlikely that significant variations in electrode response would be an explanatory factor. Two unusually high results were obtained at 9.00 and 12.00 hours on Day 3 of the study. As already stated (2.1.4) the sensor was re-assembled after three hours use. The return to more normally accepted values at 15.00 hours suggests that some electrode malfunction may have been responsible for such high results being obtained at the first two data points. High values would be obtained if the teflon membrane used was thinner than 15u or severely stretched. However, the foregoing explanation does not exclude that a variation in individual patients' corneal consumption rate on different days may also have been a contributory factor.

3.4.5 REPEAT OF DIURNAL (WAKING HOURS) STUDY

INTRODUCTION

The data of Experiment 1 (3.2.1) was analysed subsequent to carrying out Experiment 3 (3.4) and showed that frequently repeated oxygen uptake measurements produced no detectable change in the corneal requirement for oxygen. This finding permitted the waking hour study to be repeated within one 24 hour period. Possible changes in both individual oxygen consumption rate and electrode response on different days was eliminated.

3.4.6 METHOD

The clinical procedure was as described for Experiment 2 (3.4.0).

3.4.7 RESULTS

Appointment times were allocated using a Latin Square design and the results depicted in Table 8 and 8(A). Three-way analysis of variance revealed no statistically significant trend in the data (Table 9). No statistically significant variation in human in vivo corneal oxygen consumption rate was determined during the waking hours (9.00 - 21.00 hours), when the experiment was carried out over a period of one day.

The variance due to individual patient's oxygen requirements was less than that when a 48 hour period elapsed between successive measurements. The highest mean oxygen consumption rate was found to occur at 15.00 hours which agrees with the previous findings of Wigham (1978);

3.4.8 DISCUSSION AND CONCLUSIONS

A diurnal variation in the behaviour and function of certain tissues has been demonstrated, if not fully explained. There are no mitotically labile mammalian tissues investigated which have failed to demonstrate circadian rhythmicity in cell division

TABLE 8 LATIN SQUARE SHOWING CALCULATED VALUES OF APPARENT CORNEAL OXYGEN CONSUMPTION RATE FOR FIVE SUBJECTS MEASURED BETWEEN 9.00 and 21.00 HOURS OVER ONE DAY. ($ul O_2\ cm^{-2} hr^{-1}$).

Time of Day (Hours)	Order of Measurement					Mean	Yz
9.00	A 5.851	B 6.685	C 7.487	D 6.138	E 5.757	6.383	31.918
12.00	E 7.699	A 6.179	B 5.811	C 7.079	D 5.421	6.437	32.189
15.00	D 7.690	E 6.686	A 6.014	B 5.920	C 7.355	6.733	33.665
18.00	C 6.756	D 6.286	E 5.924	A 5.967	B 6.164	6.219	31.097
21.00	B 7.758	C 7.276	D 6.535	E 5.514	A 5.935	6.603	33.018
Mean	7.150	6.622	6.354	6.123	6.126		
Yl	35.754	33.112	31.771	30.618	30.632		161.887

Subjects indicated by A, B, C, D, E.

Pt's.	A	B	C	D	E
	29.946	31.580	32.070	35.953	32.338

TABLE 8(A) Results from Table 8 tabulated to Show Individual Subject Values.

Time Of Day (Hours)	SUBJECTS				
	A	B	C	D	E
9.00	5.851	6.685	7.487	6.138	5.757
12.00	6.179	5.811	7.079	5.421	7.699
15.00	6.014	5.920	7.355	7.690	6.686
18.00	5.967	6.164	6.756	6.286	5.924
21.00	5.935	7.758	7.276	6.535	5.514

Subjects indicated by A, B, C, D, and E.

TABLE 9

3 WAY ANALYSIS OF VARIANCE FOR DATA

FROM TABLE 8

Total SS	=	1060.98
Mean SS	=	1048.29
(Diurnal) SS Rows	=	0.796
(Order SS) Columns	=	3.696
(Patients) SS	=	3.891

SV	df	SS	MS	F	
Total	25	1060.98			
Mean	1	1048.29			
Due Diurnal	4	0.796	0.199	0.554	Not Significant
Due Order	4	3.696	0.924	2.574	Not Significant
Due to Patients	4	3.891	0.972	2.708	Not Significant
Error	12	4.307	0.3589		

From tables F = 0.05 (4,12) = 3.26 $\therefore$ Values not significant.

(Scheving et al. 1974). The pacemaker controlling diurnal function has not been determined in many instances. Some evidence exists for adrenergic regulation, although this has been excluded regarding the mitosis of rabbit corneal epithelium (Fogle et al. 1980).

A diurnal change in corneal thickness has been demonstrated (Hirji, 1978; Gerstman, 1972; Kikkawa, 1973, 1974), which is maximal at 6.00 a.m.; a gradual thinning occurs during the day. The initial component has been attributed to tear film evaporation on waking causing fluid to move out of the cornea by osmosis (Hirji, 1978), the tear film is hypotonic during eyelid closure due to reduced evaporation (Mishima and Maurice, 1961). Diurnal changes are reduced during periods of extended soft contact lens wear; this has been attributed to both the reduced tear film evaporation caused by the mechanical barrier created by the lens and the increased tear volume present due to tears imbibed into the lens matrix, (Hirji, 1978).

Gerstman (1972) suggested that corneal thinning was due to a gradual increase in corneal metabolism, whilst Kikkawa (1973, 1974) and Fujite (1980) have postulated that diurnal thickness changes reflect general bodily diurnal patterns which may be controlled by the steroid concentration in the blood. The exact nature of such diurnal rhythms is therefore still unclear.

Hill (1979) suggested that there will be a reduction in corneal oxygen uptake corresponding to maximal corneal thickening the passage of oxygen will be inhibited by the presence of extra fluid. From the foregoing discussion this would imply the maximum affinity for oxygen to occur during the waking hours. Maximum oxygen uptake rate was observed in the present study at 12.00 and 15.00 hours which confirmed the previous findings of Wigham, (1978). This is also consistent with the time of maximum activity for whole body respiration and function (Bell et al. 1976) Not all diurnal patterns, however, have maximum activity at the same time. Diurnal changes in corneal epithelial cell mitosis rate have been observed and reviewed (Fogle et al. 1980). The peak mitotic rate occurs around 6.00 a.m. (Bertalanffy and Lau, 1962) and may vary within a four hour period (Scheving et al. 1974) Periods of high mitotic activity in the basal cell layers would be expected to need a high oxygen requirement. This would suggest a high corneal oxygen uptake rate to occur at around 6.00 a.m. However, in man this maximum mitosis may not take place until after waking, as the maximum oxygen requirement would otherwise coincide with the reduced partial pressure of oxygen present in the eye closed situation. It may be assumed that the maximum oxygen requirement may not coincide with the visible maximum rate of cell mitosis, as changes in the rate of cell synthesis will occur both before and after.

From the foregoing, a circadian change in apparent epithelial oxygen uptake rate may be anticipated, however, the expected time of maximum oxygen consumption is not clear and the results of the present study are inconclusive.

The following experimental design limitations need to be overcome in future work.

1. No data point was at 6.00 a.m. to coincide with maximal corneal thickness and mitotic activity. Any change which may have taken place could have altered by the first data point at 9.00 a.m.

2. The interval between data points was 3 hours. The time of maximum mitosis and corneal thickening could occur over a four hour period. Sampling at one hour intervals may give a more accurate assessment.

3. The total number of subjects used was five. This was the maximum number who could be practically seen at any one data point and clinically supervised. However, due to both the large distribution of individual subject oxygen requirement (3.3.1) and the instrumental error of the sensor (3.2.1), subsequently calculated, this number would appear insufficient. Future work is moving towards the development of a disposable pre-calibrated sensor, this would enable more subjects to be seen at each data point, as the time for calibration and sterilisation would be considerably reduced.

4. Only one measurement was carried out on each subject at each data point. As repeated measurement has no effect on oxygen uptake (3.2) this number could be increased.

It may be that a diurnal change in the human in vivo corneal oxygen consumption rate does occur. The instrumental variability of the present system may be too high to detect any such changes that are present.

3.5 EXPERIMENT 4

APPARENT OXYGEN CONSUMPTION RATE IN THE IPSILATERAL AND CONTRALATERAL NON-LENS WEARING CORNEA.

3.5.1 INTRODUCTION

The cornea has an increased affinity for atmospheric oxygen following contact lens wear or eyelid closure as reported by Hill and Fatt (1964 (b)) in rabbit. This phenomenon subsequently termed "oxygen debt" has been observed in the living human eye (Hill, 1965; Jauregui and Fatt, 1971, Farris et al. 1971; Farris and Donn, 1972; Hill et al. 1973; Hill, 1979; Roscoe and Hill, 1980).

The "oxygen debt" generated by contact lens wear was compared to the oxygen consumption of the contralateral non-lens wearing eye which acted as a control (Hill, 1979; Farris and Donn, 1972; Hill, 1975).

No evidence has been established to show that a contralateral change in oxygen consumption rate does not occur following stimulation of the ipsilateral eye. Evidence for a sympathetic effect between the two eyes has been reported for various ocular parameters (Maul and Sears, 1979).

The purpose of the experiment now reported was to investigate the influence on the apparent oxygen consumption rate of the contralateral eye, induced by changes taking place in the experimental eye.

The oxygen deprivation of the cornea due to contact lens application or lid closure is maximal following a period between 30 seconds to 1 minute of covering (Hill and Fatt, 1964 (b)). Re-exposure to air allows the cornea to return rapidly to its pre-deprivation level within approximately the same period of time (Hill and Fatt, 1964 (b); Jauregui and Fatt, 1971; Hill, 1965). However, a decrease in corneal oxygen consumption at 10 minutes following contact lens removal has been described (Farris, et al. 1971; Hill, 1979). This phenomenon has been termed "reactive depression" and was attributed to the oedema produced using hard contact lenses, (Hill, 1979). Such a reactive depression was not demonstrated following soft contact lens wear (Farris and Donn, 1972).

Oxygen debt is greater when a "steep" fitting hard contact lens is worn compared to a "flat" fitting lens. By comparing the results so obtained with those calculated by passing gases of differing oxygen concentration over the cornea, the oxygen tension under a contact lens can be examined (Jauregui and Fatt, 1971). Oxygen debt varies inversely with the partial pressure of oxygen at the anterior corneal surface (Jauregui and Fatt, 1971). The oxygen debt generated during periods of contact lens wear does not

differ between the novice and the long-term contact lens wearer (Hill, 1965).

3.5.2 METHOD

Initial values for oxygen consumption rate prior to contact lens insertion were measured in seven non-contact lens wearing male (20 - 25 years) volunteer subjects, using the membrane covered oxygen sensor and procedure previously described in 3.2.2. Following measurement one eye was fitted with a Sauflon p.w. contact lens (Contact Lens Manufacturing), 0.4mm flatter than the flattest corneal meridian. In no case was corneal toricity greater than 0.1mm.

The contact lens was left in situ for five minutes. Oxygen uptake rates were measured on the lens wearing eye within ten seconds of contact lens removal, and subsequently after a further period of one minute and ten minutes. The experimental procedure was repeated on the same subjects, but oxygen consumption rates were taken from the non-lens wearing contralateral eye. The sequence of the experiment was randomised between the lens wearing and non-lens wearing eye.

3.5.3 RESULTS

The results for the lens wearing eye are displayed graphically in Figure 3.6 and tabulated in Table 10. They show

no statistically significant ('t' test) difference between oxygen consumption rates prior to lens wear, compared to values taken one and ten minutes following lens removal.

There is however, a significant increase in oxygen demand within ten seconds of lens removal ($p < 0.25$).

Table 11 shows the results obtained for the contralateral eye. This shows the same trend of oxygen consumption as for the lens wearing eye ($p < 0.1$).

3.5.4. DISCUSSION AND CONCLUSIONS

The results demonstrate that an "oxygen debt" is established after only five minutes of contact lens wear. This supports the work of Hill and Fatt (1964(b)), who showed that the cornea reached its maximum affinity for oxygen between thirty seconds to one minute of being covered. The data indicates a recovery to pre-lens wearing uptake rates within one minute of contact lens removal, which agrees with the work of Hill and Fatt (1964(b)); Hill (1965) and Farris et al (1971).

Hill (1979) however, also demonstrated that during hard lens wear a decrease in oxygen demand occurred which he termed 'reactive depression' at one and ten minutes following eight hours wear. He attributed this to the presence of corneal oedema provoked by this form of lens.

Figure 3.6

Apparent corneal oxygen uptake rate for lens wearing and non-lens wearing eye before lens wear and following lens removal

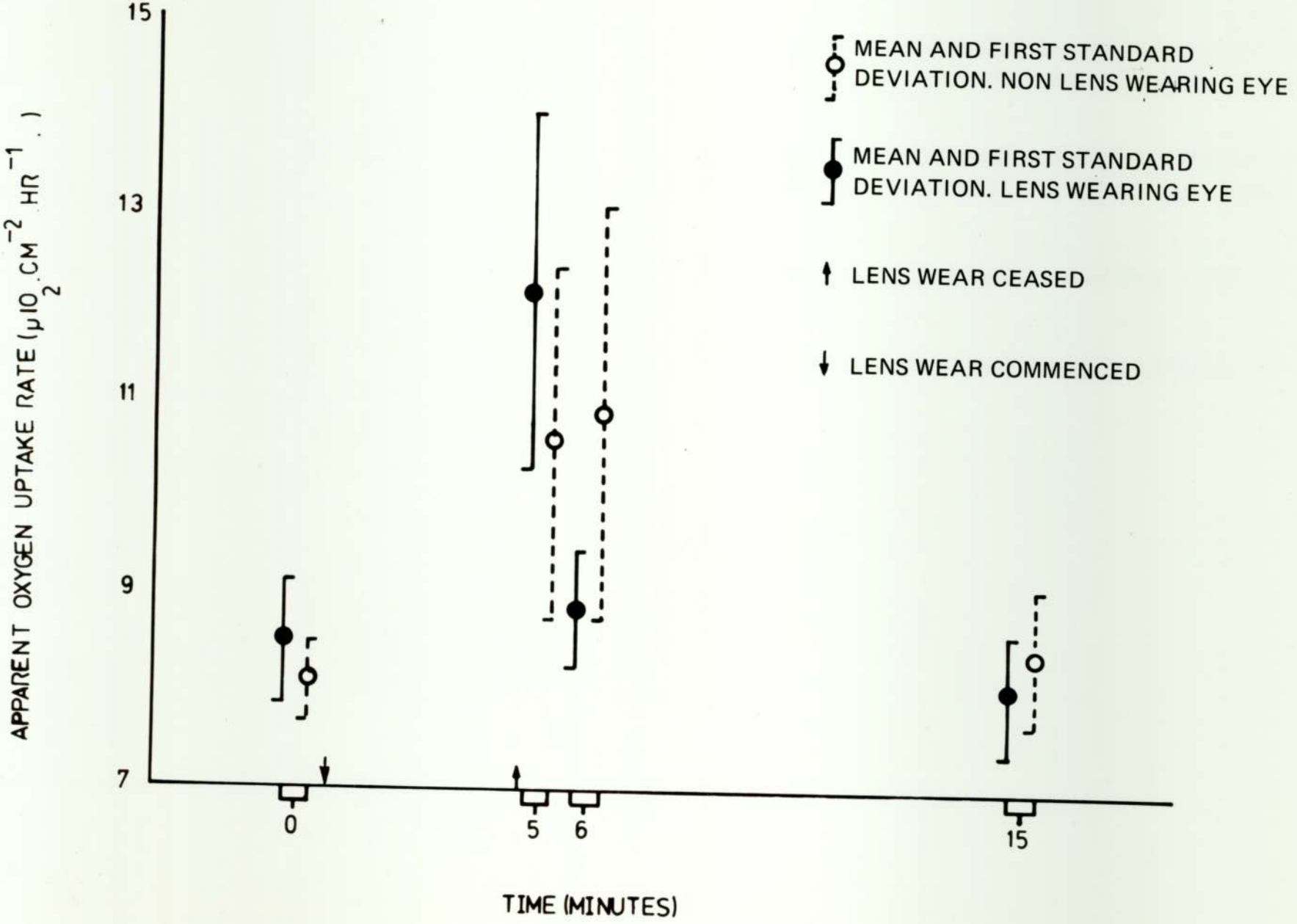

15
13
11
9
7
APPARENT OXYGEN UPTAKE RATE ($\mu l O_2 . CM^{-2} HR^{-1}$)
0
5
6
15
TIME (MINUTES)
MEAN AND FIRST STANDARD DEVIATION. NON LENS WEARING EYE
MEAN AND FIRST STANDARD DEVIATION. LENS WEARING EYE
LENS WEAR CEASED
LENS WEAR COMMENCED

TABLE 10

APPARENT CORNEAL OXYGEN UPTAKE RATES

LENS WEARING EXPERIMENTAL EYE ($ul O_2\text{-}cm^{-2}hr^{-1}$).

Subject No.	Baseline	10 Seconds After Contact Lens Removal	1 Minute After Contact Lens Removal	10 Minutes After Contact Lens Removal
1	8.167	10.710	8.390	6.935
2	8.465	11.827	8.576	9.472
3	11.283	16.602	10.345	9.619
4	8.583	12.791	9.409	8.427
5	7.641	17.237	9.445	7.197
6	8.632	8.797	9.063	6.674
7	6.986	7.290	6.423	8.599
Mean	8.536	12.179	8.807	8.131
Standard Deviation	1.348	3.722	1.232	1.207

Student 't' test. One-tailed

Baseline v 10 seconds after lens removal $t = 2.972$ Significant ($p < 0.25$)

Baseline v 60 seconds after lens removal $t = 0.795$ Not Significant

Baseline v 600 seconds after lens removal $t = 0.798$ Not Significant

TABLE 11

APPARENT CORNEAL OXYGEN UPTAKE RATES

CONTRALATERAL NON-LENS WEARING EYE ($ul O_2 cm^{-2} hr^{-1}$).

Subject No.	Baseline	10 Seconds After Contact Lens Removal	1 Minute After Contact Lens Removal	10 Minutes After Contact Lens Removal
1	7.922	8.721	8.264	8.081
2	8.241	10.033	9.801	8.632
3	8.470	12.719	18.813	7.286
4	10.050	10.585	7.959	7.380
5	7.989	9.210	9.081	9.200
6	7.655	17.061	15.191	11.380
7	6.964	6.250	6.382	7.270
Mean	8.184	10.654	10.784	8.461
Standard Deviation	0.8817	3.438	4.499	1.483

Student 't' test. One-tailed.

Baseline v 10 seconds after lens removal $t = 1.913$ Significant ($p<0.1$)

Baseline v 60 seconds after lens removal $t = 1.508$ Significant ($p<0.1$)

Baseline v 600 seconds after lens removal $t = 0.369$ Not Significant.

No statistically significant evidence of 'reactive depression' has been found in the present study, which entailed the use of a high water content soft contact lens for a short period of wear. This would be consistent with the lack of oedema produced by this form of lens over a five minute period. The data recorded in the present experiment suggests the presence of a 'sympathetic effect'. Changes in the apparent respiration rate induced by the contact lens in the experimental eye, are mimicked in the non-lens wearing fellow eye.

Visual inspection of the data for the lens wearing eye (Table 10) shows an increase in oxygen uptake rate within 10 seconds of lens removal. This is only marginal for subjects 6 and 7, and may be due to a delay in placing the oxygen sensor onto the cornea following the removal of the lens. This was sometimes encountered due to subject blinking and apprehension. Although the sensor was in position in every case within 10 seconds of the lens being taken out, there would be necessarily a drop in the oxygen requirement over this time period. All participating subjects were trained and selected for their tolerance of the experimental procedures used. In the author's view it would be difficult to ensure that the time taken between contact lens removal and placement of the oxygen sensor was less than 10 seconds in every case.

This factor is therefore a limit to the experimental design. A greater level of subject comfort may be achieved if a topical anaesthetic was used prior to the commencement of the experiment, but this has been shown to influence corneal metabolic rate, albeit the effect of Benoxinate was found to be minimal. (Augsburger and Hill, 1972).

Visual inspection of the data for the non-lens wearing eye (Table 11) shows a general increase in oxygen uptake rate to occur 10 seconds and one minute following the removal of the contact lens from the fellow eye. This is however, very marked for subjects 3 and 6. Subject 3 is further unusual, having a maximum oxygen requirement 1 minute after the cessation of lens wear. The inclusion of the results for these two subjects gives statistical significance to the overall results, but suggests that they should be interpreted with some caution.

A contralateral change in oxygen consumption causes concern over the use of the fellow-eye as a control in this type of experiment, and raises intriguing questions of the possible mechanisms regarding this event. A sympathetic increase in corneal thickness has been reported (Mandell and Harris, 1968). This was attributed to initial bilateral tear hypotonocity due to reflex tearing as a result of contact lens insertion, with a resulting

osmotic shift of fluid into the cornea. This view was supported by Fatt (1979). More recently a further demonstration of a sympathetic increase in corneal thickness has been reported (Tomlinson and Soni, 1980), although no satisfactory explanation of this phenomenon has been advanced (Tomlinson, 1981).

In addition to osmosis, regulatory mechanisms may involve both hormonal and neural pathways. Short term ipsilateral and contralateral responses to corneal irritation have been examined. The sympathetic response following paracentesis of the anterior chamber of rabbit eye appears to be largely mediated by prostaglandins (Neufeld, et al.1972; Miller et al. 1973), whereas the short term responses to topical nitrogen mustard or antidromic fifth nerve stimulation are dependent on neural pathways (Jampol et al.1975, 1976; Cole and Unger, 1973). In addition the response to laser irradiation has both neural and prostaglandin components (Unger et al. 1977). A contralateral response to nitrogen mustard applied to rabbit eye, and measured by aqueous protein level, has also been observed (Jampol and Noth, 1979); a small variable contralateral response had been previously noted (Davson and Quillam, 1947; Davson and Matchett, 1951). A contralateral response in conjunctival cytology has been demonstrated following the instillation of silver nitrate into the ipsilateral eye (Norn, 1960).

Jampol and Noth (1979) stated that both the systemic absorption of nitrogen or the release of a chemical mediator into the systemic circulation from the ipsilateral eye were unlikely; a neural pathway involving the fifth nerve was postulated as the most likely explanation. This view is supported by the previous work of Maul and Sears (1976) who showed that contralateral response to another neurally mediated stimulus, that is, antidromic fifth nerve stimulation, is blocked by contralateral fifth nerve section.

Analogy between the mechanism of trigeminal irritation and axon reflex skin vasodilation has been discussed; "pain" fibres might be in both reactions (Jampol et al. 1975; Calendar and Falkow 1953). The mediator is unknwon although a change in the contralateral aqueous concentration of Adenosine Triphosphate has been found (Maul and Sears, 1979).

The present experiment was carried out over a fifteen minute period. The contralateral increase in oxygen consumption following contact lens removal on the ipsilateral eye was observed after 10 seconds and one minute. The release of a chemical mediator into the systemic circulation from the ipsilateral eye with an effect on the contralateral eye seems unlikely with regard to the rapid response time observed. An osmotic pathway is certainly a possibility although the exact nature in which

such an osmotic shift would alter the corneal requirement is not clear. It may be that a change in the sodium ion concentration could affect the epithelial pump thus necessitating an increase in metabolism and oxygen uptake rate.

A neural pathway possibly involving the trigeminal nerve also provides a likely explanation. A contralateral increase in aqueous ATP levels has been observed one minute after stimulation of the ipsilateral trigeminal nerve resulting from an irritative response (Maul and Sears, 1979). The exact nature and implications of this finding are still not understood. However, the observation of a contralateral change within one minute fits the time scale of the present study and if ATP levels are altered it is conceivable that this may in some way modify the corneal metabolic rate and thus the need for oxygen. It now remains, however, for future work to examine this area more fully.

The results of the present study suggest a sympathetic change in corneal oxygen uptake rates may occur. This raises doubts regarding the validity of studies using one eye as a control.

3.6 EXPERIMENT 5

ALTERATIONS IN APPARENT CORNEAL OXYGEN UPTAKE RATE INDUCED AS A RESULT OF CHANGES IN THE FIT OF A HYDROGEL CONTACT LENS.

3.6.1 INTRODUCTION

A small degree of tear exchange under a hydrogel contact lens has been reported (Carter, 1972). The relative importance of such a "tear pump" in supplying oxygen to the anterior corneal surface is not known. Hard contact lenses have a low oxygen transmission and therefore most of the oxygen required for corneal metabolism is derived from tear mixing (Fatt and Hill, 1970; Fatt and Lin, 1976; Polse and Mandell, 1970).

The cornea beneath a soft contact lens derives its oxygen supply from one of three possible routes; transmission through the lens substance (Hill and Cucklanz, 1967; Fatt and St. Helen, 1971; Hill, 1977), by tear interchange beneath the lens via the tear pump mechanism, and by a combination of these two routes (Hill and Jeppe, 1975).

Clinical observations support the view that changes in the base curve of a soft contact lens affect the physiological response of the cornea. It is surmised that alterations in lens base curve will affect the tear pump mechanism under the lens (Tomlinson and Soni, 1980).

"Tight" lenses will restrict the flow of tears at the periphery allowing less interchange of tear fluid beneath the lens substance. The value of the tear pump was supported by Efron and Carney (1979) who showed the oxygen tension under a soft lens increased proportionately with blink rate, which they attributed to an active tear pump mechanism. The oxygen tension beneath the lens increased 2.6 times with a blink every five seconds compared to a no blinking situation.

Contrary to this, Polse (1979) using fluorophotometry concluded that the oxygen drived by the tear pump to be only one sixth of that obtained by transmission through the lens. Further evidence for the apparent lack of importance of tear interchange in the supply of oxygen beneath a soft lens was provided by Tomlinson and Soni (1980) and Tomlinson et al. (1981) who showed that the contribution of base curve radius to changes in corneal thickness was very small. Indirect evidence suggests that the magnitude of tear flow under a hydrogel contact lens contributes very little to the oxygen tension under the lens (Fatt and Lin, 1976; Decker et al. 1978). For most currently available soft lens designs this contribution would be in the order of 4 mmHg.

Thus, in order to prevent corneal oedema with hydrogel lens wear, the material must have a sufficiently high oxygen transmission value to meet the oxygen needs of

the cornea (Fatt and St. Helen, 1971). Polse (1979) stated that studies would be of value which examine the correlation between different lens cornea fitting relationships and their effect on tear flow rate. The experiment now reported attempted to examine the affect of hydrogel lens fit and thus "tear pumping" on corneal respiration, using the technique of oxygen debt as an indirectindicator of corneal respiratory function.

3.6.2 METHOD

Six male caucasian volunteers (18 - 33 years) were selected having corneal toricity less than 0.1 mm. Patient instruction and preparation were as described previously (3.2.2). Baush and Lomb 'B' series lenses were fitted to the subject's right eye. Lenses were selected to fit between 1.0 mm flatter and 0.25 mm steeper than the flattest corneal meridian, varying in 0.25 mm steps. The sequence in which the six lenses were fitted was different for each subject.

Each lens was left in situ for 5 minutes. Accurate centration was ensured to minimise the possible effect of the peripheral lens thickness. Apparent central corneal oxygen uptake rate was measured within 5 seconds of lens removal. Following the experiment the eye was examined with the aid of a slit lamp and fluorescein.

3.6.3 RESULTS

The results shown in Tables 12, 12(A), 13 and 14 and graphically illustrated in Figure 3.7 indicate that the "oxygen debt" generated is greatest with both a flat or steep fitting lens. The lowest "oxygen debt" is generated with lenses fitted minimally flatter than the corneal curvature. A three-way analysis of variance test using orthogonal polynomials and a Latin Square indicates that the fit of the contact lens produced a statistically significant change in the "oxygen debt" obtained ($p < 0.1$). A quadratic equation gives the best fit curve to the data for this factor.

3.6.4 DISCUSSION AND CONCLUSIONS

The "oxygen debt" is minimal when the oxygen tension at the anterior corneal surface is greatest. (Jauregui and Fatt, 1971). The results of the present study show this to occur for the lenses used, when fitted slightly flatter than the corneal curvature. The diameter and centre thickness of the lenses used, were constant, and have previously been shown to be accurate and reproducible by interferometry (El Nashar et al. 1979). However, the negative power of the lens and thus the peripheral edge thickness increased as the back optic radius increased. Clearly, any change in lens base curve necessarily required a change in both the dioptic power of that lens and it's

TABLE 12

CALCULATED VALUES FOR APPARENT CORNEAL OXYGEN UPTAKE RATES MEASURED ON SIX SUBJECTS FOLLOWING A FIVE MINUTE PERIOD OF CONTACT LENS WEAR OF DIFFERING POSTERIOR CURVATURE. ($ul O_2\ cm^{-2} hr^{-1}$).

Difference between curvature of anterior corneal surface and posterior lens curvature (mm).

Flatter						
-1.00	A 11.9	F 15.9	E 12.6	D 11.8	C 16.7	B 12.2
-0.75	B 11.0	A 8.6	F 15.8	E 12.1	D 13.8	C 16.5
-0.50	C 12.0	B 12.0	A 12.4	F 14.4	E 13.0	D 14.2
-0.25	D 12.1	C 12.8	B 12.4	A 12.8	F 12.7	E 13.9
0	E 14.0	D 15.2	C 13.2	B 13.7	A 12.6	F 12.8
+0.25 Steeper	F 16.2	E 14.4	D 13.7	C 15.3	B 13.0	A 15.0

Subjects indicated by A, B, C, D, E, F,

Pt's	A	B	C	D	E	F
Yk	73.3	74.3	86.5	80.8	80.0	87.8

= 482.7

TABLE 12(A)

RESULTS FROM TABLE 12 TABULATED TO SHOW INDIVIDUAL SUBJECT VALUES.

Difference between curvature of anterior corneal surface & posterior lens curvature (mm).

Flatter	A	B	C	D	E	F
-1.00	11.9	12.2	16.7	11.8	12.6	15.9
-0.75	8.6	11.0	16.5	13.8	12.1	15.8
-0.50	12.4	12.0	12.0	14.2	13.0	14.4
-0.25	12.8	12.4	12.8	12.1	13.9	12.7
0	12.6	13.7	13.2	15.2	14.0	12.8
+0.25	15.0	13.0	15.3	13.7	14.4	16.2

Subjects indicated by A, B, C, D, E and F.

TABLE 13

3 WAY ANALYSIS OF VARIANCE OF DATA

FROM TABLE 12

(Fit) Total SS	=	6,573.8
(Order) Mean SS	=	6,472.2
SS Rows	=	13.29
SS Columns	=	5.37
SS Pt's	=	29.9

SV	df	SS	MS	F
Total	36	6,573.8		
Mean	1	6,472.2		
Due to Fit	5	13.29	2.658	1.0023
Due to Order	5	5.37	1.074	0.053
Due to Pt's	5	29.9	5.98	2.2549
Error	20	53.04	2.652	

TABLE 14

THREE-WAY ANALYSIS OF VARIANCE AND ORTHOGONAL POLYNOMIALS

-1	-.75	-.5	-.25	0	+.25		
81.1	77.8	78.0	76.7	81.5	87.6		
-5	-3	-1	+1	+3	+5	42.3	70
+5	-1	-4	-4	-1	+5	65.4	84
-5	+7	+4	-4	-1	+5	11.5	180

Linear Component SS $\frac{(42.3)^2}{6X70} = 4.26$

Quad Component $\frac{(65.4)^2}{6X84} = 8.48$

Cubic Component $\frac{(11.8)^2}{6X180} = 0.12$

Quartic + Higher Component SS = 13.29 - 4.26 - 8.48 - 0.12
= 0.43

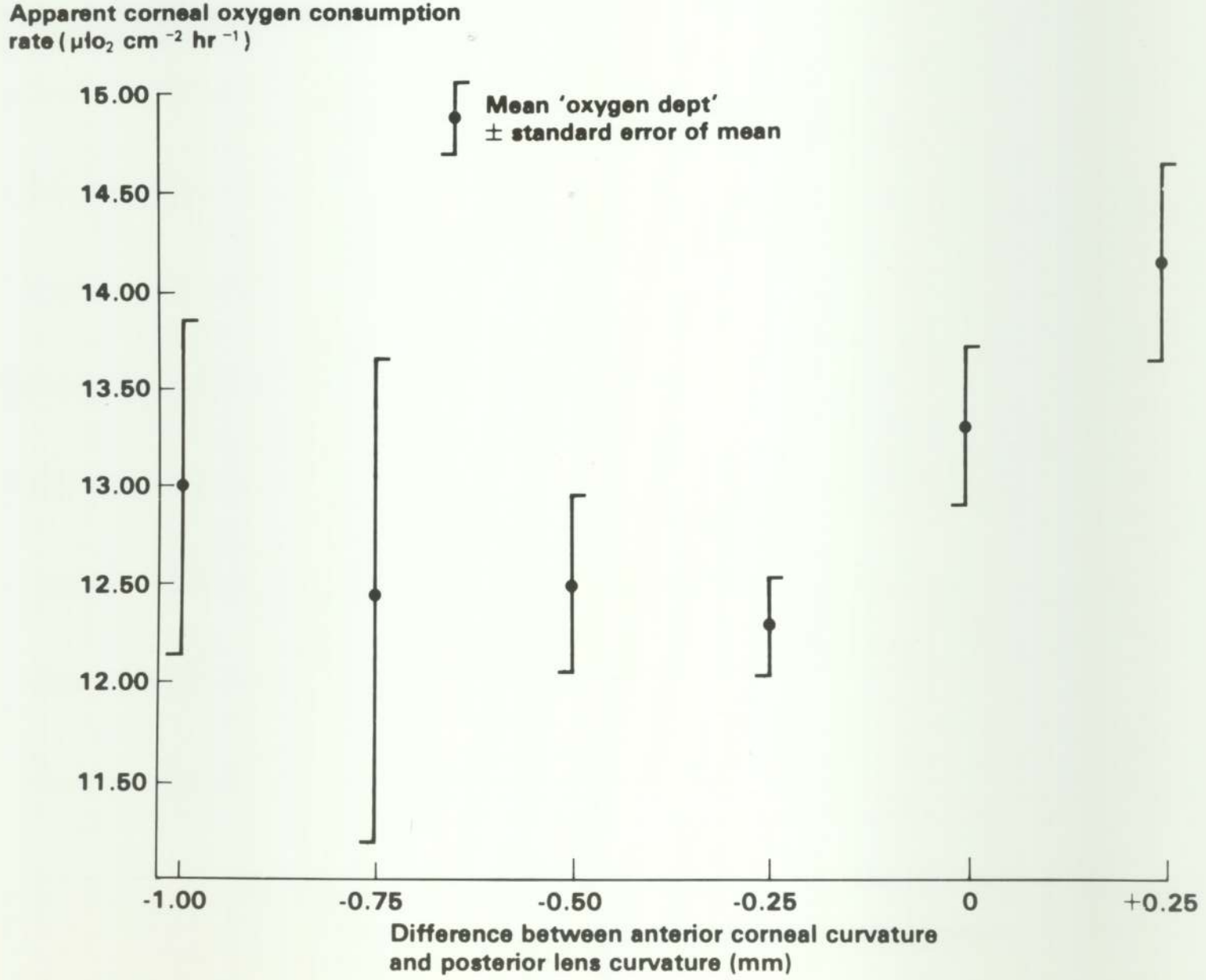
	DF	SS	MS	F	
Total	36	6,573.8			
Mean	-	6,472.2			
Due to Order	5	5.37	1.074	1	
Due to Pt's	5	29.9	5.98	2.2549	Not Significant
Due to Fit					
Linear	1	4.26	4.26	1.6063	Not Significant
Quad	1	8.48	8.48	3.1976	Significant
Cubic	1	0.12	0.12		
Remainder	2	0.43	0.215		
Error	20	53.04	2.652		

F (1,20,) = 2.9747

0.1

Figure 3.7

Changes in 'oxygen debt' produced as a result of changes in contact lens fit


Apparent corneal oxygen consumption rate (μlo_2 cm $^{-2}$ hr $^{-1}$)
15.00
14.50
14.00
13.50
13.00
12.50
12.00
11.50
Mean 'oxygen dept'
± standard error of mean
-1.00
-0.75
-0.50
-0.25
0
+0.25
Difference between anterior corneal curvature and posterior lens curvature (mm)

peripheral edge thickness. This formed a major limitation in the experimental design. Exact contraction of the contact lens was of primary importance, such that the constant centre thickness of each lens was placed over the geometric centre of the cornea.

Recentering of the lens takes place during the upward motion of the lid (Conway and Richman, 1982). Changes in peripheral edge thickness, as encountered in the present study, will therefore affect the lens motion with blinking. To minimise these effects all participating subjects were trained and selected for their tolerance of the experimental procedures used. There was no evidence of patients having noticeable tight eyelids. The experiment was carried out in the supine position to overcome lens decentering due to gravity. There was no apparent variation in subject's blink rate, albeit that this was assessed qualitatively.

The interaction of the various changes in contact lens parameters necessary for this type of experiment is therefore complex. This design limitation has been encountered in similar studies (Lowther and Tomlinson, 1981). However, steps were taken to minimise the possible variation of oxygen consumption rate which may have arisen from changing peripheral thickness and poor lens centration. The curvilinear nature of the results further implies a negligible effect due to these factors. The concept of constant centre thickness and changing edge thickness of soft lenses has been considered. An "average thickness" has been described (Sammons, 1981) which provides a mathematical average thickness and thus allows comparisons to be made between lenses of differing edge thickness.

Evidence is provided to support the theory that under certain fitting conditions a small tear-pump may operate under a soft contact lens. Such a tear pump appears to be operating at a maximum for lenses fitting slightly flatter than the flattest corneal meridian which corresponds to that widely advocated as a "best-fit" criterion by clinicians. This supports the theoretical considerations of a soft lens tear pump postulated by Kikkawa (1978, 1979). Further evidence for a soft lens tear pump mechanism has been demonstrated by monitoring changes in corneal oedema produced by changes in the contact lens base curve (Tomlinson, et al. 1981; Lowther and Tomlinson, 1981). Polse (1981) has examined the contribution of such a tear pump to corneal oxygenation and shown it to be only 15% of the total oxygen present at the tear-lens interface; this would be insufficient to prevent corneal oedema.

The present study shows that a tear pump operating under a soft contact lens can be demonstrated by the technique of oxygen debt. Whilst this supports the findings of the more widely established techniques of monitoring corneal oedema (Tomlinson, et al. 1981), there is an increasing opinion that a tear pump may have statistical but not clinical significance in soft contact lens wear (Polse, 1981; Tomlinson et al. 1981).

CHAPTER 4

OXYGEN TENSIONS AT THE ANTERIOR CORNEAL SURFACE DURING PERIODS OF EXTENDED SOFT CONTACT LENS WEAR

4 OXYGEN TENSIONS AT THE ANTERIOR CORNEAL SURFACE DURING PERIODS OF EXTENDED SOFT CONTACT LENS WEAR

4.1 INTRODUCTION

The cornea, in common with other body tissues, requires an adequate supply of oxygen (Duane, 1949; Fischer, 1930; Langham and Taylor, 1956; Freeman, 1972; Dikstein and Maurice, 1972).

The pathways of oxygen supply have been discussed previously (2:2). Obstruction of the anterior corneal surface by contact lens wear or eyelid closure produces a transient increase in oxygen consumption which has been termed 'Oxygen Debt' (Hill, 1965, 1979; Farris and Donn, 1972; Hill et al. 1973; Roscoe and hill, 1980).

The study now reported is a controlled comparative study which examines apparent in vivo human corneal oxygen uptake rates and 'oxygen debt' levels on a group of volunteer subjects who were to wear Sauflon p.w. (Contact Lenses Manufacturing) extended wear soft contact lenses over a period of eighteen months.

4.2 EXPERIMENTAL DESIGN

Basic designs for clinical studies can be categorised

into a variety of types of which three were considered:-

Paired Type

Cross-over Type

Comparative Type (Maxwell, 1968).

Paired studies have the disadvantage of a possible sympathetic response within the pairs. This may lead to masking and misinterpretation of an underlying response to a treatment. As previously mentioned in 2:5. the assumption that there is no sympathetic response between right and left eyes in each patient cannot be justifiably made (Parrish and Larke, 1981(c)).

The assumption is made in cross-over studies that when a subject is exposed to more than one treatment, the observed response is due entirely to differences between the treatments. The subject is further assumed to have recovered completely from one treatment with no 'conditioning' before a subsequent treatment commences, however there does exist the possibility of long-term changes to the eye in extended contact lens wear and a cross-over trial would be undesirable (Larke, 1981).

The experimental design of first choice is therefore a group comparative study which forms the basis of the experiment now reported.

4.3 PATIENT NUMBERS

The desirable number of observations made at each data collection point in an experiment involving the comparison of group means will depend on -

σ - the experimental error standard deviation

δ - the size of the difference between means that it is important to detect

α - the risk of asserting a difference when none exists, i.e. the probability at which the test is made

β - the risk of asserting no difference when a difference exists

τ - the type of statistical analysis

(Davies, 1967).

To provide an estimate of the subject numbers required in the present study, the following values were assumed.

$\delta/\sigma = D = 1$ $\quad\quad$ $\delta = \pm$ Standard Deviation

$\alpha = 0.1$ $\quad\quad$ $\beta = 0.2$

Double sided 'T' test

the required number of observations is at least 14. (Davies 1967).

Among the experimental design constraints must be mentioned some ethical considerations, in particular the possibility of ocular damage to the participating

subjects, and the number of subjects who could be given adequate clinical care. It therefore follows that the number of subjects involved in the study and the study duration should both be kept to a minimum.

The unpredictable number of subjects who would abandon participating in the study, for reasons unforseen at the start, would alter both the size of the initial group and the duration of the study. Previous experience suggested the need for a 40% wastage rate (Larke, 1969; Hirji, 1978). The study was therefore expected to involve an initial group size of 25 suitable volunteer subjects.

4.4 PATIENT SELECTION

Posters within the University of Aston and The City of Birmingham Polytechnic invited volunteer subjects to participate in the envisaged study. Subjects who acted as non-contact lens wearing control subjects were paid a nominal fee per visit during the study.

All volunteers were requested to attend a screening programme, the purpose of which was to produce a group with no apparent ocular anomaly or extreme ocular parameters, from the total number of potential subjects. A patient acceptance profile was devised based on previous studies (Larke, 1969; Hirji, 1978). Details

are given in Appendix 3.

On completion of the initial screening routines two groups of 26 suitable experimental subjects were randomly selected and a further 26 subjects acted as non-contact lens wearing controls.

4.5 EXPERIMENTAL PROCEDURE

The three volunteer groups were divided:-

Group A — Control Group wearing spectacles as necessary.

Group B — Experimental Group wearing Sauflon p.w. extended wear soft contact lenses stored in Physiological Saline

Group C — Experimental Group wearing Sauflon p.w. extended wear soft contact lenses used in conjunction with proprietory cleaning solutions.

A previous study of corneal thickness during periods of extended soft contact lens wear showed a difference to occur between patients using a weekly chemical contact lens cleaning routine compared to those who did not (Hirji, 1978). The study now reported was, therefore, sub-divided to examine possible changes in corneal oxygen consumption induced as a result of a contact lens cleaning routine albeit that this was carried out less frequently (see 4:6).

4.6 CONTACT LENS FITTING

Sauflon p.w. contact lenses were fitted in accordance with the manufacturer's instructions. The lenses were selected from a large stock of suitable asepticised lenses. Subjects were required to wear the 'best fit' lens overnight, after which further clinical evaluations determined the lens suitability for each patient. Any adverse ocular reaction at this stage necessarily prevented further participation by that subject in the study. Having determined the 'Best Fit' lens for each subject, the lenses were asepticised and set aside to be issued at a later date. All subjects were instructed in the emergency removal of lenses if this was deemed necessary at any stage during the study. The lenses were worn continuously from the time of issue at the start of the study, except at data collection points, when the lenses were removed to allow for measurement. The solution users group had their lenses removed for a period of 24 hours when cleaning was indicated. This was carried out with Monoclens C40 (co-polymeric oxidising preparation of Ethylene oxide, Propylene oxide and Sodium perborate). All subjects were issued with a written description of the experimental procedures which it was proposed to adopt and had to sign a consent form. Due to the unpleasant nature of placing the oxygen sensor on to the unanaesthetised cornea all patients were asked to undergo one measurement of apparent corneal oxygen uptake rate, and asked to sign a second consent form if they subsequently agreed to participate in the study.

The non-solution users group had their lenses replaced to an identical specification from stock, in the event of the lens becoming contaminated with deposits. This was done within 24 hours wherever possible. A spare lens for each patient was held in stock throughout the study.

4.7 SCHEDULE OF DATA COLLECTION AND AFTER CARE VISITS

Data collection and contact lens after care visits were carried out at the same time for convenience, and to minimise the number of visits necessary for each subject. Appointments were pre-arranged for each subject and were selected to allow examination of initial and long-term affects.

Appointments following lens issue were as follows:-

Baseline values and contact lens collection.

2	days	following	lens	insertion	$\pm$	1	day
4	"	"	"	"		"	
6	"	"	"	"		"	
8	"	"	"	"		"	
2	weeks	"	"	"		"	
3	"	"	"	"		"	
4	"	"	"	"		"	
2	months	"	"	"	$\pm$	1	week
3	"	"	"	"		"	
6	"	"	"	"		"	
12	"	"	"	"		"	
18	"	"	"	"		"	

The allocation of appointments detailed above allowed examination of possible short-term effects due to contact lens wear over the first week, increasing to

observe longer-term changes up to eighteen months following the commencement of contact lens wear. Initial appointments were scheduled $\pm$ 1 day to avoid low subject response rate if appointments otherwise would have occurred at week-ends and to allow some flexibility to avoid missing data collection points due to other reasons for non-attendance. The monthly data collection points were $\pm$ 1 week to minimise non-attendance due to holiday/ work or similar commitments. Many participating subjects were students spending much of the summer away from Birmingham and travel expenses within the British Isles were paid to encourage regular attendance.

4.8 UNKEPT APPOINTMENTS AND PATIENT WITHDRAWAL

Constant efforts were made to remind subjects of their appointments. Pre-arranged appointments within the limits given above were made where possible. During the course of the study some subjects withdrew or were rejected from the experiment. Details are shown in Appendix 7. At each data collection point seven appointment times were available between 9.00 - 17.00 hours and were allocated in a sequential manner to avoid patients attending regularly at the same time of day, albeit that no diurnal variation in apparent human in vivo corneal oxygen uptake rate has been so far demonstrated (3:2). A period of at least 24 hours elapsed between successive days of contact lens issue to allow for routine maintenance of the equipment and organisation of the collected data. No more than four patients were issued with lenses in any one day, thus lens issuing took place over five weeks. Due to the appearance

of micro epithelial cysts of the cornea after six months of lens wear the study was terminated at this point (Humphreys et al. 1980).

4.9 METHOD

At every data collection point 'oxygen debt' was measured on the right eye of each subject of the experimental groups within ten seconds of contact lens removal, using the oxygen sensor and experimental procedures previously described (3:2). The contact lens was then removed from the contralateral eye to avoid a possible sympathetic affect (3:5).

Following a recovery period of ten minutes, basal apparent corneal oxygen consumption rate was measured, also on the right eye. Patients were examined with the aid of a slit lamp. Fluorescein was only used to establish corneal damage where indicated such that prompt reinsertion of the lenses could be effected.

4.10 RESULTS

The raw data for baseline and oxygen debt values obtained at each data point are shown in Tables 15, 16, 17. The mean values are given in Table 17(a).

These results are depicted graphically;

Figures 4.1. to 4.3 show the results over the first week
Figures 4.4 to 4.6 show the results over the first month
Figures 4.7 to 4.9 show the results over the first six months.

4.10.1 From this data the following further aspects of corneal oxygen consumption rates were examined:-

1 The comparison of both pre-fitting baseline values with the basal values obtained at each data point and the degree of oxygen debt generated after 48 hours with the oxygen debt produced at each subsequent data point for both experimental groups. The results of the first week are shown in Table 18, the first month, Table 19 and the first six months, Table 20.

2 The pre-fitting baseline values for each group were subjected to d'Agastinos test to establish if each group was representative of a normal population sample. These results are shown in Tables 21, 22, 23.

A Student 'T' test showed no statistical difference between pre-fitting oxygen consumption values for each group, Table 24.

3 The comparison of both baseline values and oxygen debt between experimental groups at each data point (Table 25).

4 The comparison between baseline values and the degree of oxygen debt generated at each data point, within each group (Table 26)

5 The correlation between pre-fitting oxygen uptake rate and the degree of oxygen debt generated following 48 hours of contact lens wear in 32 subjects taken from both experimental groups. The resulting population distribution for each is shown in Figures 4.10 and 4.11.

6 The comparison of pre-fitting oxygen consumption rate with the oxygen requirement one week following the cessation of lens wear (Table 27)

4.10.2 DISCUSSION AND CONCLUSIONS

1 Main Study

The results of 'oxygen debt' and basal oxygen uptake rate over the six months wearing period are broken down into three sections which examine:-

The first week of lens wear
The first month of lens wear
The first six months of lens wear

A decrease in apparent basal corneal oxygen uptake rate occurred in both experimental groups over the first 7-14 days of the study. This observed phenomenon may represent part of an initial adaptive phase to contact lens wear. The presence of contact lens induced changes in corneal thickness during the early wearing stage have

TABLE 15

RAW VALUES, BASAL OXYGEN CONSUMPTION AND OXYGEN DEBT

NON SOLUTION GROUP

$ul O_2\ cm^{-2}\ hr^{-1}$

Pat. No:	Base line	Day 2	Day 4	Day 6	Day 8	Week 2	Week 3	Week 4	Month 2	Month 3	Month 6
1	8.891	10.081 8.293	9.200 7.603	7.297 7.702	- -	- -	11.732 7.812	10.748 6.636	10.564 7.319	7.898 6.873	7.770 7.062
2	8.049	10.413 7.707	11.203 10.281	8.619 6.134	9.558 6.429	10.089 5.381	8.396 6.608	11.354 8.342	6.939 6.299	- -	7.431 5.281
3	5.641	- -	9.648 5.951	- -	- -	10.986 9.290	10.540 8.152	10.618 7.947	9.140 8.728	7.245 5.597	7.258 6.124
4	7.461	Rejected from study									
5	13.113	10.672 9.054	13.505 7.299	8.413 5.428	- -	7.219 6.181	11.301 9.302	10.672 7.198	9.535 9.447	- -	9.599 7.384
6	5.538	Withdrawn from study									
7	6.230	8.065 8.608	12.346 6.744	11.649 7.592	9.905 8.495	- -	8.274 6.098	12.781 10.269	7.186 6.867	8.288 6.873	7.360 5.727
8	8.509	12.418 7.273	10.828 7.362	- -	- -	9.426 5.358	10.363 7.083	- -	9.006 11.762	10.225 5.999	8.869 5.217
9	6.944	8.408 8.518	4.885 7.556	- -	- -	- -	11.015 7.007	9.673 5.842	8.396 5.486	10.356 7.125	7.429 5.320
10	8.623	9.463 6.913	7.317 6.923	7.918 6.342	8.569 8.106	10.101 5.301	8.359 6.193	7.046 6.199	8.126 6.175	8.175 6.163	- -
11	9.612	Withdrawn from study									
12	7.475	Withdrawn from study									
13	6.294	Rejected from study									

Patients 14 to 26 continued overleaf.

TABLE 15 - continued

NON SOLUTION GROUP - Continued Nos. 14 to 26

Pat. No.	Base line	Day 2	Day 4	Day 6	Day 8	Week 2	Week 3	Week 4	Month 2	Month 3	Month 6
14	7.034	10.693 7.867	11.401 6.774	- -	8.404 6.178	- -	10.352 7.700	- Rejected from	- study	- 	- -
15	8.101	6.384 6.228	13.991 6.731	-	Withdrawn from study			-	-	-	-
16	5.000	- -	- -	- -	- -	6.181 7.749	9.619 7.086	10.206 5.699	8.537 4.110	5.598 4.735	7.139 6.604
17	6.332	- -	8.250 6.864	5.042 10.023	7.208 5.397	9.517 6.441	10.351 5.804	12.682 10.104	- -	6.968 5.218	9.003 5.554
18	5.674	Withdrawn from study			-	-	-	-	-	-	-
19	7.057	9.822 7.794	- -	8.979 5.893	- -	- -	11.231 10.078	8.241 5.636	8.074 11.689	8.599 5.753	11.154 6.139
20	6.435	Rejected from study			-	-	-	-	-	-	-
21	6.037	Rejected from study			-	-	-	-	-	-	-
22	6.550	6.702 5.377	7.191 6.499	7.401 6.154	7.712 5.693	- -	9.521 5.426	9.941 7.036	7.552 7.321	7.416 5.728	8.833 5.869
23	7.940	Rejected from study			-	-	-	-	-	-	-
24	5.094	11.160 9.673	11.035 7.529	11.701 8.042	13.746 7.162	7.550 5.573	10.002 6.283	8.943 6.648	8 651 6.244	6.380 4.613	12.238 6.942
25	7.944	10.103 7.851	- -	- -	10.851 8.343	8.271 6.028	- -	8.413 6.454	- -	10.261 7.064	7.634 5.627
26	8.221	9.621 5.620	- -	9.080 7.866	10.986 7.668	7.839 5.381	6.360 5.409	8.898 6.175	7.104 4.597	7.923 5.374	7.236 6.682

Upper value - Oxygen Debt.

Lower Value - Basal Oxygen Uptake Rate.

TABLE 16

RAW VALUES, BASAL OXYGEN CONSUMPTION AND OXYGEN DEBT

SOLUTION GROUP

$ul O_2\ cm^{-2}\ hr^{-1}$

Pat. No.	Base line	Day 2	Day 4	Day 6	Day 8	Week 2	Week 3	Week 4	Month 2	Month 3	Month 6
1	6.190	7.445 9.499	9.990 6.970	7.987 6.220	- -	- -	6.229 6.271	8.264 6.262	9.039 6.192	9.175 5.489	-
2	6.020	8.376 6.565	9.901 6.291	9.436 9.962	12.075 5.120	- -	9.471 6.210	8.794 5.589	8.676 4.960	- -	- -
3	7.502	10.405 7.699	8.074 -	7.342 5.528	7.809 5.991	- -	8.954 6.715	12.998 6.803	7.348 4.422	5.752 4.292	8.120 7.042
4	7.347	- -	- -	10.757 7.094	- -	8.033 6.464	9.225 9.625	8.607 6.252	9.335 4.883	9.303 6.943	- -
5	4.877	- -	- -	- -	10.363 7.675	10.986 7.521	11.155 6.491	- -	10.539 6.384	7.684 6.690	- -
6	6.369	Rejected from study									
7	9.021	9.721 -	- -	8.275 5.811	- -	- -	- -	9.719 6.637	8.022 6.064	8.146 5.791	- -
8	7.560	Rejected from study									
9	8.329	13.609 10.278	9.580 8.572	8.059 7.052	8.890 8.348	7.311 6.903	9.379 7.480	9.942 8.197	10.728 7.453	7.410 5.452	- -
10	6.326	Withdrawn from study									
11	7.512	- -	- -	- -	- -	9.095 6.361	Withdrawn from study				
12	8.442	7.412 6.294	8.574 6.504	10.395 5.671	9.356 6.217	- -	- -	8.561 6.584	11.220 7.011	- -	- -
13	7.099	- -	- -	- -	10.566 8.287	13.693 10.345	8.990 7.888	8.748 7.620	11.764 6.491	10.062 7.245	11.492 8.753

Patients 14 to 26 continued overleaf:

TABLE 16 - continued

Pat. No.	Base line	Day 2	Day 4	Day 6	Day 8	Week 2	Week 3	Week 4	Month 2	Month 3	Month 6
14	6.873	10.395 7.293	9.717 9.138	6.146 7.939	- -	- -	9.963 7.628	9.371 7.194	10.025 6.303	6.943 7.330	7.466 6.843
15	10.557	10.172 8.578	8.535 5.399	- -	- -	6.791 7.640	9.127 6.498	9.673 7.295	8.783 6.241	8.286 5.585	6.378 6.967
16	7.126	7.584 6.239	9.307 8.682	9.941 6.578	8.854 6.539	9.795 7.137	10.757 8.171	7.440 5.439	- Withdrawn from	- study	-
17	6.553	9.943 7.212	9.746 5.584	7.580 6.016	8.925 4.884	8.153 4.259	9.744 6.764	9.620 5.086	- -	4.956 4.073	- -
18	7.480	7.637 6.691	8.547 7.643	- -	7.219 5.029	- -	9.641 9.229	8.443 5.457	7.237 5.413	8.671 6.224	9.830 7.139
19	6.657	10.793 6.820	6.359 4.884	10.056 6.373	9.182 5.826	9.776 6.927	7.841 6.544	7.205 5.023	8.213 5.447	7.657 6.025	- -
20	8.488	8.719 8.664	10.056 6.936	8.481	6.764	- -	- -	10.612 7.340	7.443 5.817	- -	9.039 8.801
21	9.787	7.716 5.522	9.021 7.652			9.201 7.829	6.523 5.635	7.504 6.428		7.007 5.665	- -
22	5.133	14.193 7.003	11.097 8.227	- -	- -	10.468 9.489	16.709 9.142	10.162 8.911	12.286 6.690	6.221 4.223	- -
23	7.648	6.181 3.268	8.940 -	Withdrawn from study			- -	- -	- -	- -	- -
24	9.339	12.728 7.441	9.831 7.428	Withdrawn from study			- -	- -	- -	- -	- -
25	6.978	6.665 6.248	Withdrawn from study			- -	- -	- -	- -	- -	- -
26	9.690	Withdrawn from study			- -	- -	- -	- -	- -	- -	- -

TABLE 17

RAW VALUES, BASAL OXYGEN CONSUMPTION. - $ul O_2\ cm^{-2}\ hr^{-1}$

CONTROL GROUP

Pat. No:	Base-line	Day 2	Day 4	Day 6	Day 8	Week 2	Week 3	Week 4	Month 2	Month 3	Month 6
1	8.203	6.973	8.208	8.152	6.442	8.440	5.846	8.813	6.579	7.389	-
2	7.484	-	7.347	-	-	-	6.768	8.795	7.874	-	7.403
3	9.440	-	-	6.565	6.738	6.992	7.973	9.532	6.855	7.397	-
4	5.544	-	-	-	6.417	8.570	5.597	5.663	7.295	5.794	6.751
5	9.793	7.408	10.829	8.556	7.964	-	-	8.633	-	6.691	-
6	6.944	5.435	-	6.042	-	-	-	7.076	6.437	6.501	5.293
7	7.670	9.091	7.477	7.100	10.073	-	-	-	-	-	12.748
8	5.778	Withdrawn from study			-	-	-	-	-	-	-
9	7.408	4.972	6.618	7.952	7.165	9.539	6.719	-	8.050	6.412	-
10	6.370	7.958	-	-	-	9.828	8.850	6.329	6.842	-	-
11	7.446	-	-	-	7.248	7.743	10.665	7.152	5.616	-	
12	8.880	6.181	-	6.793	5.445	4.236	6.975	6.700	-	-	-
13	10.580	6.234	6.099	-	-	-	-	6.717	7.042	-	-
14	5.934	9.313	9.271	7.137	-	-	6.056	8.415	-	-	-
15	5.920	-	-	7.392	5.338	7.382	5.893	-	7.182	5.468	8.152
16	5.538	8.443	-	8.012	8.828	6.996	8.585	-	7.883	6.658	-
17	6.490	Rejected from study			-	-	-	-	-	-	-
18	6.591	9.874	7.310	7.469	-	6.704	7.726	9.263	6.041	6.822	7.403
19	7.147	-	-	8.695	7.808	6.374	7.297	7.393	5.949	6.925	-
20	5.480	7.154	-	-	-	6.529	-	6.995	6.772	-	-
21	5.504	6.602	6.175	-	-	-	7.052	-	-	7.082	-
22	4.703	Withdrawn from study			-	-	-	-	-	-	-
23	5.180	-	4.682	6.566	-	6.286	-	-	-	-	5.675
24	7.436	6.599	-	-	-	7.550	5.415	7.969	6.451	-	-
25	9.299	-	7.705	7.392	9.921	8.470	9.080	8.633	8.187	11.271	-
26	7.885	7.337	8.981	6.704	7.302	7.351	13.772	-	5.021	-	-

TABLE 17 (a)

Mean Values for Basal Oxygen Consumption Rate and "Oxygen Debt" over the six month period ± standard Error of Mean ($ulo_2 CM^{-2} hr^{-1}$)

	Solution		Non-Solution		Control
	Basal	Debt	Basal	Debt	Basal
Baseline	7.499	-	7.301	-	7.101
Day 2	7.136	9.478	7.626	9.571	7.304
	0.387	0.524	0.333	0.447	0.365
Day 4	7.136	9.204	7.239	10.061	7.558
	0.347	0.262	0.284	0.738	0.472
Day 6	6.888	8.806	7.117	8.609	7.408
	0.381	0.413	0.438	0.628	0.201
Day 8	6.425	9.291	7.052	9.659	7.437
	0.375	0.400	0.390	0.672	0.412
Week 2	7.356	9.291	6.268	8.717	7.436
	0.482	0.580	0.411	0.484	0.341
Week 3	7.352	9.580	7.069	9.827	7.589
	0.318	0.615	0.350	0.373	0.048
Week 4	6.595	9.156	7.156	10.035	7.618
	0.267	0.318	0.402	0.438	0.302
Month 2	5.984	9.377	7.387	8.370	6.735
	0.214	0.423	0.670	0.290	0.265
Month 3	5.787	7.661	5.931	8.102	7.034
	1.066	0.381	0.236	0.410	0.419
Month 6	7.470	8.720	6.109	8.496	7.670
	0.314	0.740	0.191	0.423	4.100

TABLE 18.

DAYS 2-8 OF CONTACT LENS WEAR

SOLUTION GROUP

Basal Oxygen Uptake Rates - Paired 'T' Test

Baseline v Day 2	t = 0.922	N/S	df = 16
v Day 4	= 0.883	N/S	= 13
v Day 6	= 0.794	N/S	= 11
v Day 8	= 1.550	($p < 0.20$)	= 10

Oxygen Debt - Unpaired 'T' Test

Day 2 v Day 4	t = 0.440	N/S	df = 33
v Day 6	= 0.910	N/S	= 29
v Day 8	= 0.248	N/S	= 28

NON SOLUTION GROUP

Basal Oxygen Uptake Rates - Paired 'T' Test

Baseline v Day 2	t = 0.043	N/S	df = 13
v Day 4	= 0.668	N/S	= 12
v Day 6	= 0.697	N/S	= 9
v Day 8	= 0.147	N/S	= 8

Oxygen Debt - Unpaired 'T' Test

Day 2 v Day 4	t = 0.564	N/S	df = 25
v Day 6	= 1.284	N/S	= 22
v Day 8	= 0.114	N/S	= 21

CONTROL GROUP

Basal Oxygen Uptake Rates - Paired 'T' Test

Baseline v Day 2	t = 0.068	N/S	df = 14
v Day 4	= 0.128	N/S	= 11
v Day 6	= 0.112	N/S	= 14
v Day 8	= 0.507	N/S	= 12

BASAL OXYGEN UPTAKE RATE AND OXYGEN DEBT COMPARED OVER DAYS 2 - 8 OF CONTACT LENS WEAR - FOR EACH GROUP

TABLE 19

WEEKS 1-4 OF CONTACT LENS WEAR

SOLUTION GROUP

Basal Oxygen Uptake Rate - Paired 'T' Test

Baseline v Week 1	t = 1.550	($p < 0.20$)	df = 10
v Week 2	= 0.006	N/S	= 10
v Week 3	= 0.334	N/S	= 14
v Week 4	= 2.291	($p < 0.05$)	= 16

Oxygen Debt - Unpaired 'T' Test

Day 2 v Week 1	t = 0.248	N/S	df = 28
v Week 2	= 0.106	N/S	= 28
v Week 3	= 0.126	N/S	= 32
v Week 4	= 0.503	N/S	= 34

NON SOLUTION GROUP

Basal Oxygen Uptake Rate - Paired 'T' Test

Baseline v Week 1	t = 0.147	N/S	df = 8
v Week 2	= 1.386	($p < 0.20$)	= 9
v Week 3	= 0.684	N/S	= 14
v Week 4	= 0.349		- 13

Oxygen Debt - Unpaired 'T' Test

Day 2 v Week 1	t = 0.114	N/S	df = 21
v Week 2	= 1.275	N/S	= 22
v Week 3	= 0.442	N/S	= 27
v Week 4	= 0.708	N/S	= 26

CONTROL GROUP

Basal Oxygen Uptake Rates - Paired 'T' Test

Baseline v Week 1	t = 0.507	N/S	df = 12
v Week 2	= 0.654	N/S	= 15
v Week 3	= 1.009	N/S	= 17
v Week 4	= 0.223	N/S	= 16

BASAL OXYGEN UPTAKE RATE AND OXYGEN DEBT COMPARED OVER WEEKS 1 - 4 OF CONTACT LENS WEAR - FOR EACH GROUP

TABLE 20

MONTHS 1-6 OF CONTACT LENS WEAR

SOLUTION GROUP

Basal Oxygen Uptake Rates - Paired 'T' Test

Baseline v Month 1	t = 2.291	(p< 0.05)	df = 16
v Month 2	= 3.108	(p< 0.01)	= 14
v Month 3	= 3.034	(p< 0.01)	= 13
v Month 6	= 0.763		= 5

Oxygen Debt - Unpaired 'T' Test

Day 2 v Month 1	t = 0.503	N/S	df = 34
v Month 2	= 0.145	N/S	= 32
v Month 3	= 2.617	(P<0.02)	= 31
v Month 6	= 0.739	N/S	= 23

NON SOLUTION GROUP

Basal Oxygen Uptake Rates - Paired 'T' Test

Baseline v Month 1	t = 0.349	N/S	df = 13
v Month 2	= 0.197	N/S	= 12
v Month 3	= 3.481	(p< 0.01)	= 12
v Month 6	= 2.455	(p< 0.05)	= 13

Oxygen Debt - Unpaired 'T' Test

Day 2 v Month 1	t = 0.708	N/S	df = 26
v Month 2	= 2.216	(p< 0.05)	= 25
v Month 3	= 2.407	(p< 0.05)	= 25
v Month 6	= 1.746	(p< 0.10)	= 26

CONTROL GROUP

Basal Oxygen Uptake Rates - Paired 'T' Test

Baseline v Month 1	t = 0.223	N/S	df = 16
v Month 2	= 0.728	N/S	= 15
v Month 3	= 0.320	N/S	= 11
v Month 6	= 1.294	N/S	= 5

BASAL OXYGEN UPTAKE RATE AND OXYGEN DEBT COMPARED OVER MONTHS 1 - 6 OF CONTACT LENS WEAR - FOR EACH GROUP

FIGURE 4.1

OXYGEN DEBT AND BASAL OXYGEN CONSUMPTION RATE OVER THE FIRST EIGHT DAYS OF EXTENDED CONTACT LENS WEAR ($ulo_2\ CM^{-2}hr^{-1}$)

SOLUTION GROUP

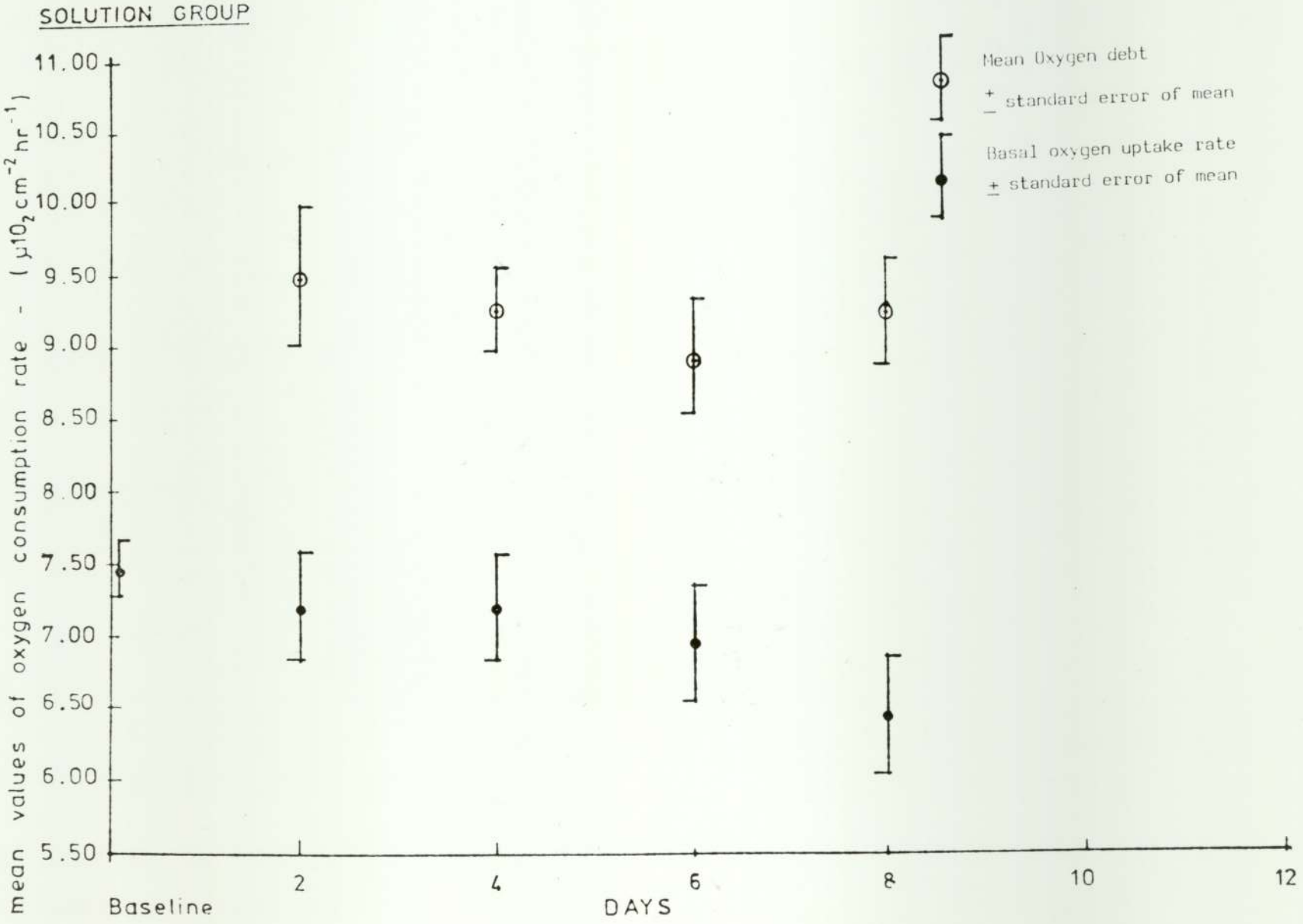

SOLUTION GROUP
Mean values of oxygen consumption rate - ($\mu l O_2 cm^{-2} hr^{-1}$)
11.00
10.50
10.00
9.50
9.00
8.50
8.00
7.50
7.00
6.50
6.00
5.50
Baseline
2
4
6
8
10
12
DAYS
Mean Oxygen debt
± standard error of mean
Basal oxygen uptake rate
± standard error of mean

FIGURE 4.2

OXYGEN DEBT AND BASAL OXYGEN CONSUMPTION RATE OVER THE FIRST EIGHT DAYS OF EXTENDED CONTACT LENS WEAR

($ulo_2 CM^{-2} hr^{-1}$)

NON SOLUTION GROUP

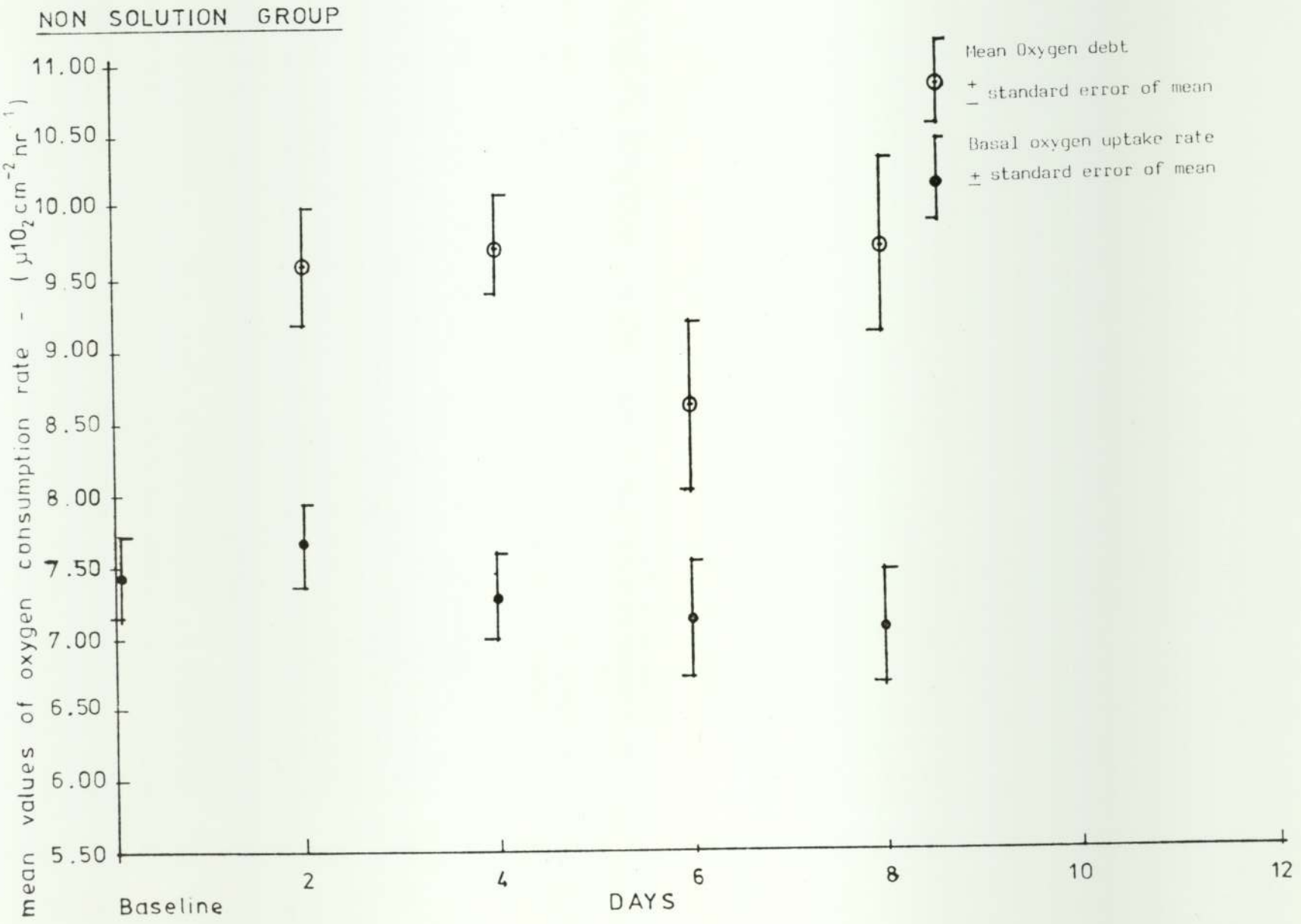

NON SOLUTION GROUP
Mean Oxygen debt
± standard error of mean
Basal oxygen uptake rate
± standard error of mean
mean values of oxygen consumption rate - ($\mu l O_2 cm^{-2} hr^{-1}$)
11.00
10.50
10.00
9.50
9.00
8.50
8.00
7.50
7.00
6.50
6.00
5.50
Baseline
2
4
6
8
10
12
DAYS

FIGURE 4.3

BASAL OXYGEN CONSUMPTION RATE OVER THE FIRST EIGHT DAYS OF THE STUDY

($ulo_2 CM^{-2} hr^{-1}$)

CONTROL GROUP

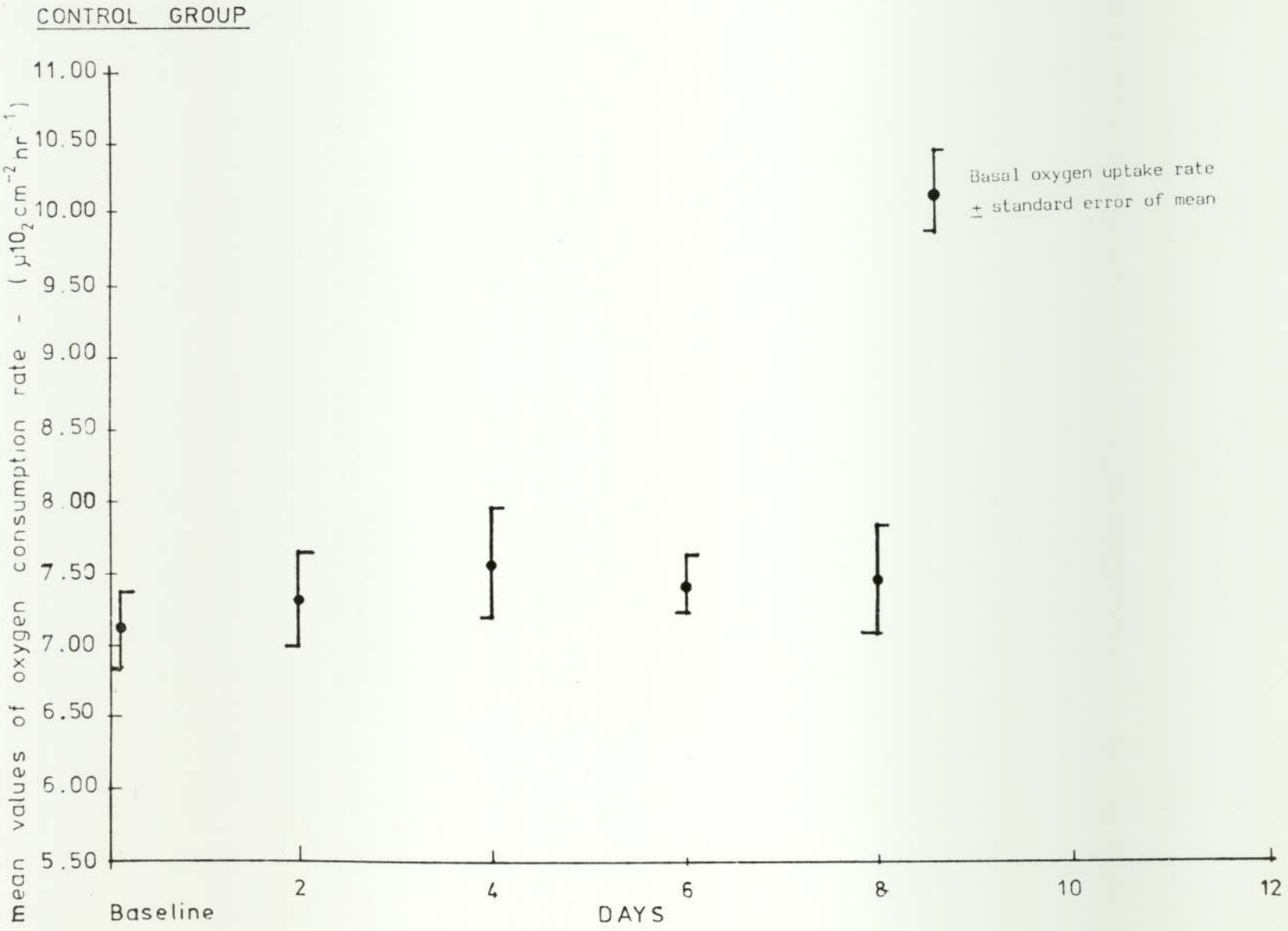

CONTROL GROUP
11.00
10.50
10.00
9.50
9.00
8.50
8.00
7.50
7.00
6.50
6.00
5.50
mean values of oxygen consumption rate - ($\mu l O_2 cm^{-2} hr^{-1}$)
Basal oxygen uptake rate
± standard error of mean
Baseline
2
4
6
8
10
12
DAYS

FIGURE 4.4

OXYGEN DEBT AND BASAL OXYGEN CONSUMPTION RATE OVER WEEKS 1 TO 4 OF EXTENDED CONTACT LENS WEAR ($ul O_2 CM^{-2} hr^{-1}$)

SOLUTION GROUP

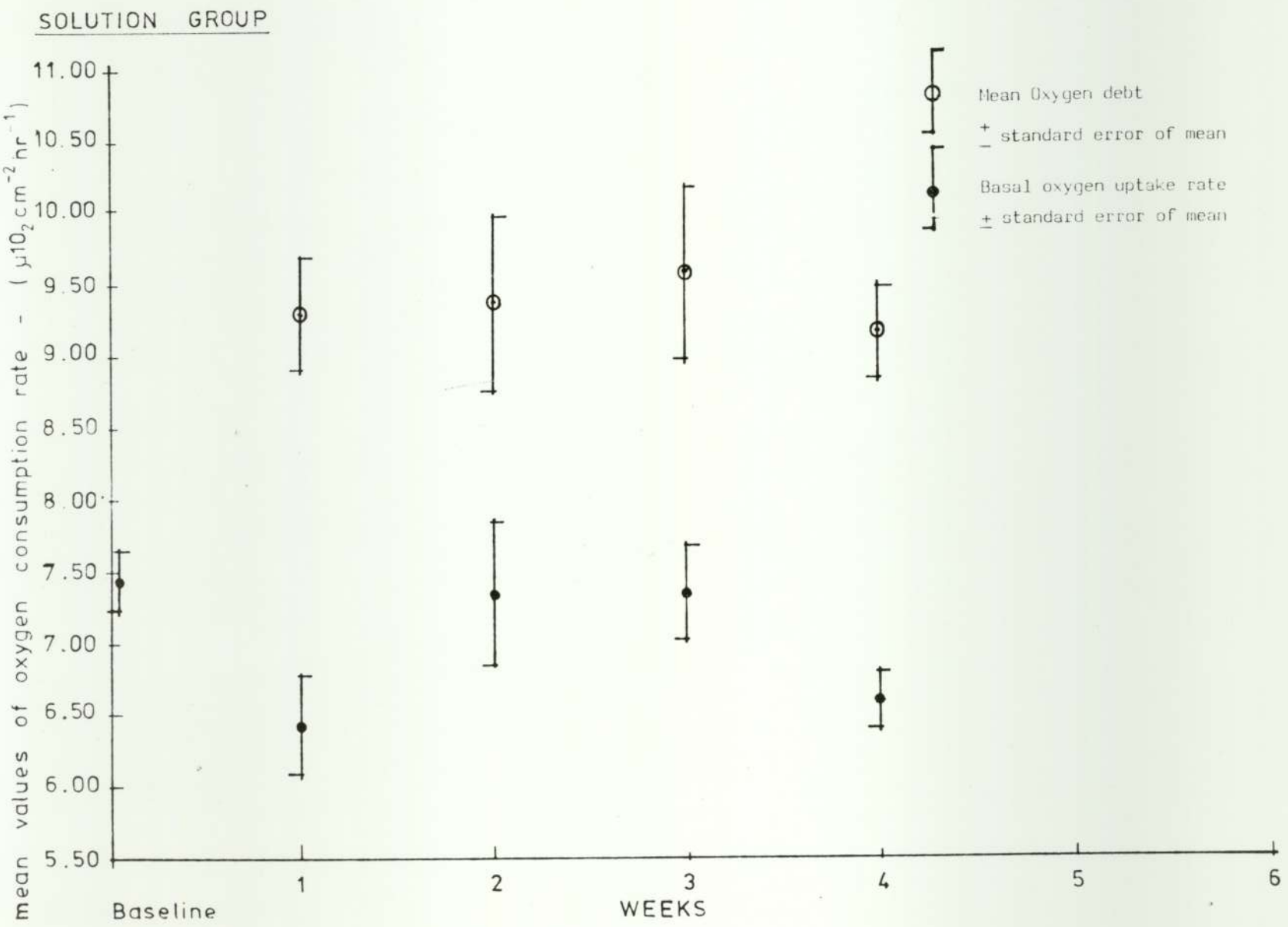

SOLUTION GROUP
11.00
10.50
10.00
9.50
9.00
8.50
8.00
7.50
7.00
6.50
6.00
5.50
mean values of oxygen consumption rate - ($\mu l O_2 cm^{-2} hr^{-1}$)
Mean Oxygen debt
± standard error of mean
Basal oxygen uptake rate
± standard error of mean
Baseline
1
2
3
4
5
6
WEEKS

FIGURE 4.5

OXYGEN DEBT AND BASAL OXYGEN CONSUMPTION RATE OVER WEEKS 1 TO 4 OF EXTENDED CONTACT LENS WEAR ($ulo_2 CM^{-2} hr^{-1}$)

NON SOLUTION GROUP

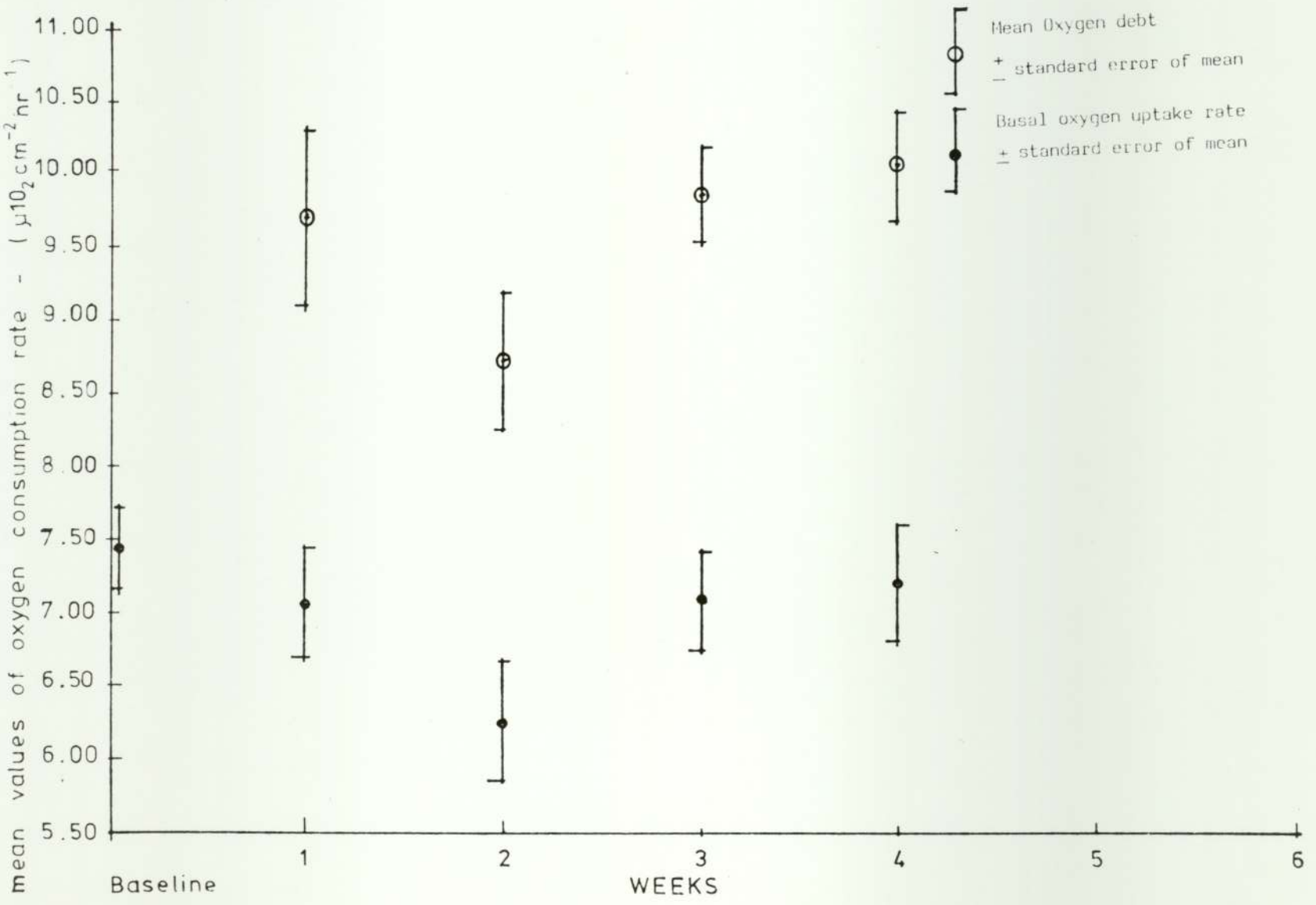

NON SOLUTION GROUP
Mean values of oxygen consumption rate - ($\mu l O_2 cm^{-2} hr^{-1}$)
11.00
10.50
10.00
9.50
9.00
8.50
8.00
7.50
7.00
6.50
6.00
5.50
Baseline
1
2
3
4
5
6
WEEKS
Mean Oxygen debt
± standard error of mean
Basal oxygen uptake rate
± standard error of mean

FIGURE 4.6

BASAL OXYGEN CONSUMPTION RATE

OVER WEEKS 1 TO 4 OF THE STUDY

($ulo_2 CM^{-2} hr^{-1}$)

CONTROL GROUP

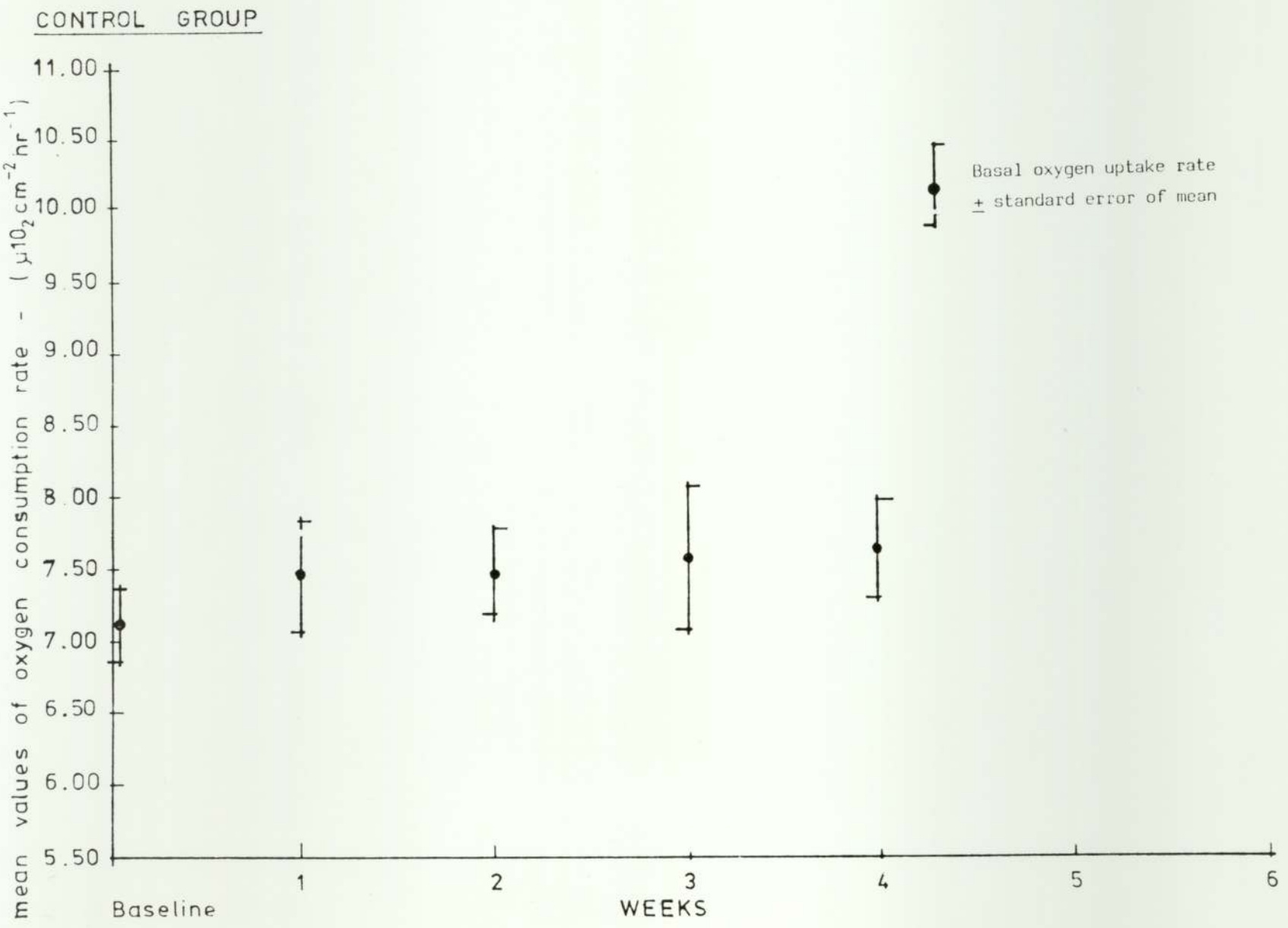

CONTROL GROUP
11.00
10.50
10.00
9.50
9.00
8.50
8.00
7.50
7.00
6.50
6.00
5.50
mean values of oxygen consumption rate - ($\mu l O_2 cm^{-2} hr^{-1}$)
Basal oxygen uptake rate
± standard error of mean
Baseline
1
2
3
4
5
6
WEEKS

FIGURE 4.7

OXYGEN DEBT AND BASAL OXYGEN CONSUMPTION RATE OVER MONTHS 1 TO 6 OF EXTENDED CONTACT LENS WEAR ($ulo_2CM^{-2}hr^{-1}$)

SOLUTION GROUP

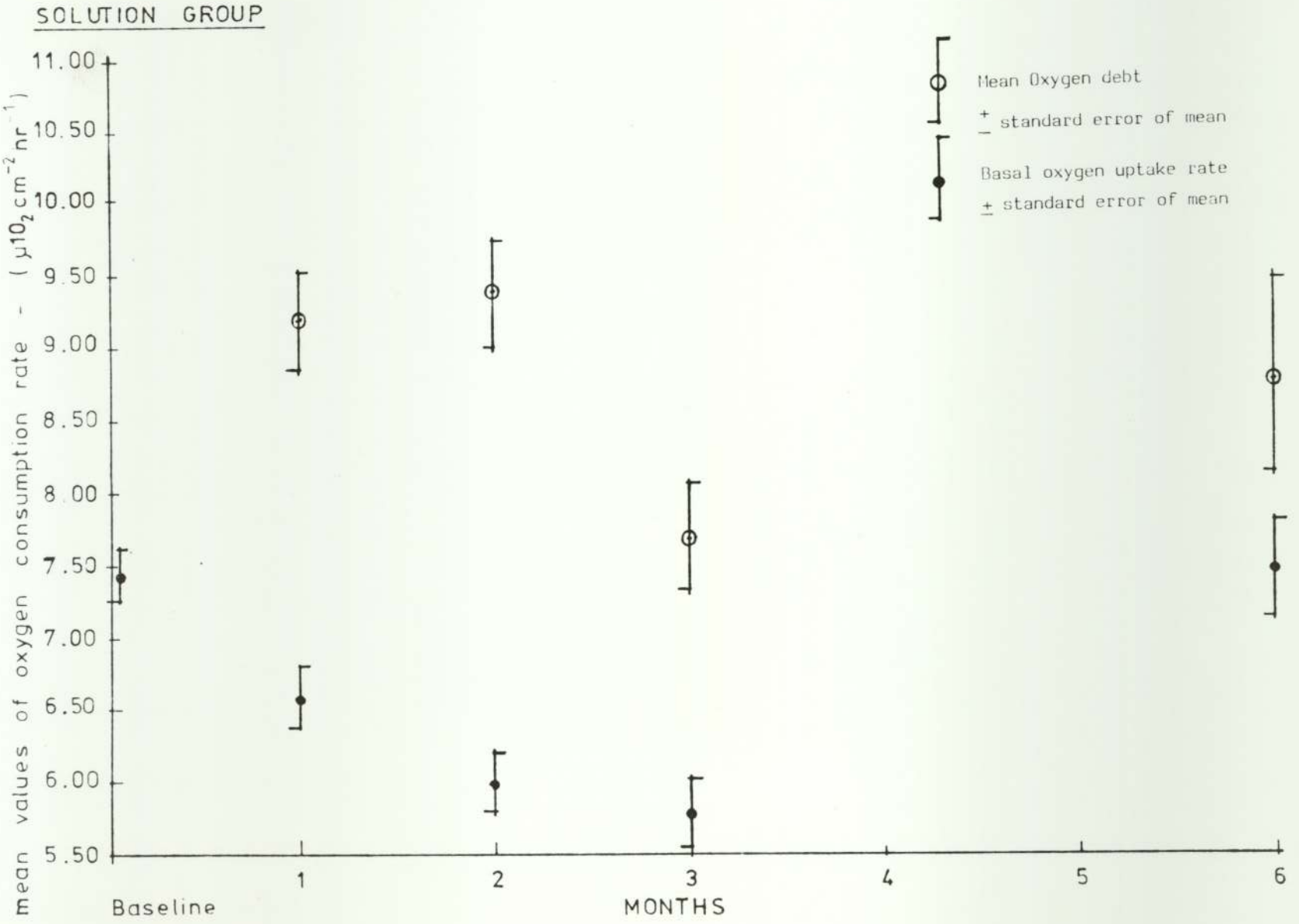

SOLUTION GROUP
Mean Oxygen debt
± standard error of mean
Basal oxygen uptake rate
± standard error of mean
mean values of oxygen consumption rate - ($\mu l O_2 cm^{-2} hr^{-1}$)
11.00
10.50
10.00
9.50
9.00
8.50
8.00
7.50
7.00
6.50
6.00
5.50
Baseline
1
2
3
4
5
6
MONTHS

FIGURE 4.8

OXYGEN DEBT AND BASAL OXYGEN CONSUMPTION RATE OVER MONTHS 1 TO 6 OF EXTENDED CONTACT LENS WEAR ($ulo_2CM^{-2}hr^{-1}$)

NON SOLUTION GROUP

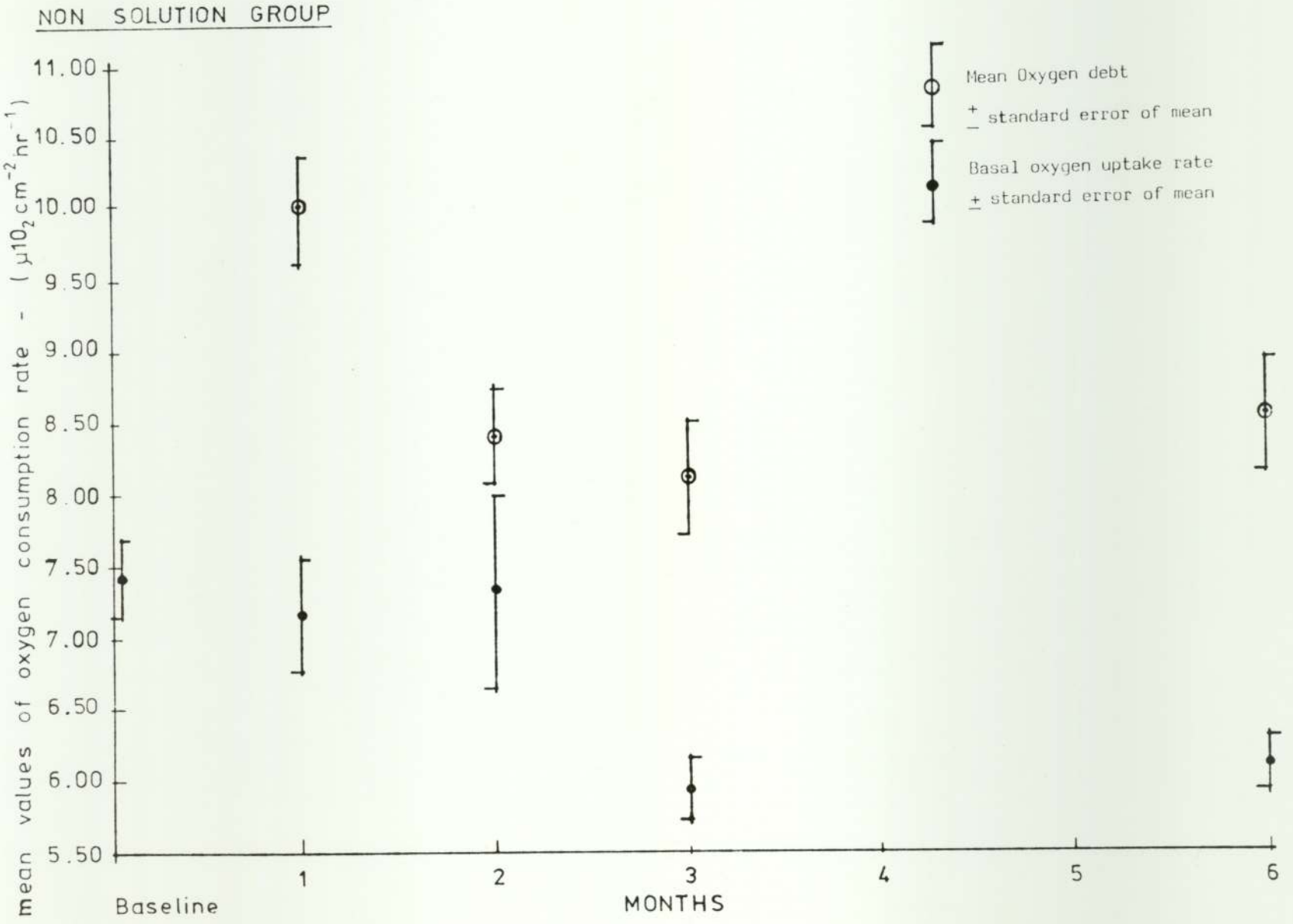

NON SOLUTION GROUP
Mean Oxygen debt
± standard error of mean
Basal oxygen uptake rate
± standard error of mean
Mean values of oxygen consumption rate - ($\mu 10_2 cm^{-2} hr^{-1}$)
11.00
10.50
10.00
9.50
9.00
8.50
8.00
7.50
7.00
6.50
6.00
5.50
Baseline
1
2
3
4
5
6
MONTHS

FIGURE 4.9

BASAL OXYGEN CONSUMPTION

RATE OVER MONTHS 1 - 6

OF THE STUDY. ($ulo_2 CM^{-2} hr^{-1}$)

CONTROL GROUP

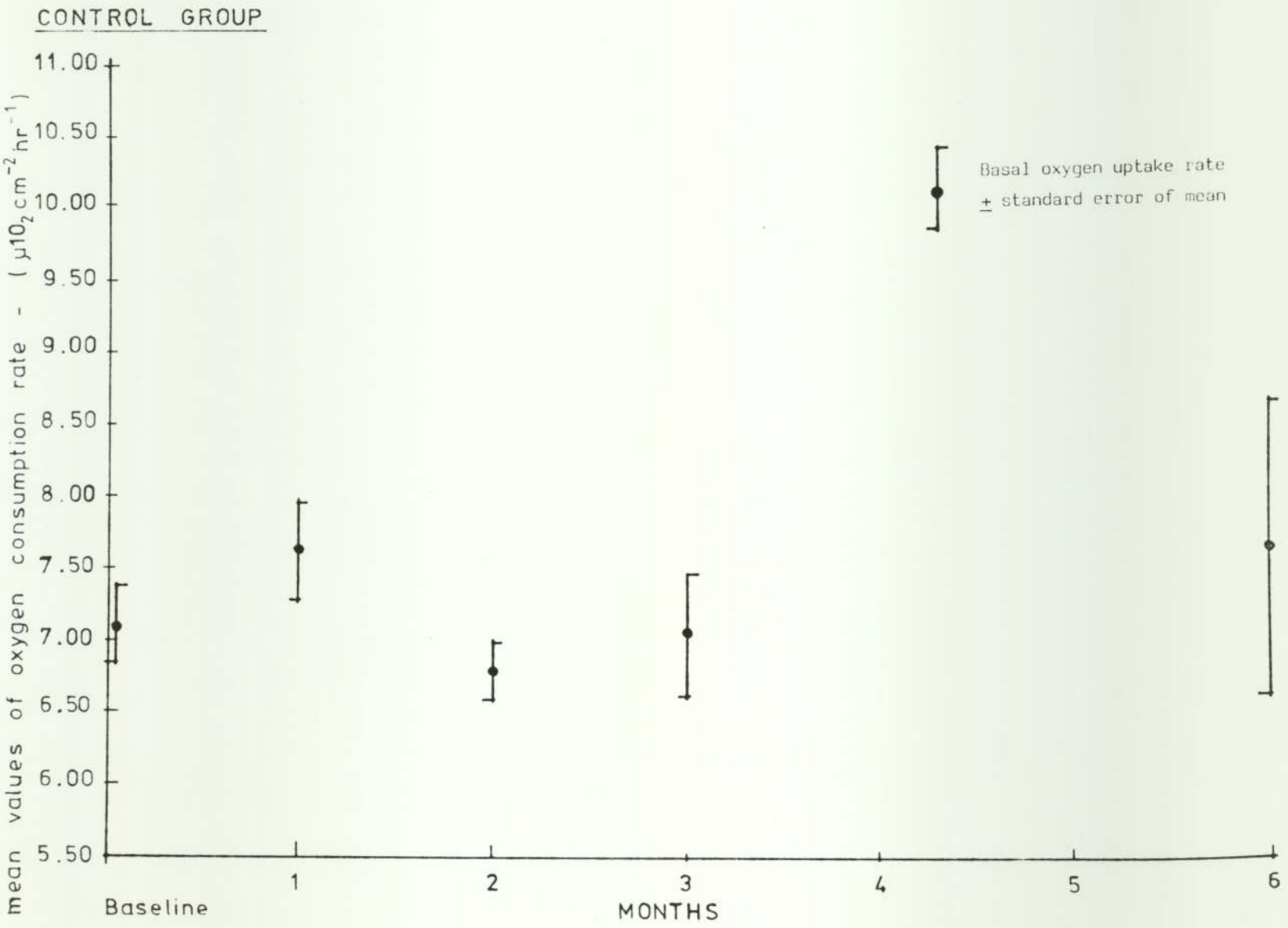

CONTROL GROUP
11.00
10.50
10.00
9.50
9.00
8.50
8.00
7.50
7.00
6.50
6.00
5.50
mean values of oxygen consumption rate - ($\mu 10_2 cm^{-2} hr^{-1}$)
Basal oxygen uptake rate
± standard error of mean
Baseline
1
2
3
4
5
6
MONTHS

TABLE 21

D'Agastino's Test for Normality

Non Solution Group Baseline Values

Grouped Oxygen Uptake Rate. $ulo2cm^{-2}hr^{-1}$	O_2 Uptake Rate $\bar{x}i$	Observed Frequency fi	$fi\bar{x}i$	$fi\bar{x}i^2$
$\leq$ 4.5				
4.5 - 5.5	5	2	10	50
5.5 - 6.5	6	7	42	252
6.5 - 7.5	7	6	42	294
7.5 - 8.5	8	5	40	320
8.5 - 9.5	9	4	36	324
9.5 - 10.5	10	1)	20	200
$\geq$ 10.5		) 2 1)		
		Efi = 26	Efi$\bar{x}$i = 190	Efi$\bar{x}$i^2 = 1440

$$SS = 1440 - \frac{(190)^2}{26} = 51.53846$$

T = 267 D = 0.28053

Since D is neither $\leq$ 0.2570 nor $\geq$ 0.2872 (p 0.01) the null hypothesis (Ho) of population normality cannot be rejected.

TABLE 22

D'Agastino's Test for Normality

Solution Group Baseline Values

Grouped Oxygen Uptake Rate $ul O_2\ cm^{-2}\ hr^{-1}$	Oxygen Uptake Rate $\bar{x}i$	Observed Frequency fi	i	$fi\bar{x}i$	$fi\bar{x}i^2$
< 4.5					
4.5 - 5.5	5	2	1-2	10	50
5.5 - 6.5	6	4	3-6	24	144
6.5 - 7.5	7	7	7.13	49	343
7.5 - 8.5	8	9	14.22	72	576
8.5 - 9.5	9	1	23	9	81
9.5 - 10.5	10	3	24-27	30	300
> 10.5					
		$Efi = 26$		$Efi\bar{x}i = 194$	$Efi\bar{x}i^2 = 1494$

$$SS = 1494 - \frac{(194)^2}{26} = 46.46154$$

$T = 247.00$ $\quad$ $D = 0.27333$

Since D is neither $\leq$ 0.2570 nor $\geq$ 0.2872 ($p > 0.01$) the null hypothesis (Ho) of population normality cannot be rejected.

TABLE 23

D'Agastino's Test for Normality

Control Group Baseline Values

Grouped Oxygen Uptake Rate. $ulo2cm^{-2}hr^{-1}$	O_2 Uptake Rate $\bar{x}i$	Observed Frequency fi	$fi\bar{x}i$	$fi\bar{x}i^2$
$<$ 4.5				
4.5 - 5.5	5	3	15	75
5.5 - 6.5	6	8	48	288
6.5 - 7.5	7	7	49	343
7.5 - 8.5	8	3	24	192
8.5 - 9.5	9	3	27	243
9.5 - 10.5	10	2	20	200
$>$ 10.5		Efi = 26	Efi$\bar{x}$i = 183	Efi$\bar{x}i^{-2}$ = 1341

$$SS = Efi\bar{x}i^{2} - \frac{(Efi\bar{x}i)^2}{12} = 52.96154$$

$$T = E\ (i - \frac{n+1}{2})\ \bar{x}i = 265.50$$

$$D = \frac{T}{\sqrt{n^3\ SS}} = \frac{265.50}{\sqrt{(26)^3 \times 52.96154}} = 0.27518$$

Since D is neither $\leq$ 0.2570 nor $\geq$ 0.2872. ($p > 0.01$) the null hypothesis (Ho) of population normality cannot be rejected.

TABLE 24

Paired Student 'T' Test Comparing pre-Fitting Values of Corneal Oxygen Consumption Rate of the Control Group to those of both Experimental Groups

Control Group	v	Solution Group	t = 0.869	N/S
			df = 50	
Control Group	v	Non Solution Group	t = 0.745	N/S
			df = 50	

TABLE 25

Comparison of Basal Corneal Oxygen Uptake Rates Between Solution Group and Non Solution Group at each Data Point

			(2-Tailed) Unpaired 'T' Test
Day 2	t = 0.937	df = 29	N/S
Day 4	0.227	25	N/S
Day 6	0.395	20	N/S
Day 8	1.150	18	N/S
Week 2	1.700	19	($p<0.20$)
Week 3	0.599	28	N/S
Week 4	1.201	29	N/S
Month 2	2.113	26	($p<0.05$)
Month 3	0.386	25	N/S
Month 6	3.818	18	($p<0.01$)

Comparison of Oxygen Debt Values Between Solution Group and Non Solution Group at each Data Point

			(2-Tailed) Unpaired 'T' Test
Day 2	t = 0.129	df = 31	N/S
Day 4	1.185	27	N/S
Day 6	0.269	20	N/S
Day 8	0.491	18	N/S
Week 2	0.881	19	N/S
Week 3	0.343	28	N/S
Week 4	1.576	29	($p<0.20$)
Month 2	1.900	26	($p<0.10$)
Month 3	0.787	25	N/S
Month 4	0.278	18	N/S

TABLE 26

Solution Group

Comparison of Baseline Oxygen Uptake Rate and the Oxygen Debt Generated at each Data Point for the Solution Group

	Paired 'T' Test		
Day 2	t = 4.467	df = 16	($p < 0.001$)
Day 4	6.850	13	($p < 0.001$)
Day 6	3.582	11	($p < 0.01$)
Day 8	5.838	10	($p < 0.001$)
Week 2	4.613	10	($p < 0.001$)
Week 3	4.323	14	($p < 0.001$)
Week 4	8.215	16	($p < 0.001$)
Month 2	10.922	14	($p < 0.001$)
Month 3	6.993	13	($p < 0.001$)
Month 6	2.393	7	($p < 0.10$)

Non Solution Group

Comparison of Baseline Oxygen Rate and the Oxygen Debt Generated at each Data Point for the Non Solution Group

	Paired 'T' Test		
Day 2	t = 4.741	df = 13	($p < 0.001$)
Day 4	3.684	12	($p < 0.01$)
Day 6	1.788	9	($p < 0.20$)
Day 8	4.539	8	($p < 0.01$)
Week 2	4.069	9	($p < 0.01$)
Week 3	9.598	14	($p < 0.001$)
Week 4	11.563	13	($p < 0.001$)
Month 2	1.541	12	($p < 0.20$)
Month 3	8.006	12	($p < 0.001$)
Month 6	5.832	13	($p < 0.001$)

FIGURE 4.10

POPULATION DISTRIBUTION OF APPARENT BASAL CORNEAL OXYGEN UPTAKE RATE IN 32 VOLUNTEER SUBJECTS

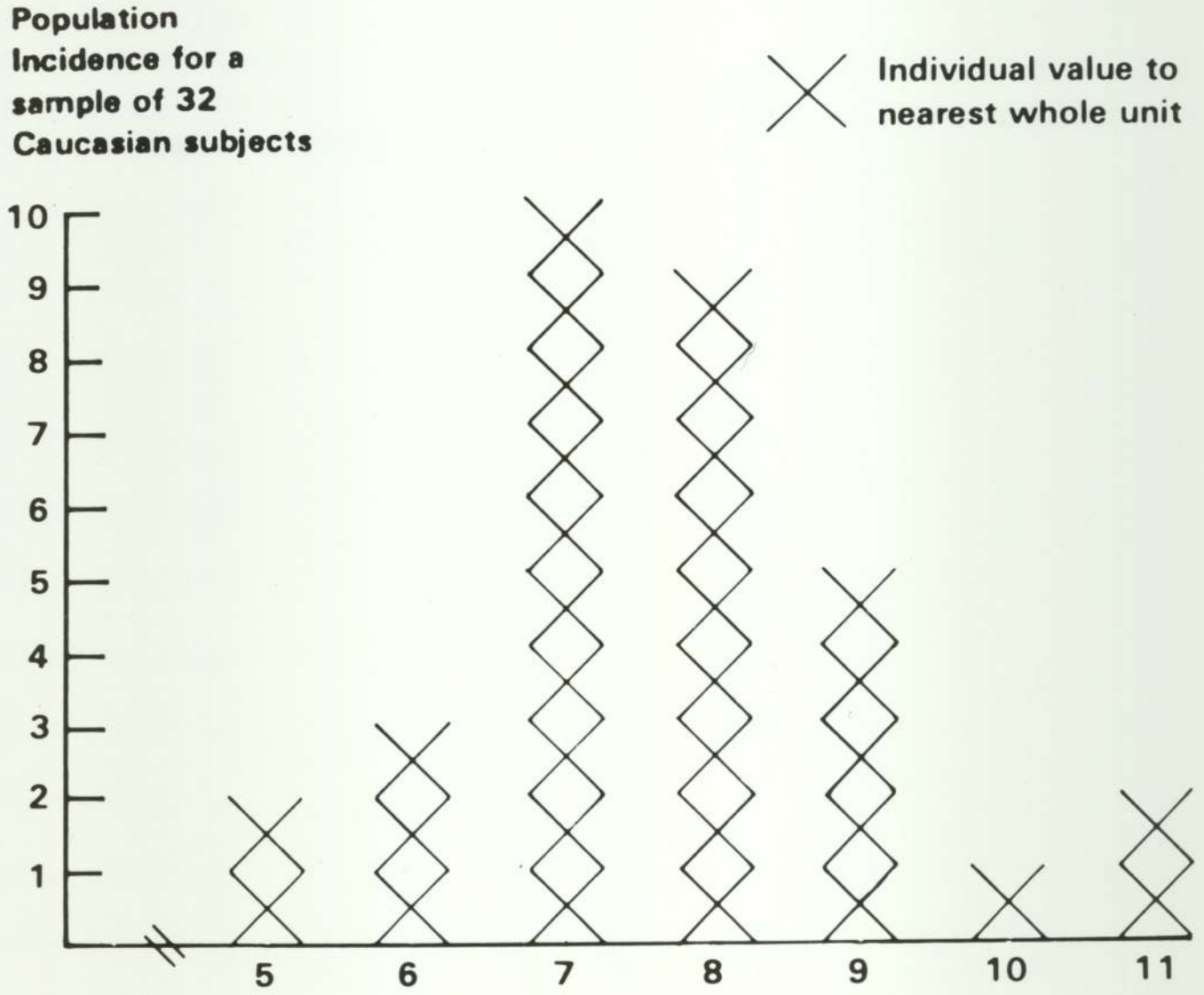
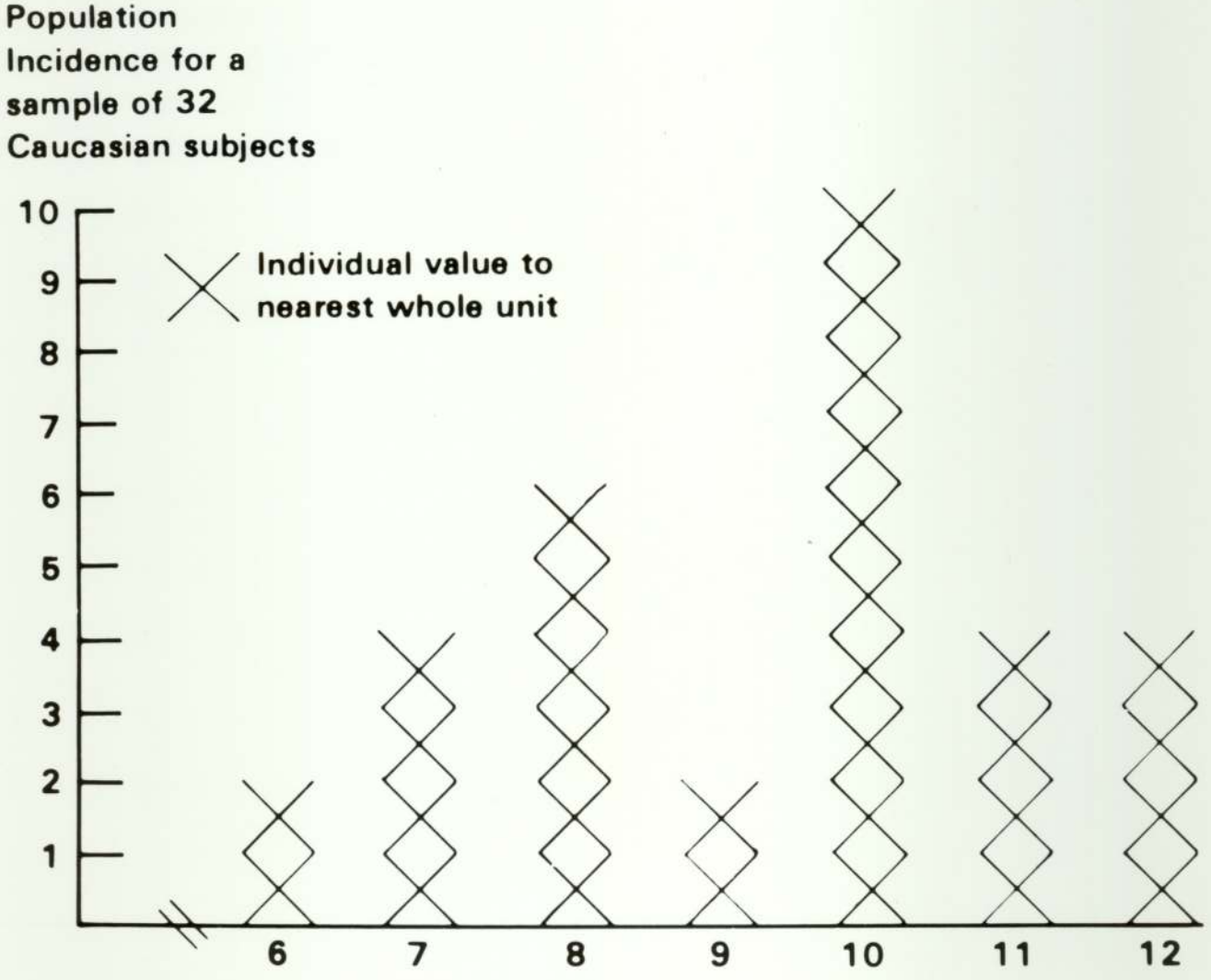

Population
Incidence for a
sample of 32
Caucasian subjects
Individual value to
nearest whole unit
10
9
8
7
6
5
4
3
2
1
5
6
7
8
9
10
11
Apparent in vivo human corneal oxygen uptake rate
($\mu 10_2$ cm^{-2} hr^{-1})

FIGURE 4.11

POPULATION DISTRIBUTION OF 'OXYGEN DEBT' GENERATED FOLLOWING 48 HOURS OF CONTACT LENS WEAR IN 32 VOLUNTEER SUBJECTS



Apparent in vivo human corneal 'oxygen debt' following 48 hours extended Soft Contact Lens wear

($\mu 10_2 cm^{-2} hr^{-1}$)

TABLE 27

A Pre Fitting Baseline Values of Corneal Oxygen Uptake Rates	-v-	B Values for Corneal Oxygen Uptake Rate - 1 Week After Cessation of lens wear.

Patient Group & Number	A	B
SOL 5	4.877	13.029
NON SOL 9	6.944	6.533
SOL 1	6.190	7.808
SOL 21	9.787	7.045
SOL 17	6.553	6.708
NON SOL 7	6.230	6.747
SOL 13	7.019	7.096
SOL 14	6.873	7.103
SOL 18	7.480	7.352
NON SOL 8	8.509	7.692
NON SOL 5	13.113	8.470
SOL 20	8.488	9.085
SOL 7	9.021	7.675
NON SOL 25	7.944	5.690
NON SOL 17	6.332	6.716

Paired 'T' Test - 2 tailed

t = 0.058

df = 14 Not Significant.

Pre-fitting Baseline values of corneal oxygen uptake rates compared to those obtained one week after the cessation of lens wear in subjects with micro epithelial cysts, in both experimental groups.

been reported (Mandell and Harris, 1968; Mandell et al. 1970; Mandell and Polse, 1969; Miller, 1968). Due to the linear relationship that exists between corneal thickness and corneal water content (Hedbys and Mishima, 1966), changes in thickness may represent the magnitude of oedema (Bailey and Caney, 1973). Oedema may result from an osmotic imbalance due to tear hypotonicity (Schoessler and Hill, 1969), which may arise from the excessive lacrimation produced in the early stages of contact lens wear (Harris and Mandell, 1969; Lowther et al. 1970). Schoessler and Lowther (1971) demonstrated an initial osmotically induced epithelial oedema. A hypotonic tear shift due to the early wearing stage of hard lenses has been reported (Terry and Hill, 1977), this lasted for up to ten days.

An increase in central corneal thickness occurring over the first week of extended contact lens wear has been observed (Humphreys, 1981). This may be due to osmotic changes, although hypoxia at the anterior corneal surface may be a contributory factor; the contribution of each mechanism has been discussed (Fatt and Chaston, 1981) based on the previous work of Sarver et al. (1981). The basal oxygen uptake rates measured in the present study were carried out 10 minutes following lens removal. The observed decrease in oxygen consumption within the

first two weeks corresponds to an increase in corneal thickness (Humphreys 1981). Hill (1979) suggested that the corneal demand for oxygen would be reduced in the presence of oedema due to the resistance offered to the path of oxygen by the extra fluid present.

Thus, initial corneal oedema may be contributory in reducing the corneal demand for oxygen. This would explain the initial reduction in oxygen uptake rate and the return to near pre-fitting values as corneal thickness decreased by week 2.

Examination of the monthly data reveals a second decrease in oxygen consumption which is greatest for both experimental groups, three months after the commencement of lens wear. It is likely that this represents a second unrelated decrease in oxygen consumption, not explainable in terms of initial contact lens induced oedema.

An explanation for this event may be attributable to:-

1 Changes in the structure or rate of cell mitosis of the epithelium

2 Seasonal variation - changes in temperature
- changes in light intensity or duration

3 Metabolic adaptation to the reduced oxygen environment.

1 A variation in the number of epithelial cell layers in rabbit of between five and seven has been

observed (Hill, 1978). More recently, the continuous wear of a hard lens for 24 hours caused epithelial damage; the thickness of the monkey epithelium was reduced to one cell layer thick in places (Bergmanson and Chu, 1982). Albeit that this type of lens worn continuously would provide considerable corneal insult; it is conceivable that wearing an extended soft lens for three months could cause some reduction in the number of cell layers. Krejci and Krejcova (1973) demonstrated structural changes in rabbit epithelium following a fifteen week period of hydrophilic lens wear. This was established by the reduced time for cell death to occur when the excised tissue was placed in culture medium.

A reduction in epithelial cell number would possibly reduce the demand for oxygen, although this would be of significance only if the high oxygen consuming basal cells were affected. It is more probable that only the superficial cell layers in contact with the contact lens may be damaged (Bergmanson and Chu, 1982).

Structural changes on fish respiratory epithelium have been observed due to continuous mechanical abrasion and hypoxia; fusion occurs between adjacent lamellae and the oxygen consumption is reduced (Herbert and Merkens 1961; Hawkes, 1982). The intracellular spaces are narrowed in the anoxic toad bladder (Crocker et al. 1970). The exact physiological nature of such changes have not been postulated. The occurrence of a similar event in

man in response to the mechanical pressure or hypoxia induced by a contact lens, may result in a reduced oxygen demand.

A change in cell structure and volume may occur with a variation in oxygen tension levels. This may arise from changes in the sodium ion permeability of the cell membrane in relation to the activity of the membrane ion pump. After re-establishing normal oxygen tensions following a period of relative anoxia, a 20% shrinkage in human in vivo epithelial structure was noted (O'Leary et al. 1981). Under certain conditions, mitochrondrial structure will alter in respect of shape volume, and cell wall permeability. It has been suggested that the conformational changes which occur in mitochondria may be intimately connected to the energy transformations that occur during substrate oxidation (Loewy and Siekewitz, 1978). A reduction in both cell membrane and mitochondrial activity may therefore induce a reduced demand for oxygen. A change in the rate of epithelial cell mitosis may also influence oxygen demand. Epithelial mitosis rate has been shown to alter in response to excitement or painful stimuli (Friedenwald and Buschke, 1944). It is conceivable that the mechanical barrier of an extended wear lens, will limit the normal rate at which the superficial epithelial cells slough off into the tear film; this may have an inhibitory feedback to reduce the

rate of mitosis (Perris, 1982).

The outline of mitotic control mechanisms of adult mammalian tissues have emerged in recent years (Bullough, 1972(a)(b)). Evidence exists for a tissue-specific negative feedback system. The post-mitotic (ageing) cells can feedback information to the mitotic basal cells by a tissue-specific antimitotic messenger molecule; termed a chalone (Bullough, 1962). In stable condition of normal chalone concentration each cell mitosis results in one mitotic and one post mitotic cell. If the chalone concentration increases there is a decrease in the number of basal mitotic cells. Most of the current work has been carried out on epidermal cells, althougn chalone systems are universally present in mammalian tissues. A review of this work and a detailed account of the systems has been presented (Bullough, 1975).

The exact nature and mode of action of chalone chemistry is not yet realised. Mitotic homeostatic control has been observed and it may be that if present in ocular tissues, inhibition of post mitotic cells by a contact lens may reduce the rate of cell mitosis in order to maintain constant tissue thickness. A reduction in basal cell activity would reduce the need for atmospheric oxygen.

2 An alternative explanation may be considered in terms of seasonal variation; due to both increased day-light duration and intensity, and temperature (Perris, 1981). Albeit that no statistical variation occurred

throughout the duration of the study for the control group, visual inspection of the data reveals a slight but similar pattern to that obtained for the experimental groups. The collected data at three months following lens wear, was that obtained nearest to mid-summer's day. Since the cornea is both metabolically torpid and avascular, its main source of heat must be environmental (Mapstone, 1968), although this view has been questioned (Freeman and Fatt, 1973).

Changes in the ambient temperature produce a linear change in corneal temperature (Shwartz, 1965; Mapstone, 1968; Freeman and Fatt, 1973). Temperature is one of the fundamental parameters of tissue metabolism: the cornea has a reduced relative metabolic activity when ambient temperature is low (Freeman and Fatt, 1973). It has been suggested that sustained reduced anterior eye temperature may involve an adaptive process to increase local temperature; requiring a thermal feedback mechanism (Freeman and Fatt, 1973). However, the results of the present study show a decrease in oxygen consumption corresponding to the expected maximum in ambient temperature during the study and thus a temperature effect would not be a satisfactory explanation.

The effect of light on metabolic function has recently been reviewed (Hollwich, 1979); studies suggest that light influences carbohydrate metabolism (Hollwich and Dieckhues, 1967; Halberg et al. 1960; Elfvin et al. 1955). However, the period of maximum light intensity and duration

also corresponds in the present study to the minimum oxygen uptake rate, and this also does not provide a suitable explanation for the observed results.

3 A further explanation for the second observed drop in oxygen consumption rate may be given in terms of a biological feedback control, regulating metabolic function (Atkinson, 1965; Umbarger, 1964). It is now established that even in the simplest organisms, complex regulatory circuits play an essential role governing the rate of flow of metabolites through various pathways as the synthesis of proteins and other macromolecules (Monod et al. 1963).

Although the numerous metabolic pathways of a normal cell are biochemically independent, they are closely inter-connected and co-ordinated with regard to the rates of synthesis of their products. Much of this co-ordination is derived from regulatory enzymes which operate at critical steps in the sequence of metabolic reactions; through these enzymes a mechanism exists for the close co-ordination of metabolism (Gerhart and Schachman, 1965).

The time course of physiological variation in metabolic rate varies with the nature of the environmental change (Prosser, 1958). Three periods can usually be identified; an initial shock reaction lasting seconds or minutes, a stabilised rate for minutes or hours, and acclimatisation or compensation over a period of days or months (Prosser and Brown, 1961). In the process of acclimatisation

to hypoxia there is developed an ability to maintain a normal intensity of the oxidising exchange of substances despite the lowering of the pressure of the oxygen in the surrounding environment; the basis of this ability is due to the increase in the effectiveness of the oxidising - reducing enzymes of the system at the cellular level (Barbashova, 1964). As early as 1878 the hypothesis was advanced that a lowering of metabolic rate was a mechanism involved in the struggle of an organism against an oxygen deficiency (Bert, 1878). More recently, a lowering of oxygen consumption rate by tissue isolated from rats and kept at an oxygen environment of 10 per cent has been demonstrated (Duckworth, 1961), although prolonged exposure to hypoxia may not always affect metabolic rate (Stickney and Van Liere, 1953).

During periods of contact lens wear the production of carbon dioxide will increase; this may build up and diffuse into the pre-corneal tear film (Wigham, 1978). A reduction in oxygen consumption has been observed in cold blooded animals following an increase in carbon dioxide tension, this has now been demonstrated in some mammals (Fenn and Rahn, 1965).

A similar circumstance in man would provide an alternative explanation in terms of adaptation at the cellular level for the observed trend in the data.

The living cell is a steady-state system, maintained by an undirectional flow of metabolites, which is flexible

to ensure the constancy of the organisms internal environment, despite wide variations in the external environment (Harper et al. 1979). The control of cellular metabolism centres on the regulation of enzyme type, amount and level of activity (Loewy and Siekevitz, 1978).

Lactate dehydrogenase (L.D.H.) has been termed an iso-enzyme; it can occur in multiple forms all having the same specific enzymic activity, but which can be separated from one another by physical means. Each form is made up of a different arrangement of the four component polypeptide chains which may be of the heart type (H) and/or the muscle type (M), yielding the possibility of five different combinations corresponding to the five iso-enzymes found in nature. The kinetic properties of each form are compatible with the physiological requirements of the host tissue (Market, 1963). Characteristic changes in the iso-enzyme pattern occur in different diseases, for example, a change in the iso-enzyme pattern of cardiac muscle has been observed due to myocardial infarction (Harper et al. 1979; McDonald et al. 1977). L.D.H. converts pyruvate to lactate and vice-versa. In tissues with prevailing aerobic metabolism L.D.H. iso-enzymes are built up from mainly (H) sub-units (Kahan and Ottovay, 1975(a). These sub-units are relatively inactive when pyruvate is present in excess- their reaction rate is high however for the reverse process, i.e. lactate is readily dehydrogenated to pyruvate.

Pyruvate is easily converted to lactate by the L.D.H. iso-enzyme containing mainly (M) sub-units and is present in tissues with mainly anaerobic metabolism and a high rate of cell mitosis (Kahan and Ottovay, 1975(a). Their activity is suppressed by excess of lactate.

Of the metabolic pathways operating in the cornea, the Kreb's Cycle is the most efficient; consequently studies have examined the effect of oxygen deprivation on the concentration of specific oxygen dependent enzymes; in particular succinic dehydrogenase and lactate dehydrogenase. Changes in enzyme concentration occur with alterations in oxygen availability and time of deprivation (Hill et al. 1974; Uniacke and Hill, 1972; Lowther and Hill, 1973; King et al. 1971; Augsburger et al. 1972; Uniacke et al. 1972; Rengstorff and Hill, 1974).

L.D.H. iso-enzyme patterns obtained in the pre-corneal tear film may provide information regarding the metabolism of the corneal epithelium (Haeringen and Claussius, 1976; Kahan and Ottovay, 1975 (a)(b)). The bulk of tear L.D.H. originates from the cornea (Kahan and Ottovay, 1975(a)). Changes in this L.D.H.content of the tears with metabolic disorders such as diabetes mellitus and various forms of cataract have been observed (Kahan and Ottovay, 1975(a); McDonald et al. 1977). Different proportions of L.D.H. iso-enzymes in the tear fluid have been determined using pyruvate and lactate as substrates (LDHp and LDH1); the ratio of anaerobic to aerobic metabolism in the corneal epithelium can be given by the expression $\frac{LDHp}{LDH1}$ (Hudomel et al. 1980), and in normal eyes was found to have a mean

value of 3.96.

Similarities between corneal and lenticular metabolism have been suggested (Foulks et al. 1979). Nuclear cataract produced a significant increase in the $\frac{LDHp}{LDHl}$ ratio (mean 6.11). It was suggested that this showed adaptation at the intracellular level to reduced oxygen supply and nutrition (Humodel et al. 1980). Such a change in the L.D.H. pattern implicates an increase in the lactate concentration, albeit that the exact dynamics of the pyruvate-lactate system are not readily identifiable (Bailey, 1981).

The catalytic efficiency of an enzyme is affected by changes in the concentration of substrates and products of an enzymatic reaction. An increase in product concentration will tend to slow down its rate of formation maintaining an equilibrium. The mechanism of this 'feedback inhibition' has been described (Monod et al. 1963). An increase in the lactate, pyruvate concentration may therefore limit its rate of formation by the Glycolytic pathway; thus reducing the demand for oxygen utilisation (Bailey, 1981).

Most of the glucose in the cornea is incompletely oxidised to lactic acid under normal atmospheric availability of oxygen. In rabbit, this value has been given as 84%; lactic acid can be consumed in some tissues, but it is not known how it is used in the cornea (Fatt, 1978). The high rate of production of lactic acid is normally

associated with insufficient oxygen for complete oxidisation of glucose. The cornea however is a thin tissue with its cellular layers in intimate contact with oxygen - containing fluid; and the high concentration of lactic acid "must be considered an unsolved problem in corneal physiology" (Fatt, 1978). The possibility of an adaptive change in the cytochrome system has also been reported in the literature; and an increase in the cytochrome content following hypoxia has been observed (Lawrie, 1953; Fox, 1954), although contradictory findings have been noted (Stickney and Van Liere, 1953). Such an increase in cytochrome activity may facilitate the utilisation of the available oxygen in hypoxia (Barbashova, 1964). An adaptive change in L.D.H. iso-enzyme pattern or cytochrome activity may produce a change in the glycolytic pathway due to hypoxia, thus reducing the oxygen consumption rate. Such a change would produce a satisfactory explanation for the observed drop in corneal oxygen demand after three months of lens wear.

The results of the present study show both initial and long term adaption to contact lens wear. Hypothesis have been put forward to explain these findings in terms of chemical, environmental, intracellular and structural adaptation. The 'Solution' experimental group showed a near return to pre-fitting values at six months. This may be indicative of a recovery, but due to the presence of microepithelial cysts, and the poor patient attendance at this point this may not be a real event. The over-riding conclusion must

be that the temporal profile in baseline oxygen uptake rate and oxygen debt in extended contact lens wear cannot be explained at the present time.

The following experimental design limitations were encountered:-

(a) There was no incentive to encourage regular patient attendance. Consequently, data was not available for some subjects at various stages throughout the study. A subsequent investigation (Callender, 1980) required an initial payment for the provision of lenses which was refunded at the end of the study, if all data collection appointments were kept.

(b) Only one opportunity was available to obtain 'oxygen debt' values at each data collection point. The sensor had to be in contact with the cornea within 10 seconds of lens removal. Failure to achieve this resulted in a loss of data at that time. Clearly, with subject apprehension, blinking and potential irritation, this was not possible on every occasion.

(c) Only one value for basal oxygen uptake rate was taken, 10 minutes following lens removal. Due to the lack of effect of repeated measurement on oxygen consumption, it would enhance accuracy if an increased number of measurements were obtained.

4,10.3 Further Considerations of Oxygen Consumption Rate

Basal oxygen uptake rates were compared between the two experimental groups at each data point. A statistically significant difference was found after 2 weeks, 2 and 6 months. Both groups showed a reduction in oxygen demand in the first two weeks; this was minimal after 1 week for the solution group and at 2 weeks for the non-solution group. At this point in time no recourse to cleaning solutions had been necessary and therefore no explanation as to the difference in the rate of change in oxygen consumption between the groups is offered. After six months, the patient attendance was low for the solution group and this may have affected the results giving an apparent rise to near pre-fitting values.

Oxygen debt values were also compared between the two groups. A statistically significant change was noted after 1 and 2 months. The second decrease in oxygen demand occurred after two months for the non-solution group, thus more rapidly then for the solution users. After three months the mean values for both groups were minimal and similar.

Due to the overall similarities which exist between the two groups in respect of both basal and oxygen debt values, it does not appear that the use of a proprietory cleaning

solution has any affect on corneal oxygen consumption or debt.

The frequency of cleaning was determined clinically and by patient requirement. It was necessarily infrequent and thus contrasts to the regular cleaning regime used in the previous work on corneal thickness (Hirji, 1978). It is therefore concluded that the infrequent use of a proprietory cleaning solution does not affect the corneal demand for oxygen.

The obtained values for oxygen debt were compared to the basal oxygen demand at each data point and for both experimental groups. The 'oxygen-debt' was higher and statistically significant at each point. The oxygen debt values generally mirrored the changes in basal values. This suggests that the degree of 'oxygen-debt' generated is proportional to the basal metabolic rate during lens wear.

A correlation of pre-fitting oxygen uptake rate and the degree of 'oxygen-debt' generated following 48 hours of extended contact lens wear was examined to determine if a subject with a high pre-fitting oxygen demand necessarily produces a high 'oxygen debt'. Previous work by Terry and Hill (1977) confirmed a direct correlation between pre-fitting tear tonicity and the degree of lens induced hypotonicity needed to produce oedema.

No correlation was found in the present study; this may be due to additional factors influencing the oxygen tension at the anterior corneal surface during contact lens wear, thus modifying the amount of 'oxygen debt' produced.

Lens fit has been shown to alter the degree of 'oxygen debt' produced in one form of soft contact lens wear (Parrish and Larke, 1981(b)). Contact lens thickness and mobility may also be contributory factors, both in the experimental eye under observation and the contralateral eye, as a sympathetic change in apparent corneal oxygen uptake rate has been demonstrated (Parrish and Larke, 1981(c). Further changes in oxygen tension under a contact lens due to blinking have been observed (Fatt and Hill, 1970).

The 'oxygen debt' produced by contact lens wear can therefore be influenced by many external factors; each one capable of changing the oxygen tension under a contact lens at the anterior corneal surface, and thus the degree of 'oxygen debt' generated. At the present time the affect of each of these factors has not been individually determined. However, the measurement of 'oxygen debt' still provides an indirect indicator of corneal oxygen utilisation under a contact lens.

There was no correlation between pre-fitting oxygen uptake rates and the degree of 'oxygen debt' generated after 48 hours contact lens wear ($r = 0.07$) in 32

volunteer subjects. The population distribution of both the pre-fitting levels and the 'oxygen debt' produced were found to conform to a normal distribution ($p > 0.01$) (D Agastino's test for normality) and are shown graphically in Figures 4.10 and 4.11).

The study was scheduled to be carried out over eighteen months. Due to the presence of epithelial microcysts it was terminated at the six month data collection point. A comparison of pre-fitting values for oxygen demand with those obtained one week after the cessation of lens wear was carried out, on fifteen volunteer subjects, a detailed description of the recovery from microcysts has been reported (Humphreys et al. 1980).

There was no statistically significant variation between pre and post wearing oxygen demand. This suggests that the presence of micro corneal cysts does not affect the corneal requirement for oxygen. The cysts were distributed in the superficial epithelial layers; as such they would be unlikely to affect the high oxygen consuming basal cells, and this is consistent with the observed results. If contact lens wear had been continued, involvement of the posterior epithelium or Bowman's membrane may have occurred (Humphreys et al. 1980); clearly this may have affected the oxygen consumption rate.

CHAPTER 5

THE CORNEAL ENDOTHELIUM

5.1. Introduction

Endothelial cells are derived embryologically from para-axial mesoderm tissue and as such may be described as mesothelium or mesenchymal epithelium. The term corneal mesothelium has been suggested to be more appropriate than corneal endothelium, as an endothelial cell is one which lines a blood or lymph-filled channel (Smith and Copenhauer, 1944). The term endothelium does not therefore correctly describe the posterior corneal cells. This consideration was presented by Verhoeff and cited by Lloyd, (1944).

The term endothelium is, however, widely accepted and will be retained in this work.

The endothelial layer has been shown to be the major tissue in controlling the relative degree of stromal hydration (Mishima and Kudo, 1967; Maurice, 1972). Corneal deturgescence is maintained through the activity of a metabolically driven pump (Dikstein and Maurice, 1972). The normal state of corneal deturgescence reflects the balance between fluid that is drawn into the stroma, and fluid which is actively pumped back into the aqueous. If the pump is impaired, corneal oedema ensues. The adult human endothelium is a finite group of cells in which mitotic cell division in vivo does not routinely occur (Shaw et al. 1978; Stocker, 1971), although cell division in rabbits has been demonstrated (Bron and Brown, 1974

Van Horn et al. 1977(a)). When endothelial cells are destroyed, the remaining cells enlarge and spread out reforming the uniform cellular monolayer (Doughman, et al. 1976). The ability to re-establish morphologic continuity has been interpreted as a spreading potential or healing reserve (Bron and Brown, 1974; Bourne et al. 1976; Laing et al. 1976(a)).

The ability to reverse corneal oedema has been termed the endothelial functional reserve (Shaw et al. 1978). The limits of the endothelial functional reserve may be approximated by cell loss, or a change in the pumping capacity of the endothelium or both. Such a cornea may appear clinically normal. Further insult or stress however may produce decompensation. Endothelial decompensation has become established as a primary factor in the pathogenesis of corneal oedema.

Monitoring endothelial cell density may give the first signs of endothelial decompensation. However, cell density can undergo a marked reduction without compromising corneal hydration or increasing corneal thickness. A thin clear corneal graft has been maintained by an endothelium having only 13% of the cells considered normal for its age (Laing et al. 1976 (b)).

5.2. THE DEVELOPMENT OF ENDOTHELIAL OBSERVATION

Observation of the cornea using the slit lamp was reported as early as 1911 (Gullstrand, 1911). One of the earliest

references to human in vivo endothelial observation has been attributed to Vogt (1919; 1920; 1921). During the following 20 years, concepts of slit lamp methods of observation including specular illumination developed and have been reported (Berliner, 1943). Advances were made regarding ocular photography, and the first satisfactory photographs of the optical corneal cross section were produced by Goldman (1938; 1940).

Maurice (1968) described the first specular microscope and produced photographs of endothelium from in vitro rabbit eye. The instrument required contact with the epithelial surface, but provided magnifications of up to 400X, thus considerably exceeding those obtained with the slit lamp. Brown (1970), constructed a clinical photomicroscope; contact with the anterior corneal surface was not necessary but the resultant magnification was only in the order of 10X - 20X. This instrument was applied clinically in a study of grafted corneae (Bron and Brown, 1974). Hoefle et al. (1970) used the specular microscope of Maurice in the non-contact mode to photograph endothelium in vitro and measure corneal thickness. Improvements in the specular microscope for in vitro use were made (Leibowitz et al. 1974). Laing et al (1975) were the first to obtain high magnification photographs of the endothelium in vivo. The first clinical specular microscope was reported by Laing et al. (1975; 1976(a)). The use of clinical specular microscopy during routine examination was subsequently described (Bourne et al. 1977; Bourne and Kaufman. 1976(a)).

More recent developments have centred on increasing the available field of view in order to obtain a larger number of cells per photograph. Varying techniques have been employed (Maurice, 1974; Koester et al. 1979, 1980; Sherrard, 1979; Roberts, 1980; Roberts et al. 1981; Sherrard and Buckley 1981(a)(b)). Further developments of the macrophotography approach to produce a non-contact specular microscope have been made (Holm, 1978; McCarey, 1978; Bigar et al. 1978(a), (b), 1979). Most contact specular microscopes can be used in a non-contact mode by using a long working distance objective lens. Non contact methods generally use a slit lamp to project a thin slit of light onto the endothelium. The specular reflection from the endothelium can then be photographed. Changes in the corneal endothelium following soft contact lens insertion have been monitored using a similar technique (Zantos and Holden, 1977; Holden and Zantos, 1978). More recently changes following hard contact lens wear have been reported (Barr and Schoessler, 1980).

The advantages and disadvantages of contact and non-contact specular microscopy are summarised in Table 28.

5.2.1. OPTICAL PRINCIPLES

The detailed optical pronciples of specular microscopy have been well reported (Bourne and Enoch, 1976; Laing, et al. 1979(a); Laing 1980; Olsen 1979(a)).

TABLE 2.8

CONTACT SPECULAR MICROSCOPY

Advantages

1. Better corneal fixation stability.
2. Easier scanning over cornea.
3. Better resolution.
4. Higher magnification.

Disadvantages

1. Patient tolerance.
2. Epithelial contact/potential damage
3. Cannot be used following recent surgery.
4. Applanation artefacts/posterior corneal rings. (Sherrard and Buckley, 1980).

NON-CONTACT SPECULAR MICROSCOPY

Advantages

1. Good patient tolerance.
2. Less risk of corneal damage.
3. Quicker to use.

Disadvantages

1. Microsaccardic eye movements.
2. Difficult to obtain direct analysis of results.
3. Only small region of specular reflection.
4. Lower magnification.
5. Objective less powerful/less resolution.

The microscope objective is separated in half. Incident light in the form of a narrow slit at nearly normal incidence is passed down one side. Following its reflection at the posterior corneal surface it returns along the other side and is collected by the objective lens. Most light is transmitted into the aqueous humour. A small fraction of light, approximately 0.2% is reflected from the aqueous humour - endothelial interface (Laing et al. 1979(a)). In the normal cornea, nearly all of this light is specularly reflected and collected by the objective lens of the photomicroscope. When focussed on the posterior corneal surface, an image of the endothelium is so formed. Applanation produces partial stabilisation of the cornea enhancing the quality of the image (Olsen, 1979(a); Bourne and Enoch, 1976). (Figure 5.1).

Estimates of cell density will vary between contact and non-contact modes of observation due to changes in the anterior corneal surface as a result of applanation.

Example 1

Assume applanation of the cornea produced a magnification of unity 1X

and u_c = Refractive index of cornea = 1.376

r_c = Radius of non applanated cornea 7.8mm (0.0078m)

t_c = Thickness of cornea = 0.5mm (0.0005m).

Then

$$\frac{1}{l_1} = \frac{u_c - 1}{r_c} - \frac{u_c}{t_c}$$

Therefore $\frac{1}{l_1} = \frac{1.376-1}{0.0078} - \frac{1.376}{0.0005} = 3.63372 \times 10^{-4}$

Therefore Magnification $= \frac{h_1}{h} = \frac{7.8-0.36337(mm)}{7.8-0.5} = 1.0178$

There is an increase in magnification of 1.78% due to the curvature of the anterior corneal surface.

By the same principle an increase in corneal thickness or refractive index will also increase the apparent magnification.

Example 2

Assume tc changes from 0.5mm to 0.7mm.

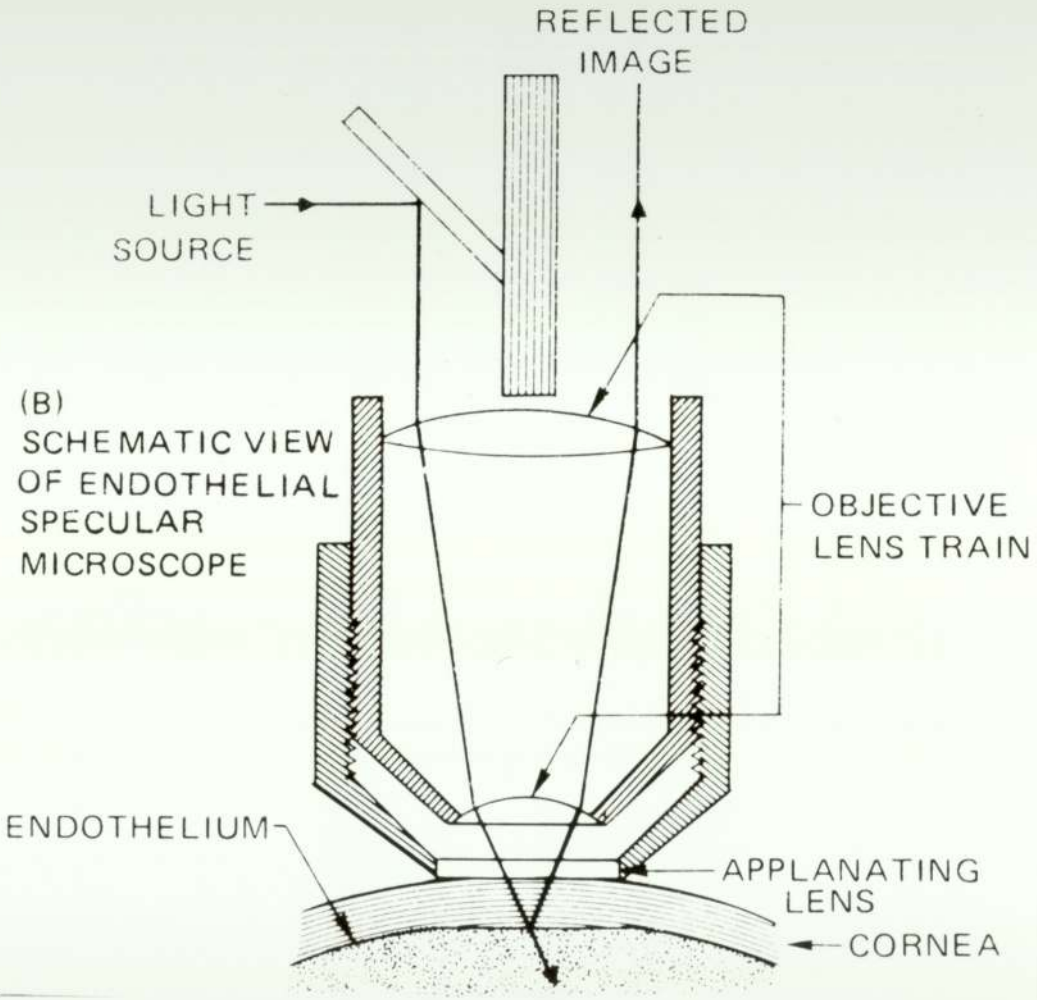
$\frac{1}{l} = \frac{uc-1}{rc} - \frac{uc}{tc}$ - Magnification $\frac{h_1}{h} = \frac{7.80.52}{7.8-0.7} = 1.0253 = 2.53\%$

There is an increase in magnification due to increased corneal thickness.

Figure 5.1

Schematic view of endothelial specular microscope

After Knight et al. 1978



After Knight, P.M., Link, W.J., Kaufman, H.E. - The Corneal Endothelium, Jan. 1978, Heyer Schulte Medical Optics Centre.

5.3. APPLICATION OF SPECULAR MICROSCOPY

Estimates of endothelial cell densities have been obtained using both contact and non-contact specular microscopy (Laing, et al. 1975; Olsen, 1979(b)). A decreased endothelial cell population with age has been demonstrated (Sperling, 1978; Blackwell et al., 1977; Laule, et al 1978; Sturrock et al 1978; Laing et al 1976(b); Bourne and Kaufman, 1976(a); McCarey, 1979; Majima et al. 1979; Hiles et al. 1979; Shaw et al. 1978; Blatt et al. 1979, Capella, 1971).

Since 1975, specular microscopy has become widely used and established as a technique to observe changes in endothelial cell density and cell morphology. It has been used extensively to monitor changes in cell density following cataract surgery (Cheng et al 1977(a); Forstot et al. 1977; Hirst et al. 1977; Tragakis et al. 1977; Sugar et al 1978 (b); Laing et al. 1979(b); Olsen, 1980(a); Miyake et al. 1978; Culbertson et al 1979; Majima et al. 1979; Gallin et al. 1979; Abbott and Forster, 1979), and also following cataract extraction together with intra ocular lens implantation (Abbot and Forster, 1979; Bourne et al. 1979; Sugar et al. 1978(a)(b); Kaufman and Katz, 1976, 1977; Forstot et al 1977; Bourne and Kaufman, 1976(b); Katz et al. 1977; Aquavella and Shaw, 1977; Stark et al 1977; Gallin et al. 1979; Stanley et al. 1980; Cheng et al. 1977(a),(b); Irvine et al. 1978; Hoffer and Phillipi, 1978: Kimura,1978; Rao et al 1978(a); Hirst et al, 1977; Binkhorst et al. 1977 (a)(b), 1978). Cell loss following intra ocular lens

implantation has also been reported using scanning electron microscopy (Katz et al 1977, Kaufman and Katz, 1976). Cell loss due to phakoemulsification (Kelman, 1967) in the surgical treatment of cataract has also been reported using scanning electron microscopy (Polack and Sugar, 1976, 1977). Relatively few specular microscopy studies of the affects of phakoemulsification have been reported (Bourne and Kaufman 1976(d); McCarey et al 1976; Irvine et al. 1978; Chansel and Polack, 1976; Bourne 1978; Sugar et al. 1978(a); Culbertson, et al. 1979; Gallin et al. 1979).

Specular microscopy has also been used to examine endothelial cell densities and morphology prior to, and following keratoplasty (Laing et al 1976(a); Rao et al. 1978(b); Olsen, 1979(b); Sato, 1978; Culbertson et al. 1979; Maloney et al. 1979; Bourne and O'Fallon, 1978; Ruben et al. 1979; Abbot and Forster, 1979; Ruusuvaara, 1979, 1980; Bourne and Kaufman 1976(c); Olson and Levenson 1977).

Considerable endothelial cell damage can result following surgical intervention of the anterior segment. Attempts to isolate the cause of such damage included a review of the surgical procedures used. Cellular damage has, however, been reported due to irrigating solution commonly used during surgery or applied topically to the cornea (Coles, 1975; Van Horn et al 1977(b); Edelhauser et al. 1976; Green et al 1977; Gonnering et al 1979; Waltman et al 1977; Hull et al. 1975; Cohen et al 1979; Weekers and Dethinne 1978; Lavine et al. 1979; McCarey et al. 1976; Edelhauser et al.1975; 1976; 1978; Vannas et al. 1978).

Endothelial damage has also been observed following the introduction of air into the anterior chamber (Leibowitz et al. 1974), anterior uveitis (Olsen, 1980(b))., viral induced toxicity (Wilcox, et al. 1958), raised intra ocular pressure (Svedbergh, 1975; Olsen, 1980(c); Irvine 1956; Vannas et al. 1977; Stocker, 1971; Setala and Vannas, 1978), osmotic dehydration of the cornea, (Sherrard, 1976; 1978), external mechanical trauma (Bourne et al 1976) keratoconus (Laing et al. 1979(c), and ultrasound (Olson et al. 1978 (a)(b)).

5.3.1. EVALUATION OF ENDOTHELIAL SPECULAR MICROGRAPHS

Endothelial observation using a specular microscope provides sufficient cellular detail to allow a qualitative evaluation of cell morphology. A classification of the variety of morphological structures observed in endothelial photographs has been proposed (Laing, 1979, Laing et al. 1979(b); Sherrard, 1978).

Endothelial photography taken with a slit lamp can undergo increased magnification by photographic enlargement, (Holden, 1978), or projection enlargement (Parrish and Larke, 1981(a)).

Such magnification however, does not increase cellular detail and has been described as empty magnification (Laing, 1980). However, it does provide sufficient detail to allow quantitative assessment of the endothelium. A variety of morphological parameters may be quantified,

including cell area, cell density, cell perimeter, average cell diameter, and length of cell side. Cell area and cell density are the most widely reported measurable parameters in determining endothelial status. Several methods exist for the determination of cell parameters.

1. COMPARISON ANALYSIS

Using this system, the obtained endothelial cell pattern is compared to a similar pattern of known size. This can be carried out by direct endothelial observation or by a comparison of endothelial negatives against a comparison sizing chart.

2. FIXED FRAME ANALYSIS

This is the simplest method of cell counting (Bourne and Kaufman, 1976(a)). For this purpose endothelial cell pictures are projected onto a frame of known size. Cells partially in the frame are counted on two borders only utilising a symmetry principle. Provided the number of boundary cells is small compared to the total number of cells contained within the frame and cellular pleomorphism is not large, an accurate estimate of cell density can be obtained.

3. VARIABLE FRAME ANALYSIS

This is a more sophisticated method of cell analysis and determines the mean cell area (Laing, et al. 1976(a). The variable area occupied by an integral number of cells

is measured. A digitizer is used to trace around the boundary of the cells being analysed. Cell density is obtained by dividing the total number of cells by the area of the frame. The same method had been used in individual cell analysis in order to determine the size of single cells (Laing, 1976 (a)(b)).

Using either fixed or variable frame analysis the presence of extensive cellular pleomorphism may result in an endothelial cell density which is not representative of the entire endothelial population.

4. AUTOMATED CELL ANALYSIS

The technique of automated cell analysis, replaces manual counting with a method of electronic scanning to detect cell borders, and thus to develop an estimate of cell density. Such a system requires sharp contrast between cell boundaries and cell contents and contrast enhancement may be necessary.

5.4.

APPARENT ENDOTHELIAL CELL DENSITY DURING TWENTY MONTHS EXTENDED SOFT CONTACT LENS WEAR.

5.4.1. Introduction

Endothelial cell loss, often considerable, may result following cataract extraction and intra ocular lens

implantation. One commonly used alternative form of optical correction in aphakia is extended soft contact lens wear.

The study now reported was designed to develop a method of endothelial cell observation and photography that would permit the monitoring of endothelial cell densities in a group of extended soft contact lens wearing subjects over a twenty month period.

Patient selection, grouping, contact lens fitting and instruction have been described in 4.2. - 4.4.

5.4.2. APPARATUS

Initial attempts were directed to construct a contact specular microscope. Illumination and observation systems were produced by a Köhler illuminator (Vickers Instruments). The objective was divided into two by a blackened card such that light passed down one side and was reflected back from the cornea along the other side. A rotating silvered mirror was adjusted to allow the light to follow this course. An adjustable focus applanating dipping cone (Nikon) was attached to the illuminator.

The system allowed observation of in vitro pig eye endothelium. The field of view was small and the amount of light collected by the objective lens minimal. Adjustments were carried out to the angle and intensity of both incident and reflected light. With the optimum adjustments cellular structure could not be clearly seen on in vivo human or

rabbit eye.

It was decided not the continue the development of this apparatus in favour of the adaptation of the photo slit-lamp (Zantos and Holden, 1977; Holden 1978; Holden and Zantos 1978; Barr and Schoessler, 1979).

An Olympus OMI camera was attached to a black metal extension tube which fitted onto one 10X eye-piece of a Nikon photo slit lamp. (Figure 5.2 and 5.3). This allowed photography through the eye-piece thus giving increased magnification compared to that obtained using the normal camera attachment which photographs only through the objective lens of the slit lamp.

5.4.3. METHOD

A high resolution colour transparency film was used. (Kodachrome 64). The slit lamp electronic flash unit was set at maximum intensity and the slit lamp diaphragm fully opened. The standard 50mm Olympus camera lens was left in situ and together with the slit lamp eye piece was focussed at infinity. Albeit that no topographical change in endothelial cell density or variation between right and left eyes has been demonstrated in normal corneae, (Laule et al. 1978; Hoffer 1979; Kaufman et al. 1966; Blackwell et al. 1977; Sturrock et al. 1978), the nasal aspect of the right cornea in every case was used. Each subject was asked to fixate about 20 degrees temporally. The observation

Figure 5.2

Olympus OM1 Camera with Holden-Zantos attachment

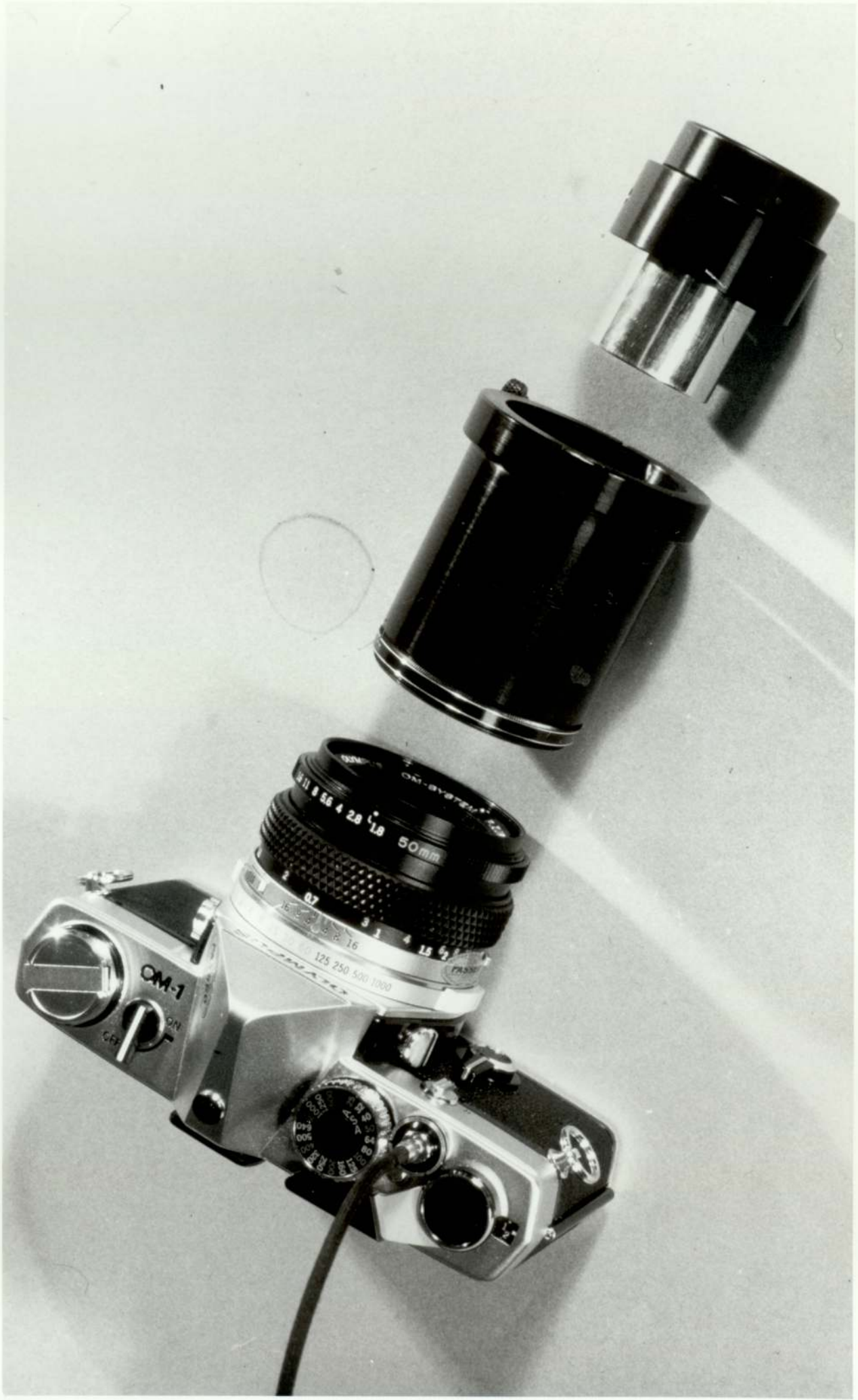
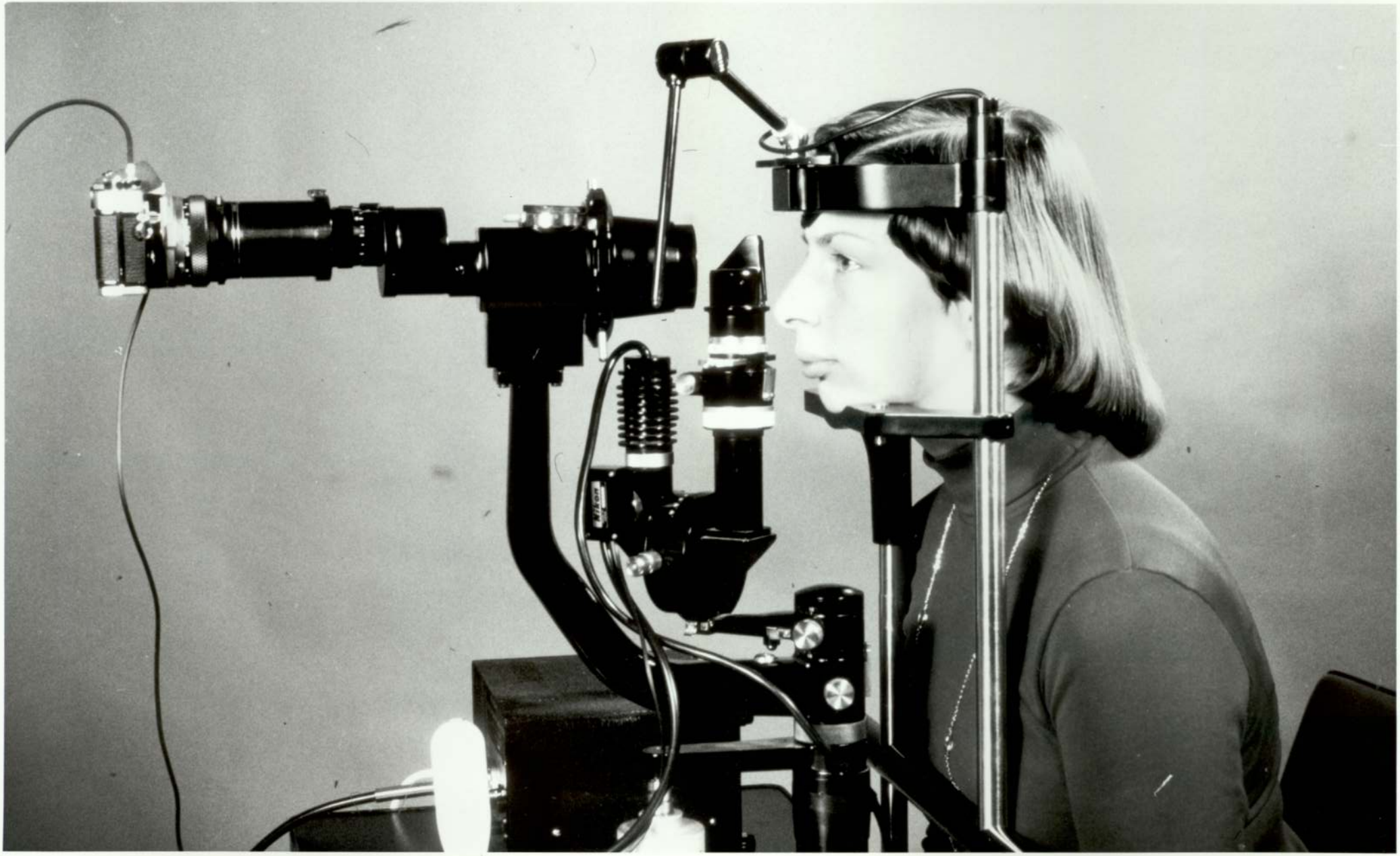

OM-1
ON
OFF
50mm

Figure 5.3.

Practical arrangement for endothelial photography with Nikon photo slit lamp



system was normal to the anterior corneal surface. The illumination system was set about 40^{o} nasally. (Figure 5.4 (a), (b), (c).

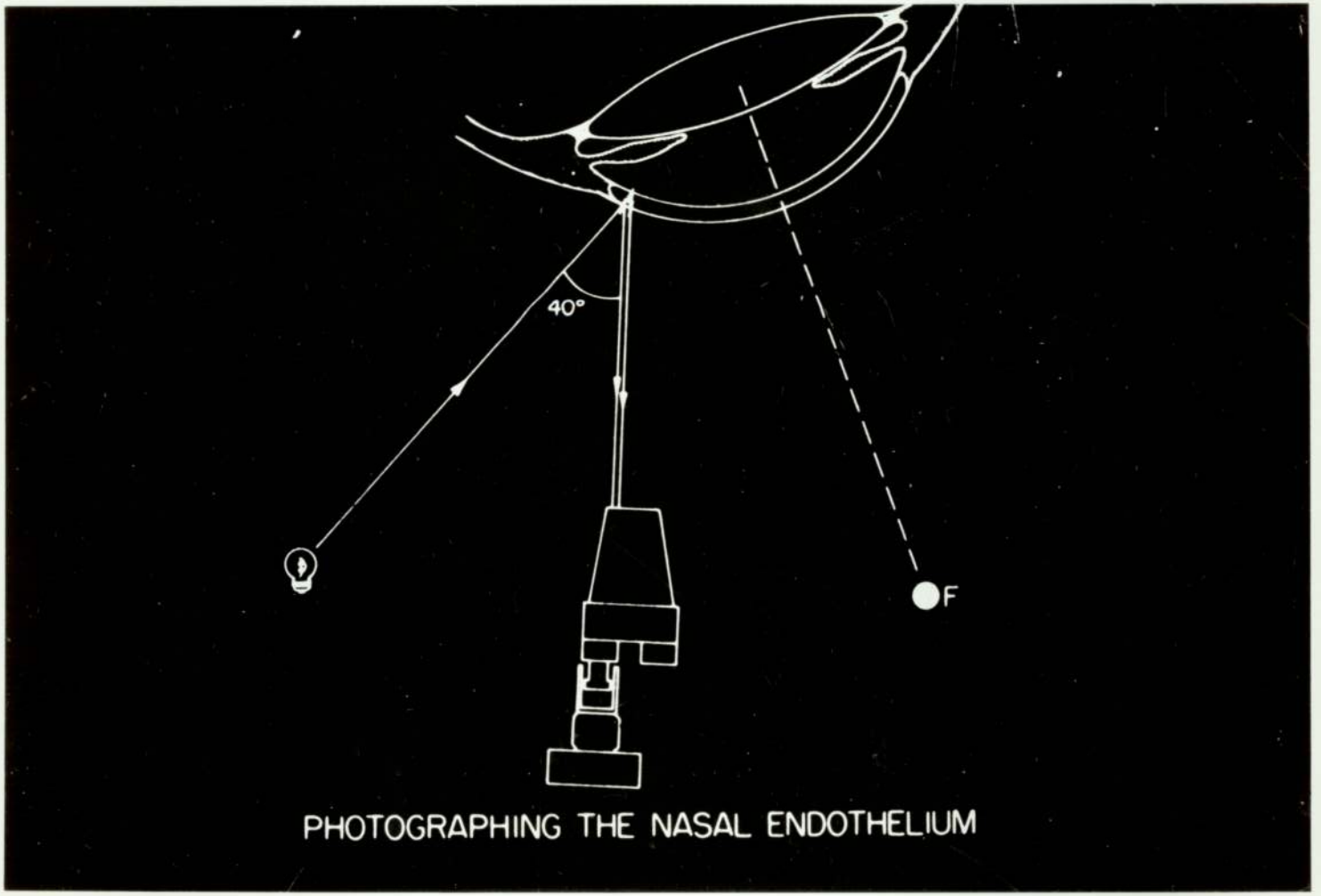
Under low magnification the required endothelial area was observed as a dull patch adjacent to the bright anterior corneal reflex. The magnification was increased to 35X allowing the endothelial mosaic to be visible. Fine adjustment to the focus, and angles of observation and incident light were carried out as necessary. Five photographs were taken for each subject at each visit in order to provide a strong possibility of obtaining at least one transparency depicting clear cellular outline. This number had previously been determined as the optimum, balancing quality of transparency against financial cost (Sherrard, 1979). The overall magnification of the system was obtained by photographing a graticule of known dimension, placed at the anterior corneal plane. The linear magnification on the transparency was found to be 7X. A second graticule was placed in the eye piece of the slit lamp and thus was superimposed on all transparencies. The length of one pre-determined scale division was checked to be of constant length on all transparencies used for all cell counting, in order to ensure constant magnification between different films. In order to increase the size of the image on the transparencies for cell counting Holden (1978) described a method of enlargement by re-photographing the endothelial area using Kodachrome 64 colour transparency film. However, a projection system was favoured. A Zeiss microfilm reader was adapted to house a 29X objective lens. Each transparency was enlarged by projection.

Figure 5.4(a)

Diagrammatic representation of Holden-Zantos Technique for Endothelial Photography of the Nasal Endothelium

F is Fixation Target

After Holden and Zantos



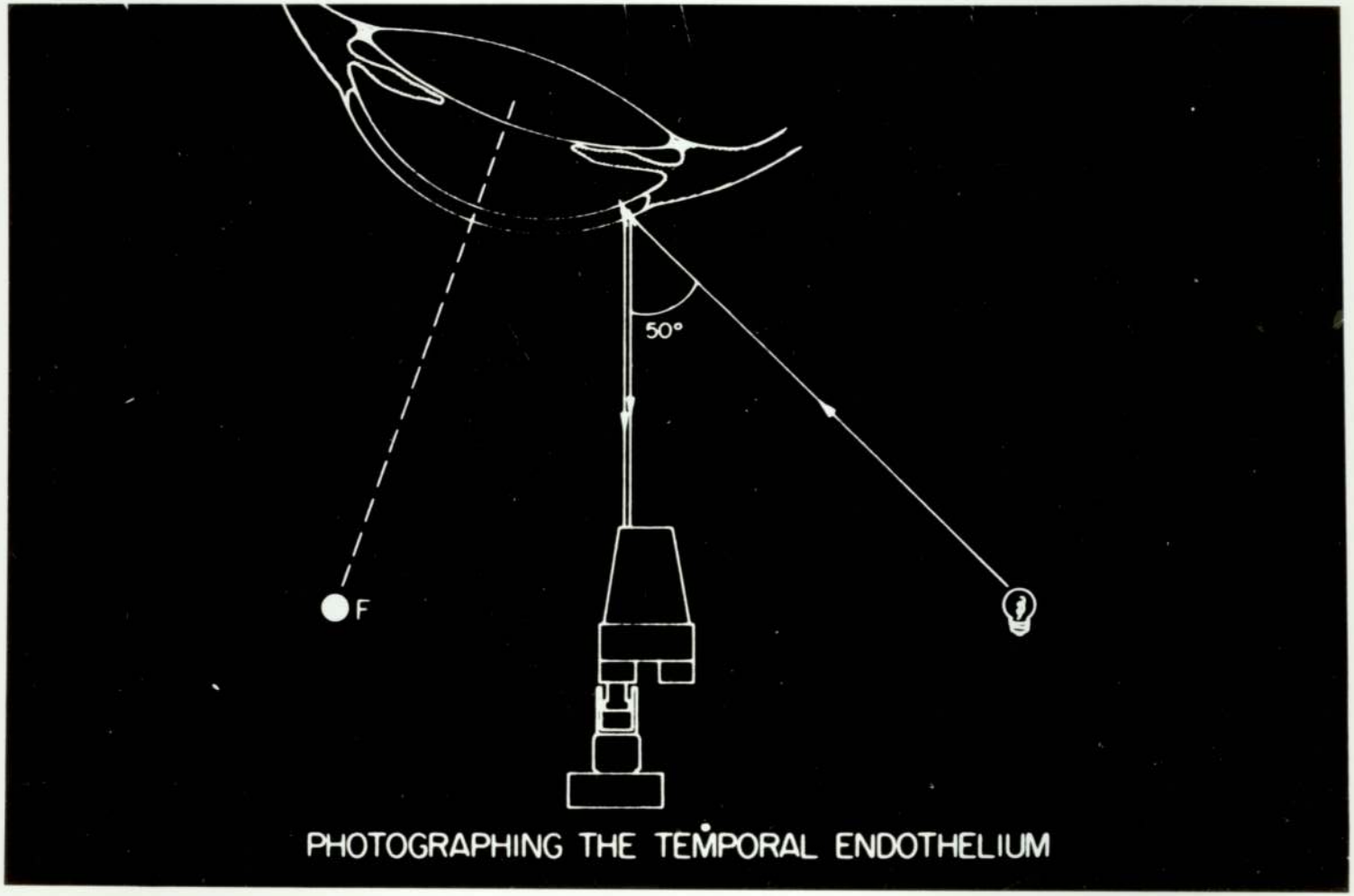
After Holden, B.A., Zantos, S.G., The Holden-Zantos Technique for Endothelial and High Magnification Slit Lamp Photography, Bausch & Lomb Educational Service.

Figure 5.4.(b)

Diagrammatic representation of Holden-Zantos Technique for Photography of the Temporal Endothelium.

F is Fixation Target

After Holden and Zantos



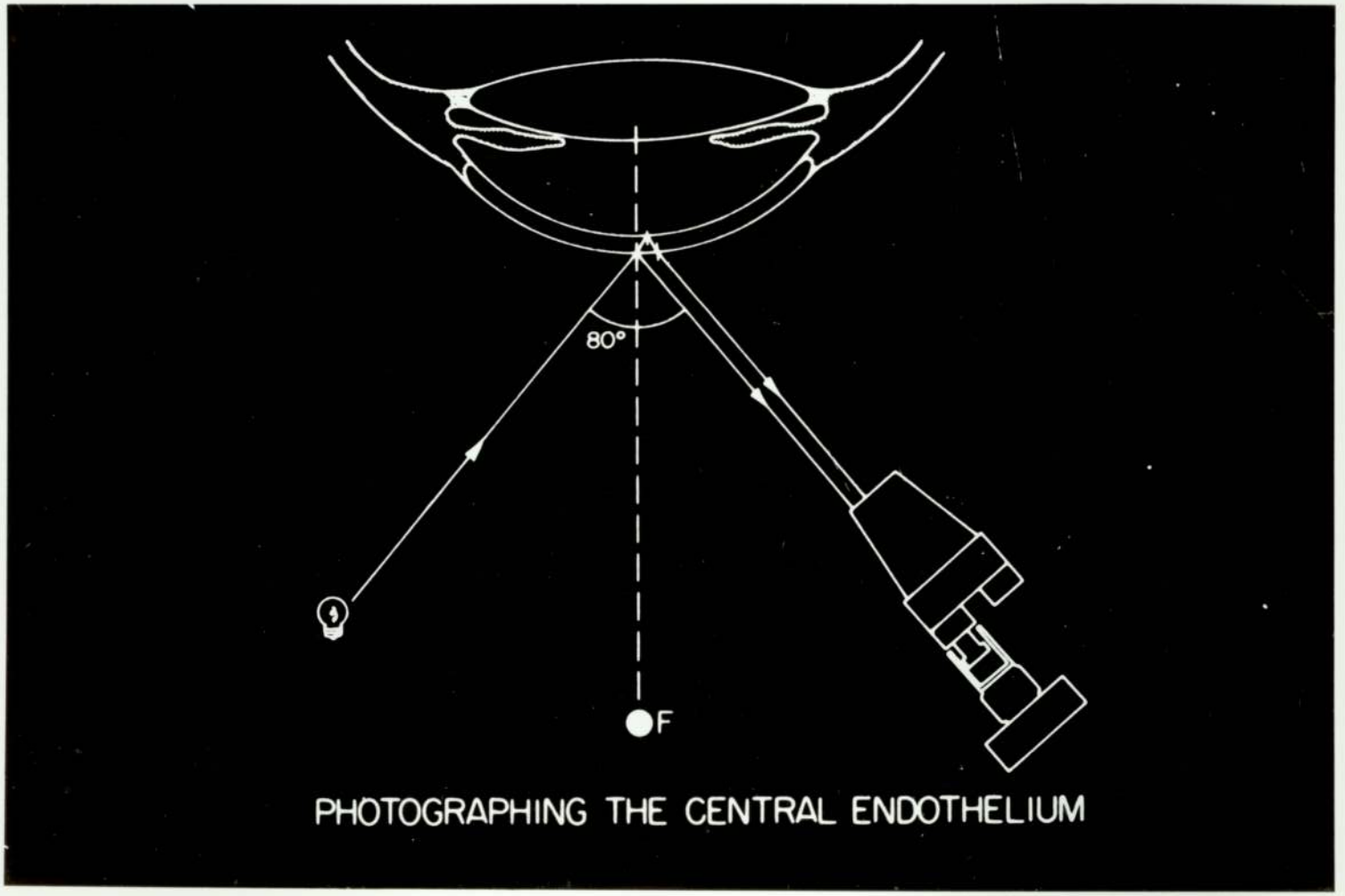
After Holden, B.A., Zantos, S.G., The Holden-Zantos Technique for Endothelial and High Magnification Slit Lamp Photography, Bausch & Lomb Educational Service.

Figure 5.4(c)

Diagrammatic representation of Holden and Zantos Technique for Photography of the Central Endothelium.

F is Fixation Target

After Holden and Zantos



After Holden, B.A., Zantos, S.G., The Holden-Zantos Technique for Endothelial and High Magnification Slit Lamp Photography, Bausch & Lomb Educational Service.

A graticule of known dimensions was photographed and enlarged under exactly the same conditions as the endothelial cells of a normal cornea. The final overall magnification of the projected image was found to be 196X.(Figure 5.5. and 5.6)

5.4.4. METHOD OF CELL COUNTING

A fixed frame cell counting method was used. A square of side length 24.5 mm was placed over the projected screen, and the number of cells enclosed by the rectangle was counted. Cells touching the left-hand side and top of the square were excluded. A square of half the size was used on transparencies having only a small area of clearly defined cells. The largest square was used whenever possible to include the greatest number of cells. In order to minimise counting errors the counting frame was placed on a clear celluloid sheet and each cell marked with an ink dot as it was counted.

A similar method of cell counting and projection enlargement has been described (Sturrock et al. 1978; Hoover and McAulife, 1979).

The area of the counting frame represented 1/64th of a millimetre of corneal area. The cell count in the frame was therefore multiplied by a magnification factor of 64X in order to express cell density in cells per square millimetre of cornea.

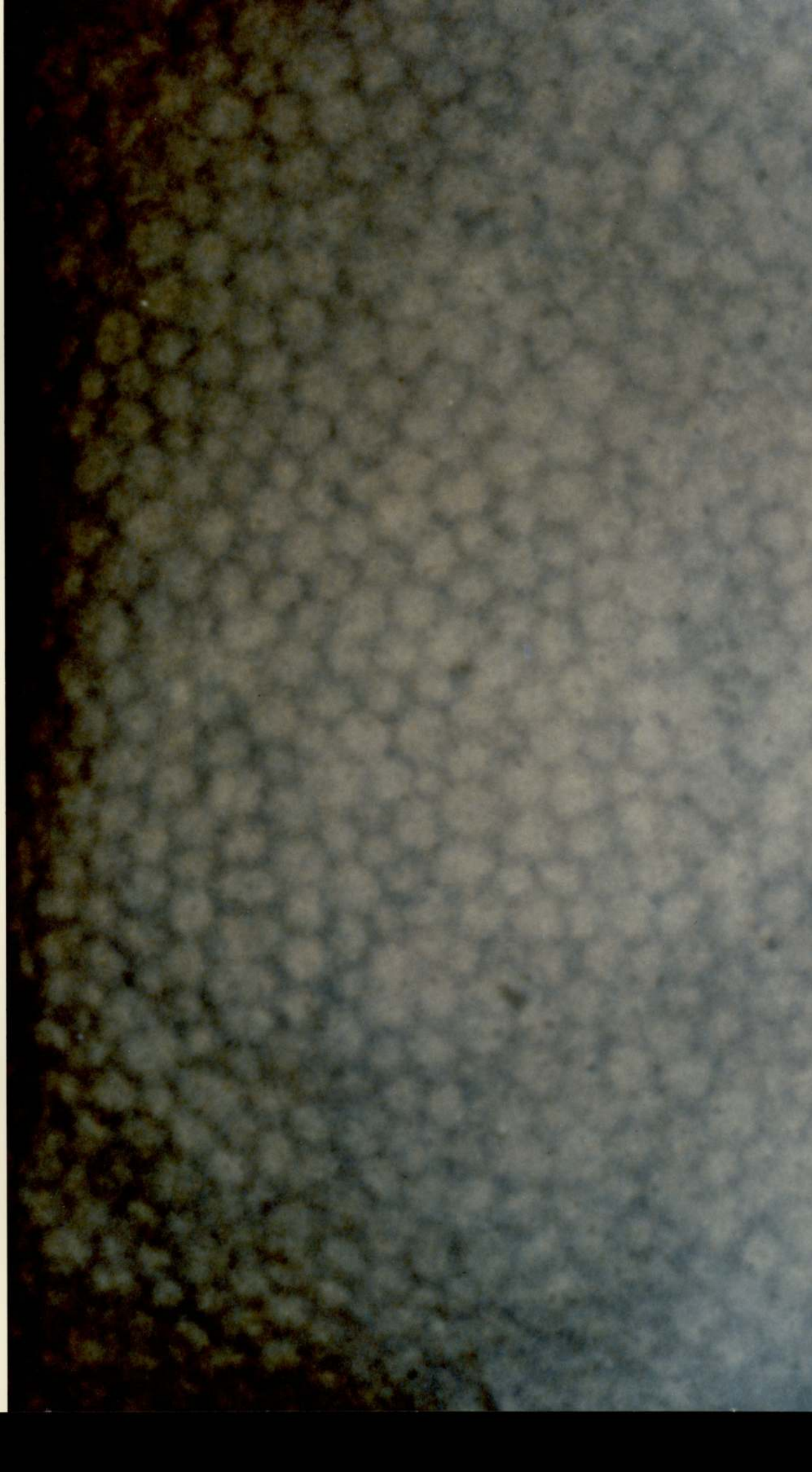
Figure 5.5.

Zeiss microfilm reader showing counting square for determining endothelial cell densities

24.625mm² = 1/64 sq mm cornea
17.5

Figure 5.6.

Human corneal endothelium photographed through Nikon photo slit lamp with a Holden Zantos attachment.



In order to assess cell counting precision by this method, five transparencies were each counted ten times in a random sequence. The coefficient of variation was subsequently determined. Automated cell analysis was considered, but insufficient contrast between cell borders and contents prevented the use of this system (Akers, 1980).

For each patient at each data collection point the transparency depicting the highest resolution of cell outline was selected. Each transparency was counted five times in a random sequence. The mean of all five readings were obtained in each case. The positioning of the counting square was also random and thus slightly differing areas of the transparency may have been counted at each time.

Data collection was taken prior to contact lens wear and at 3, 6, 12, 18 months thereafter. The study however, was terminated after six months due to appearance of microepithelial cysts (Humphreys et al. 1980). At the 3 and 6 month data collection point endothelial photography was carried out without the contact lens in situ. This avoided possible changes in magnification due to lens power and thickness, and also allowed greater clarity of cell outline especially in subjects whose contact lenses had surface deposits.

5.4.5. RESULTS

The precision of the projection method of endothelial cell counts gave a coefficient of variance of 5.55%, and the results are tabluated in Table 20.

Apparent endothelial cell densities were obtained for 68 subjects prior to contact lens wear. Values ranged between 2124 and 3315 cells per square millimetre with a standard deviation of 272, and mean of 2652. The results follows a normal distribution ($P < 0.01$) D-Agastino's Test for Normality (Zar, 1974), and are shown in Table 20 and Figure 5.7. Endothelial cell densities during six months of extended soft contact lens wear as shown in Table 31 (a), (b) and (c).

Paired 'T' testing of the data revealed no statistically significant difference between results obtained prior to contact lens wear and at 3 or 6 months following the commencement of the study. (Tables 32, 33 and 34). or between solution and non-solution groups (Table 35 & 36).

5.4.6. CONCLUSION

A system has been developed which allows endothelial cell densities to be determined using a photo slit lamp. Magnification is obtained by a projection method, thus eliminating the need for photographic enlargement. Cell counts obtained by this method show a good degree of reproducibility. The mean value for endothelial cell density prior to contact lens wear compared well to those ontained previously (Bourne et al 1976; Azen et al, 1980).

Extended soft contact lens wear produced no stastically significant change in endothelial cell density over a six month period compared to pre lens wearing values. This is in contrast to the cell loss observed following intra ocular lens implantation in the optical correction of aphakia, and supports the recent work of Ahmed (1980). No difference in endothelial cell density was observed between solution and non-solution groups.

TABLE 29

PRECISION OF ENDOTHELIAL CELL COUNTING

SUBJECTS INDICATED BY A,B,C,D,E.

	A	B	C	D	E	
	2560	2816	2752	2880	2880	
	2752	2880	2688	3008	3008	
	2880	2624	2688	2944	2752	
	2624	2624	2624	2752	2880	
	2496	2624	2688	2880	2496	
	2560	3008	2368	2880	2880	
	2560	2624	2560	3008	3136	
	2816	2496	2944	2944	2816	
	2752	2560	2752	2752	2432	
	2752	2944	2560	2880	2880	
Mean $\bar{x}$ =	2675	2720	2662	2892	2816	cells per sq.mm.
Standard Deviation =	130	176	151	89	213	
Coefficient of Variance =	4.89%	6.49%	5.69%	3.09%	7.58%	

Average Co-efficient of Variation = 5.55%

TABLE 30

POPULATION DISTRIBUTION - ENDOTHELIAL CELL COUNT

for 68 Volunteer Subjects

D'AGASTINOS TEST FOR NORMALITY

Groups Cells per sq. mm.	fi	$\bar{x}i$	fixi	$fixi^2$
2000 - 2250	6	2125	12750	27093750
2250 - 2500	12	2375	28500	67687500
2500 - 2750	26	2628	68250	179156250
2750 - 3000	15	2875	43125	123984375
3000 - 3250	8)			
> 3250	1) 9	3125	28125	87890625

Efi = 68 Efixi = 180750 $Efixi^2$ = 485812500

$$SS = 485812500 - \frac{(180750)^2}{68} = 5363051.5$$

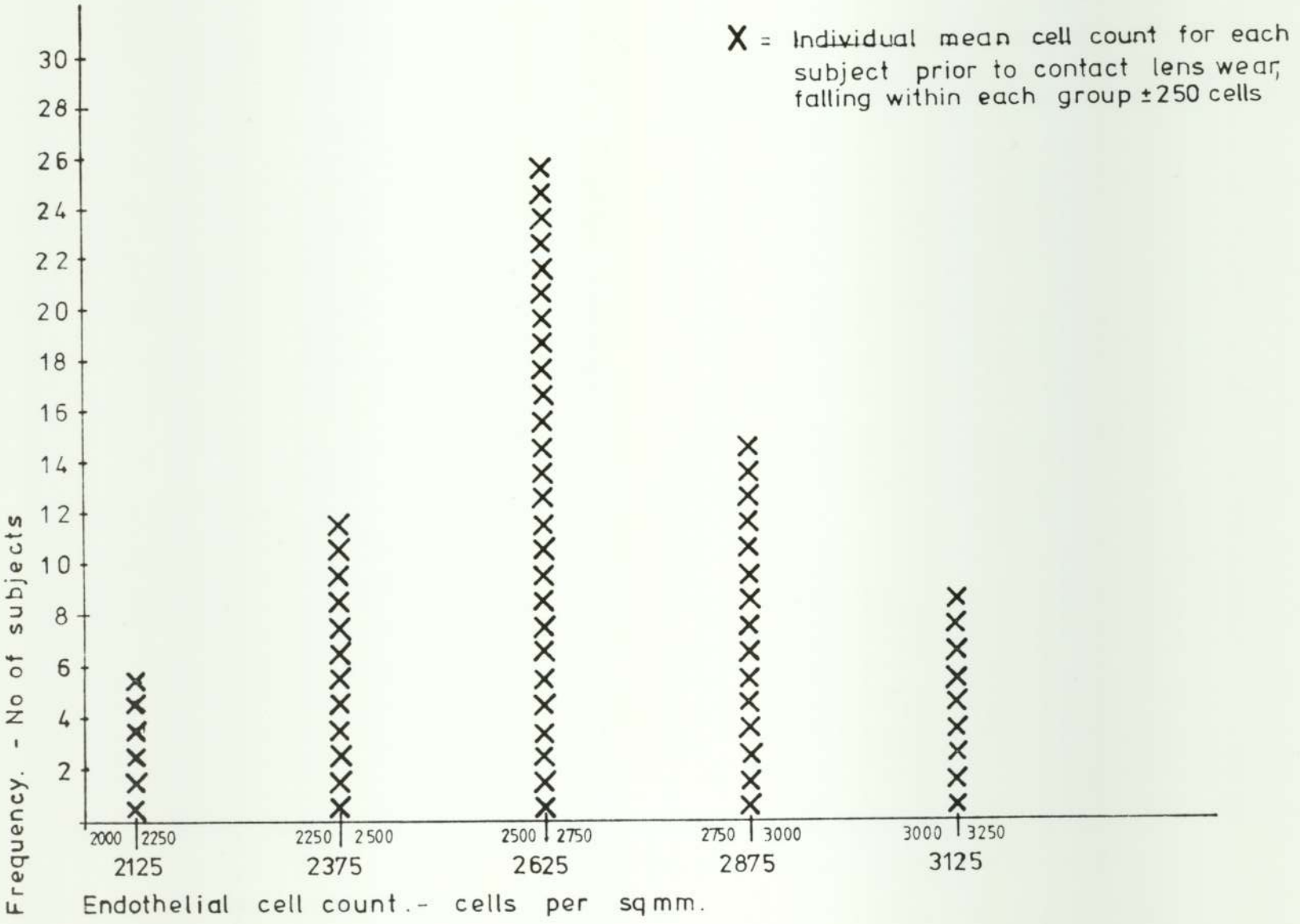
$$T = E\left(i - \frac{n+1}{2}\right)\bar{x} = 357375$$

$$D = \frac{357375}{\sqrt{(68)^3 \times 5363051.5}} = 0.27520$$

Since D is neither $\leq$ than 0.2687, nor $\geq$ 0.2872, the null hypothesis of population normality cannot be rejected.

Figure 5.7.

Population Distribution of
Endothelial Cell Density


X = Individual mean cell count for each subject prior to contact lens wear, falling within each group ±250 cells
Frequency. - No of subjects
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
2000 2250
2125
2250 2500
2375
2500 2750
2625
2750 3000
2875
3000 3250
3125
Endothelial cell count.- cells per sq mm.

ENDOTHELIAL CELL COUNT - CELLS PER SQ MM. CONTROL GROUP

Pat. No:	Baseline					Three Months					Six Months					MEAN VALUES		
																Base line	3 Mths	6 Mths
1	2624	2560	3008	2368	2712						2368	2496	2624	2496	2560	2662	-	2508
2	2560	2496	2560	2688	2688											2598	-	-
3	3072	3008	2752	2816	2560	2624	2752	2560	2624	3200						2841	2752	-
4	3456	3456	3200	3200	3264	3200	3072	3200	3200	3007						3375	3135	-
5	2240	2560	2752	2496	2496											2508	-	-
6	3520	3008	3328	2752	2752											3072	-	-
7	2752	2688	2688	3200	2752	2944	2688	2752	2948	3008						2816	2868	-
8	2368	2452	2304	2662	2368											2462	-	-
9	3200	3008	2816	3008	3008						2816	2944	2496	2624	2752	3008	-	2726
10	2715	3200	2816	3008	3880	2816	2816	2944	3136	2816						3131	2905	-
11	3008	3392	2880	2624	2560											2892		
12	2560	2560	2048	2560	2112	2048	2560	2048	2560	2624	2752	2624	2560	2560	2880	2368	2368	2675
13	2240	2816	2624	2304	2176	2688	2768	3072	2368	2368						2432	2572	-
14	2304	2240	2176	2240	2048	2304	2304	2240	2176	2048	2432	2304	2624	2688	2432	2201	2214	2496
15	2560	2752	2752	2624	2880	2752	3008	2880	2816	2560						2713	2803	-
16	3200	3008	3136	2944	2944	2688	3008	2880	3200	3136	3136	2944	3008	2944	3136	3046	2982	3033
17	2816	2624	2560	2752	2752						2816	2560	2624	2560	2752	2700	-	2662
18	2688	2560	2752	2434	2560	2560	2624	2434	2688	2560	2434	2434	2688	2560	2688	2598	2573	2560
19	2048	2112	2176	2240	2176	2304	2112	2176	2048	2176						2150	2163	-
20	2752	2304	2752	2816	2624	2560	2624	2816	2752	2560	2752	2304	2624	2624	2368	2469	2662	2543
21	2880	2752	2944	2560	2880	2560	2624	2944	2880	2752	2560	2624	2752	2880	2944	2803	2752	2752
															Mean $\bar{x}$ =	2707	2673	2661
															N =	21	13	9

TABLE 31(a)

ENDOTHELIAL CELL COUNT - CELLS PER SQ MM. SOLUTION GROUP

Pat. No:	Baseline					Three Months					Six Months					Mean Values		
																Base line	3 Mths	6 Mths
1	2816	2944	3072	3008	2816	2624	2816	2944	2944	3008	2560	2624	2560	2752	2624	2931	2867	2624
2	2432	2368	2560	2496	2432						2688	2560	2048	2432	2432	2457	-	2432
3	3008	3072	3264	3008	3008	2944	3008	2816	3200	2072	2944	3008	2944	3008	28k6	3072	3008	2944
4	2752	2624	2432	2624	2688	2560	2496	2432	2496	2560						2624	2508	-
5	2432	2496	2816	2368	2560	2752	2560	2624	2624	2496	2752	2624	2880	2944	2752	2534	2611	2790
6	2432	2434	2176	2304	2112	2560	2560	2368	2368	2432						2291	2457	-
7	2560	2304	2560	2496	2560	2560	2752	3200	3008	2560	2624	2624	2624	2624	2624	2496	2816	2624
8	2816	2752	2688	2560	2816						2688	2752	2880	2752	2688	2726	-	2752
9	2496	2432	2176	2432	2176	2624	2560	2688	2304	2432						2343	2521	-
10	2880	3136	2816	2432	2880	2944	2752	2624	2624	2560	2816	2688	2368	2560	2624	2827	2700	2611
11	2752	2816	2496	2496	2688											2649	-	-
12	2240	2432	2048	2176	2112	2048	2624	2432	2368	2624	2048	2368	2112	2112	2432	2201	2419	2214
13	2112	2368	2560	2752	2304						2368	2345	2048	1934	1984	2419	-	2145
14	2752	2688	2688	2624	2688	3072	3008	3072	2880	880						2688	2982	-
15	2624	2496	2240	2816	2816	2816	3072	2944	2816	2316	2560	2752	2625	2880	2816	2598	2892	2756
16	2176	2368	2048	2048	2304											2188	-	-
17	2368	2570	2944	2752	2560											2636	-	-
18	2496	2432	2176	2240	2368	2496	2880	2624	2560	2560						2342	2624	-
19	2368	2624	2752	2432	2560	2368	2816	2368	2432	2560						-	2508	-
20	2492	2560	2570	2624	2492	2560	2624	2688	2560	2560	2752	2688	2560	2624	2752	2545	2598	2675
21	2880	3008	2752	2880	2496	2624	2880	2624	2560	2432						2803	2624	-
22	2880	3008	2944	2752	2880											2892	-	-
23	2880	2752	2880	2752	2816						3072	2560	2688	2880	2752	2816	-	2790
24	2816	2752	2752	2560	2816	2560	2752	2624	2816	2560	2432	2560	2688	2432	2368	2739	2662	2496
															Mean $\bar{x}$ =	2601	2674	2604
															N =	23	16	13

TABLE 31(b)

ENDOTHELIAL CELL COUNT - CELLS PER SQ MM. NON SOLUTION GROUP

Pat. No:	Baseline					Three Months					Six Months					Mean Values		
																Base line	3 Mths	6 Mths
1	2880	2880	3008	3008	2432											2841	-	-
2	2560	2752	2880	2624	2496											2662		-
3	2880	2880	2716	2688	2560	2432	2688	2752	2880	2752	2880	2752	2880	2752	2880	2764	2700	2828
4	3008	3072	2624	2688	2944	2432	2368	2496	2432	2624	2560	2624	2624	2496	2112	2867	2470	2483
5	2560	2688	2688	2560	2560											2611	-	-
6	2944	2560	2752	2944	2816											2803	-	-
7	2240	2112	2240	2176	2048	2368	2752	2560	2560	2688	2304	2368	2560	2688	2752	2163	2585	2534
8	2880	2496	2880	2624	2688	2816	3238	3072	2944	2944	2944	2880	2304	2560	2624	2713	3020	2662
9	2304	2112	2624	2112	2240	2304	2368	2112	2624	2240	2560	2688	2112	2240	2240	2278	2329	2368
10	2816	3136	3136	3072	3072	2496	2560	2880	2688	2688	2944	3200	2816	2816	2624	3046	2662	2880
11	2752	2560	2752	2560	2688	2368	2560	2880	2688	2560	2752	2624	3136	2816	2368	2662	2611	2739
12	2560	2560	2816	2752	2752	2560	2880	2688	2560	2496	3200	2944	2496	2560	2688	2688	2636	2777
13	3264	3200	3136	3072	3200											3174	-	-
14	2112	2112	2048	2048	2304											2124	-	-
15	2880	2752	2944	2624	2624	2560	2560	2560	2560	2560	2880	2880	2624	2432	2624	2764	2560	2688
16	2816	2880	2624	2524	2624											2713	-	-
17	2560	2752	2304	2560	2432											2521	-	-
18	2112	2368	2240	2048	2560	2560	2624	2240	2304	2304	2560	2496	2752	2368	2752	2265	2406	2585
19	3008	2624	2496	2560	2944	2944	3200	3200	2880	2624						2726	2969	-
20	2816	2560	2816	2944	2880	2560	2816	2944	2880	2944						2803	2828	-
21	2048	2560	2624	2752	2560	2240	2432	2368	2432	2560	2560	2560	2688	2688	2304	2508	2406	2560
22						2848	3008	2752	2688	2432							2745	-
23	2624	2560	2752	2496	2560	2624	2624	2560	2496	2624	2752	2560	2496	2752	2624	2598	2585	2636
24	3200	2560	3072	3008	3200						3200	3072	2816	2944	2008	3008	-	3008
25	2368	2304	2208	2112	2368	2240	2432	2368	2112	2112	2368	2144	2208	2144	2368	2272	2252	2246
															Mean $\bar{x}$ =	2648	2610	2642
															N =	24	16	14

TABLE 31(c)

TABLE 32

Baseline Values of Endothelial Cell Density Compared to Those Obtained After Three and Six Months

PAIRED STUDENT 'T' TEST

TWO-TAILED

Solution Group					
Baseline	-v-	3 Months	Baseline	-v-	6 Months
2391		2867	2931		2624
3072		3008	2457		2432
2624		2508	3072		2944
2534		2611	2534		2790
2291		2457	2496		2624
2496		2816	2726		2752
2343		2521	2828		2611
2828		2700	2201		2214
2201		2419	2419		2145
2688		2982	2598		2726
2342		2624	2545		2675
2545		2598	2884		2790
2803		2624	2739		2496
2739		2662			
2598		2892			

t = 1.8233 Significant ($p<0.1$) t = 0.9350 - Not Significant

df 14. df 12.

TABLE 33

Baseline Values of Endothelial Cell Density
Compared to Those Obtained After Three and Six Months

PAIRED STUDENT 'T' TEST

TWO-TAILED

Control Group

Baseline	-v-	3 Months	Baseline	-v-	6 Months
2841		2752	2662		2508
3315		3135	3008		2726
2816		2868	2368		2675
2368		2368	2201		2496
2432		2572	3046		3033
2201		2214	2700		2662
2713		2803	2598		2560
3046		2982	2619		2534
2598		2573	2803		2752
2150		2163			
2649		2662			
2803		2752			
3131		2905			

t = 0.8635 - Not Significant
df 12.

t = 0.1021 - Not Significant
df 8.

TABLE 34

Baseline Values of Endothelial Cell Density
Compared to Those Obtained After Three and Six Months

PAIRED STUDENT 'T' TEST

TWO-TAILED

Non Solution Group

Baseline	-v-	3 Months	Baseline	-v-	6 Months
2764		2700	2764		2828
2867		2470	2867		2483
2163		2585	2163		2534
2713		3020	2713		2662
2278		2329	2278		2368
3046		2662	3046		2880
2662		2611	2662		2739
2688		2636	2688		2777
2764		2560	2764		2688
2265		2406	2265		2585
2726		2969	2508		2560
2803		2828	2598		2636
2508		2406	3008		3008
2598		2585	2272		2246
2272		2252			

t = 0.115 Not Significant t = 0.5762 Not Significant

df 14. df 13.

TABLE 35

A Comparison of Cell Densities Obtained After Three Months Between Solution and Non-Solution Groups

UNPAIRED STUDENT 'T' TEST

TWO-TAILED

Solution Group v Non Solution Group at 3 Months.

Solution Group	-v-	Non Solution Group
2867		2700
3008		2470
2508		2585
2611		3020
2457		2329
2816		2662
2521		2611
2700		2636
2419		2560
2982		2406
2892		2969
2624		2828
2508		2406
2598		2585
2624		2252
2662		2745

t = 0.903 Not Significant
df = 30.

TABLE 36

A Comparison of Cell Densities Obtained After Six Months Between Solution and Non Solution Groups

UNPAIRED STUDENT 'T' TEST

TWO-TAILED

Solution Group v Non Solution Group at 6 Months.

Solution Group	-v-	Non Solution Group
2624		2828
2432		2483
2944		2534
2790		2662
2624		2368
2752		2880
2611		2739
2214		2777
2145		2688
2756		2585
2675		2560
2790		2636
2496		3008
		2246

t = 0.455 Not Significant

df = 25.

CHAPTER 6

CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

6 CONCLUSIONS

6.1 APPARENT OXYGEN UPTAKE RATE

The possibility of measuring apparent human corneal oxygen uptake rate and more recently "oxygen debt" has promoted a considerable amount of research interest. The methods, calculations and instruments reported in the literature are all subject to variations and mathematical assumptions which renders the obtained values relative and not absolute; as such they are useful for comparative studies.

Having considered the difficulties of the existing measurement techniques, this study was concerned with the modification of an existing apparatus to improve both accuracy and reproducibility of measurement. The apparatus was subsequently used on human in vivo cornea to assess:-

1. Instrumental variability - repeatability
2 Population distribution of oxygen uptake rate in normal subjects
3 Diurnal (waking hour) variation in oxygen uptake rate.

The degree of "oxygen debt" generated by a contact lens is inversely proportional to the oxygen tension that existed under the lens at the anterior corneal surface (Jauregui and Fatt, 1971).

The measurement of "oxygen debt" could therefore be used as an indirect indicator of corneal metabolic function during contact lens wear. Using this measurement the effect on corneal respiration was observed due to:-

1 Changes in the fit of one form of soft contact lens

2 Changes in the non-experimental - contralateral eye

Evidence was provided to support the theory of a small tear pump operating under a soft contact lens and for a sympathetic alteration in corneal metabolic rate.

The longer term monitoring of corneal oxygen uptake rate during a six month period of extended soft contact lens wear was carried out.

Evidence was provided for both a short and long term decrease in oxygen consumption rate. Hypotheses were advanced to explain the observed results, although the evidence was insufficient to allow any one such explanation to be accepted. The significance of the results therefore remains unclear.

There was insufficient evidence to suggest any variation in corneal oxygen requirement between the solution and non-solution groups. Further, there was no correlation between pre-lens fitting oxygen requirement and the degree

of oxygen debt produced after 48 hours.

The study was discontinued after six months due to the presence of micro-epithelial cysts of the cornea in all participating experimental subjects (Humphreys et al. 1980). There was no statistically significant difference between pre-fitting oxygen requirement and values obtained on twelve such subjects one week following the cessation of lens wear.

6.2 ENDOTHELIAL CELL DENSITIES

An existing method of endothelial cell counting using a photo slit lamp was modified, together with a projection system of enlargement. Endothelial photography was carried out to determine:-

1 Variability of the system

2 Population distribution of endothelial cell density in normal subjects

3 Variation in endothelial cell density during a six month period of extended soft contact lens wear

The results demonstrated an acceptable level of instrumental variability and the endothelial cell densities obtained conformed to a normal distribution. There was no statistically significant decrease in endothelial cell

density as a result of extended contact lens wear in either of the experimental groups.

6.3 SUGGESTIONS FOR FURTHER WORK

6.3.1 Corneal Metabolic Rate

Future developments must initially be directed towards reducing the variability of the measurement system.

This may be achieved by:-

(a) Reducing the diameter of the platinum cathode from 25u to 15u in diameter. The reduction in cross sectional area will increase the electrode reaction time; the time of sensor contact with the corneal will therefore be reduced.

(b) The sensor output will decrease, as a result of reducing the cathode diameter, by approximately one half (Treherne, 1982). The use of a screening cable and subsequent amplification of the signal may be necessary.

(c) The previous use of the hydrogel sleeve surrounding the sensor to provide easier handling and thus stability on the cornea may not be required. The overall diameter of the electrode can be considerably reduced to 1.5mm and the body made thinner and longer for better handling (Parker, 1982); this together with the

potential reduction in corneal contact time will permit a reduced area of contact with the cornea for a shorter period of time. This would enhance subject comfort and also allow a topographical study of oxygen consumption rate to be undertaken.

(d) The cathode can be embedded in quartz glass instead of the currently used epoxy-resin. This will allow a more stable electrode response and quicker and more accurate calibration (Trenerne, 1982).

(e) The sensor output could link directly into a computer interface board. The output signal would be sampled electronically. This would allow a large number of data points to be obtained from a short period of corneal contact. A direct digital read out of oxygen consumption could be produced. This would increase the accuracy of evaluating the sensor output and eliminate the need for much of the peripheral equipment.

(f) It may be possible to manufacture the electrode tip as a pre-calibrated disposable unit of the type currently used to monitor blood oxygen tensions (Parker, 1982). This would eliminate the remainder of the peripheral equipment. The constant calibration would further enhance the accuracy of the system.

The suggestions made, would suggest the development of a smaller sensor, easier and quicker to use, with enhanced subject acceptability. The need for laboratory peripheral

equipment would be removed, and the electronic assessment of the output signal would improve the accuracy of the measurement as a whole.

Following the successful modifications in sensor design, the following aspects of human in vivo corneal oxygen uptake rate could be examined:-

(1) The variability of the new design of sensor.

(2) Re-examination of diurnal (waking hour) study from 6.00 - 21.00 hours. Data points should be every hour with five readings at each point.

(3) Re-examination of sympathetic change in oxygen consumption rate, using larger subject numbers, and observing the oxygen uptake in the contra-lateral eye whilst a contact lens is in situ in the ipsilateral eye.

(4) Examination of oxygen uptake following the topical instillation of Adrenalin and varying concentrations of saline in order to determine the relative effects of each with regard to an explanation of a sympathetic effect.

The possibility of long-term adaptation of corneal metabolic rate to extended contact lens wear may have significance clinically. It may be further examined by

(a) Tear film iso-enzyme analysis

(b) Fluorescence specular microscopy

(c) Tear film - lactate levels

The different proportion of LDH iso-enzymes in tear fluid can be determined (Wroblewski and La Due, 1955; Wacker et al. 1956). The tear fluid iso-enzyme pattern could be monitored during prolonged periods of extended soft contact lens wear to examine possible changes which may occur. The iso-enzyme pattern in the tear fluid holds information regarding the metabolism of the corneal epithelium. (Haeringen and Claussius, 1976; Kahan and Ottovay, 1975(a)(b)).

A fluorescene specular microscope has been described (Laing, et al. 1980). Such an instrument can localise and measure the relative amounts of naturally occurring and induced fluorochormes in the cornea. Results showing the distribution of naturally occurring pyridine nucleatides and fluoroproteins have been described (Laing, 1980). The use of an instrument in the monitoring of metabolic intermediaries may provide further information regarding corneal metabolic function both with and without contact lens wear.

Adaptation to a reduced oxygen environment by the cornea may alter the amount of lactate produced. Tear lactate levels during prolonged periods of extended soft contact lens wear may therefore provide additional information regarding possible changes in corneal metabolic pathways (Wigham, 1978).

6.3.2 CORNEAL ENDOTHELIUM

Transient endothelial blebs in the corneal endothelium have been observed following periods of soft contact lens wear in unadapted patients. This has been attributed to anoxia (Holden and Zantos, 1980). Whilst such blebs would not appear to be of clinical significance, they may represent a further insight into the understanding of endothelial physiology. Future work may examine the effect of changes in tear tonicity and varying oxygen tensions in the aetiology of blebs.

COMPUTER PROGRAMME FOR CALCULATING OXYGEN CONSUMPTION RATE FROM THE RAW DATE

```
LIST
STP4

10  REM CALCULATION OF O2 CONSUMPTION RATE AND CORRELATION COEFFICIENT
20  PRINT " TYPE IN VALUES OF X"30
30  INPUT N
40  PRINT "TYPE IN X AND Y VALUES ALONG LINE"
50  MAT  INPUT A[N,2]
60  MAT  PRINT A
70  PRINT "ARE VALUES OF X,Y CORRECT? TYPE YES OR NO"
75  DIM B$[5]
80  INPUT B$
90  IF B$="YES" THEN 120
100  PRINT "RE-TYPE DATA"
110  GOTO 40
120  PRINT "OK RUNNING"
135  LET T=S=H=J=K=M=P=0
140  FOR I=1 TO N
150  LET T=T+A[I,1]
160  NEXT I
170  FOR I=1 TO N
175  LET S=S+(LOG(A[I,2]*1.55))/2.303
190  NEXT I
200  FOR I=1 TO N
210  LET H=H+(A[I,1]-T/N)*((LOG(A[I,2]*1.55))/2.303-S/N)
220  NEXT I
225  FOR I=1 TO N
230  LET J=J+(A[I,1]-T/N)*(A[I,1]-T/N)
240  NEXT I
250  LET R=H/J
260  LET Q=-2.303*R*184.81
261  FOR I=1 TO N
262  LET K=K+((LOG(A[I,2]*1.555))/2.303-S/N) ** 2
263  NEXT I
281  PRINT "SUM XY"," SUM X2"," SUM Y2"," SLOPE"," Q"
283  PRINT H,J,K,R,Q
285  PRINT LIN(2)
286  PRINT " CORRELATION COEFFICIENT IS ";H/SQR(J*K)
310  LET M=SQR(J/N)*SQR(1-(H/SQR(J*K)) ** 2)
320  PRINT "STANDARD ERROR OF ESTIMATE X IS"M
325  LET P=SQR(K/N)*SQR(1-(H/SQR(J*K)) ** 2)
326  PRINT "STANDARD ERROR OF ESTIMATE Y IS"P
335  END
```

APPENDIX 2

Determination of response time of oxygen electrode with changes of electrolyte (seconds)

Potassium Hydroxide	Saturated Potassium Chloride	Sodium Chloride
2.2	1.9	1.8
2.2	2.1	2.0
2.1	2.1	1.9
2.3	2.0	1.9
2.0	2.0	2.2
2.1	2.1	2.0
1.9	2.0	2.0
2.1	2.3	2.2
1.8	2.1	2.3
1.7	1.9	1.9
1.9	2.2	1.9
2.0	2.0	2.3
2.0	2.1	2.0
2.3	2.0	2.0
2.4	1.7	2.0
2.1	2.0	1.8
2.0	1.9	2.1
2.0	2.0	2.0
2.1	2.2	2.0
2.1	2.0	2.0
$\bar{x}$ = 2.07	$\bar{x}$ = 2.03	$\bar{x}$ = 2.02
S = 0.17	S = 0.13	S = 0.14

APPENDIX 3

PATIENT ACCEPTANCE PROFILE

1. Patient not younger than 18 or older than 34
2. Male or Female
3. Patient has not received eye injury or operation
4. Patient has not had some recent injury (within the last six months) ocular medication nor worn contact lenses.
5. Patient is not at present receiving any form of drug for ocular use
6. Patient is not at present receiving any form of drug for systemic use (excluding oral contraceptives)
7. Patient has not had some form of orthoptic treatment (principally strabismus leading to abnormal eye positions)
8. Patient does not suffer from "Hay-Fever"
9. Patient does not suffer from Asthma, allergic dermatitis or other allergic conditions
10. Patient is not subject to recurrent or persistent red eyes, repeated styes, intolerance to light, watery eyes, scaley eye lids, repeated colds
11. Patient is not at present receiving psychiatric care
12. Patient is of caucasian extract
13. Patient does not have abnormal lid/cornea relationship
14. Patient has myopia of -1.00 dioptres to -8.00 dioptres either eye
15. Patient does not have astigmatism greater than 1.50 dioptres in either eye
16. Patient has visual acuity of not less than 6/6 either eye with spectacles
17. Patient does not have a pathological or congenital anomaly of the cornea shown by slit lamp examination in either eye
18. Patient does not have fluorescein staining of the cornea as indicated by slit lamp examination of either eye
19. Patient does not have rose bengal staining of the cornea/bulbar conjunctiva as indicated by slit lamp examination in either eye
20. Patient does not have a pathological or congenital anomaly of either eye as shown by ophthalmoscopy
21. Patient does not have a Tarsal conjunctival abnormality

APPENDIX 4

DEPARTMENT OF OPHTHALMIC OPTICS

SOFT LENS RESEARCH

Determination of corneal oxygen consumption

The aim of the experiment is to measure the rate of oxygen consumption by the corneal epithelium, with a view to producing a clinical procedure for assessing corneal trauma during contact lens wear.

You will be asked to lie back in the examination chair and a small micro oxygen electrode will be placed on to your cornea. This will be kept in position for about 30 - 45 seconds. On subsequent visits the procedure adopted will be exactly the same but you will be asked to wear a soft contact lens during the experiment on to which the electrode will be placed.

Should you wish the experiment to stop at any time please tell the practitioner concerned and this will be done immediately.

J R Larke

APPENDIX 5

UNIVERSITY OF ASTON IN BIRMINGHAM

DEPARTMENT OF OPHTHALMIC OPTICS

SOFT LENS RESEARCH

DECLARATION to be signed by experimental patients on initial registration.

I have read the notes for guidance and information of patients attending the Soft Lens Research Clinic, and have received and read a written description of the experiment in which I am to take part.

I hereby agree to act as a volunteer experimental subject.

Signed . . . . . .

Date . . . . . .

NAME
(Block Capitals) . . . . . .

ADDRESS . . . . . .

. . . . . .

. . . . . .

. . . . . .

J.R.L. 14

APPENDIX 6

THE UNIVERSITY OF ASTON IN BIRMINGHAM

SOFT LENS RESEARCH

CLINICAL EVALUATION OF SOFT CONTACT LENSES

AGE : _____

FIDENTIAL (To be completed by patient)

Mr. Official
Miss Patient
Mrs. No: _____

name _____ Other names _____

e Address _____ Term Address _____

_____ _____

_____ _____

: _____ Tel: _____

upation: _____

e of regular Optician (if any) Family Doctor

_____ _____

ress _____ Address _____

_____ _____

_____

ual "correction"
ring at present: Spectacles ☐ Contact Lenses ☐ Both ☐ None ☐

spectacles worn:- Age when spectacles first prescribed _____

l time ☐ Part time ☐ All distances ☐ Long distance ☐ Close work ☐

contact lenses worn:- Every day ☐ Intermittantly ☐ Infrequently ☐ Discontinued ☐

n was your last eye examination? _____

e you any complaints on the performance of your present lenses? _____

ily History: Are there any instances of eye diseases* in a

Grandparent ☐
Parent ☐
Brother/sister ☐
None known ☐

e.g. *Glaucoma ☐
Retinal Detachment ☐
Cataracts ☐
Blindness or very poor sight of unknown origin. ☐

Personal History

General Health:

- Good ☐
- Indifferent ☐
- Poor ☐

Any past:

- Eye disease ☐
- Eye Injury ☐
- Neither ☐

Any eye treatment other than glasses

- Yes ☐
- No ☐

Do you suffer from any of the following conditions:-

Frequent colds	☐	Red eyes	☐
Catarrh	☐	Red eyelids	☐
Sinus trouble	☐	Scaly eyelashes	☐
Hay fever	☐	Styes	☐
Asthma	☐	Sore or gritty eyes	☐
Food allergies	☐	Itching eyes	☐
Drug allergies	☐	Watering eye(s)	☐
Boils, abscesses	☐	Sticky eyes	☐
Pimples, Acne	☐	Discharging eyes	☐
Lip cold sores	☐	Intolerance to light	☐
Headaches/Migraine	☐	Double vision	☐
Dandruff	☐	Intermittent "steamy" vision	☐

Are you at present taking any regular pills, tablets or medicines prescribed by your doctor?
(please state what, if known)

- Yes ☐
- No ☐

Other relevant comments:

APPENDIX 7

UNCOLLECTED DATA

Apparent Oxygen Uptake Rate

Solution Group

2 patients were rejected from the study.
7 patients withdrew themselves from the study.
18 data points lost due to apparatus malfunction.
16 data points lost due to poor quality result.
18 data points lost due to patients non attendance.

Non-Solution Group

5 patients were rejected from the study.
4 patients withdrew themselves from the study.
15 data points lost due to apparatus malfunction.
15 data points lost due to poor quality result.
12 data points lost due to patients non attendance.

Control Group

1 patient was rejected from the study.
2 patients withdrew themselves from the study
14 data points lost due to apparatus malfunction.
31 data points lost due to poor quality results.
31 data points lost due to patients non attendance.

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