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NEUROPATHOLOGY AND SENSITIVITY IN THE KERATOCONIC CORNEA

A Thesis

submitted by

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for the degree of

DOCTOR OF PHILOSOPHY

Department of Optometry

The City University

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ABSTRACT

Corneal touch threshold (CTT) was investigated by aesthesiometry in patients with keratoconus, with and without contact lens wear. Using a matching control group it was established that CTT was significantly higher for the central corneal position in keratoconus. No difference in CTT was found in four peripheral corneal positions in keratoconic and normal corneas. Central CTT correlated inversely with central corneal curvature and central corneal thickness. Central corneal curvature was the most significant single factor to correlate with central CTT and indicates that CTT increases (sensitivity reduces) as the cornea steepens. Corneal surface irregularity, as measured by mire image distortion, correlated positively with central CTT as did corneal scarring. Central CTT did not show a relationship with duration of the disease nor the visibility of the corneal nerve fibres.

Lid margin touch thresholds (LTT) were investigated for the central position on the lower and upper eyelid margins. No statistical differences were found between keratoconic and normal eyes nor between upper and lower eyelid margins. The magnitude of LTT was in the order of that established for the peripheral corneal CTT.

Innervation of the human corneal stroma and epithelium was investigated by light and electron microscopy in the central and mid-peripheral positions. All nerve bundles were located in the anterior two thirds of the corneas. In keratoconic corneas mid-peripheral stromal nerve bundles were disorganised and irregular taking up the shape of the adjacent collagen lamellae. Nerve bundles had a regular oval appearance in the control corneas. In both groups Schwann cell cytoplasm was sparse and of varying degree of electron density; axon varicosities were not uncommon and axon content with respect to organelles were similar.

The axon density showed large variation in keratoconic specimens and averaged more than threefold that of control specimens for stromal and epithelial nerves.

The control corneas showed a greater proportion of large diameter stromal axons than in keratoconic corneas. This result was reversed for epithelial axons.

The results are discussed with respect to the disease process and influence on tactile sensitivity.

CHAPTER 1

INTRODUCTION

1.1 CORNEAL SENSITIVITY.

1.1.1 Measurement of Corneal Touch Threshold (CTT).

The standard clinical method for testing corneal sensation is by using a piece of cotton wool. Although the technique is in common use, due to its simplicity, it does not provide any information on the fine grades of sensation.

Von Frey (1894) first introduced a semi-quantitative method for testing corneal tactile sensitivity. He used horse hairs of variable length and diameter and achieved a rough grading of sensitivity this way. Boberg-Ans (1955,1956) improved this technique considerably by using nylon filaments of known length and diameter, and hence turned tactile sensitivity testing into a quantifiable measure which could be used as a relatively simple clinical tool. Cochet-Bonnet (1960) used the idea of Boberg-Ans and produced a commercially available instrument which has been used extensively in research and clinical studies.

Aesthesiometry, as described by Cochet-Bonnet (1960) & Millodot (1969), is performed by touching the cornea with

the tip of the filament ensuring that the filament is normal to the corneal surface on contact. The approach should be slow with a constant speed and terminated at the first detectable bending of the filament (approximately 5°). Withdrawal of the filament should be at the same speed, and the whole procedure repeated approximately six times including at least one approach without contact to test the reliability of the responses.

Several sources of error are present.

Temperature & humidity.

Boberg-Ans (1956) and Millodot & Larson (1967) showed that the coefficient of elasticity of the nylon filament is affected by changes in ambient temperature and humidity and concluded that such environmental factors should be controlled.

Contact angle.

The effect of the force applied by the nylon filament will depend upon the area of contact. The standard monofilament has a diameter of 0.12 mm and if applied normally to the corneal surface will cover approximately 10 basal epithelial cells or 3 surface epithelial cells. The force will be concentrated over a smaller area if the filament is applied at an angle other than normal to the surface cells giving rise to a concentration of the pressure.

Bending of the filament.

Boberg-Ans (1956) showed that increasing the bending of the filament also increase the force applied, and Millodot & Larson (1967), when repeating the experiment, found an exponential effect of the force by bending the filament. The force approximately doubled when the bending of the filament was increased from 5º to 15º. The table of pressure-values supplied with the Cochet-Bonnet aesthesiometer was in good agreement with the values found by Millodot & Larson for the minimum angle of bending of 5º.

Speed of approach.

The time during which the elastic powers of the nylon filament act is of great importance for the perceptability of the stimulus (Boberg-Ans 1956). An increase in the speed of application might increase the stimulus many times. In this way a quick application in the form of "jabbing or pricking" may be felt very distinctly but the continous pressure by the same force may not. Meurs et al (1979) divided the force applied by the aesthesiometer into kinetic- and elastic energy. They expressed the force in a formula in which the force is dependent upon a certain relationship between mass and velocity. For practical purposes this means that the cornea should be touched with a constant and very slow motion. Repeating the applications rapidly may lead to summation of the effect which can result in the stimulus reaching above threshold levels.

These sources of error make the procedure difficult to carry out with the instrument handheld, and research reports using the aesthesiometer handheld should be interpreted with caution. Millodot (1973) modified the technique by mounting the aesthesiometer on a table which provided control of X,Y,Z movements, and thereby improved the repeatability of the measurements. By controlling the experimental setup in this manner the error does not usually exceed ± 5% (Millodot & Earlam 1984).

Other instruments.

Nafe and Wagoner (1937) and Franceschetti (cited in Cochet & Bonnet, 1960) substituted Von Frey's hairs with metal rods but these were cumbersome and not as popular as the aesthesiometer produced by Boberg-Ans (1955) using nylon. Slight alterations were made to the Cochet-Bonnet aesthesiometer by Beuerman et al (1978) and various other instruments have been produced, some more accurate than others. Surgical nylon sutures of different thicknesses have been mounted on metal rods (Lele & Weddell 1956), an electronic aesthesiometer using a platinum wire (Larson 1970) and a corneal sensitometer used by Schirmer (1963) which was a spring-gauge aesthesiometer. Hamano (1960) introduced a corneal sensibiliometer where electricity stored in a condenser was transmitted to the cornea by means of a silver chloride electrode having a cotton thread at its

tip. Voltage could be changed from 0 to 180 volts. Using a metal "contact pin", Draeger (1984) produced an electronic aesthesiometer in which the pressure could be varied continously, thereby obtaining a dynamic measurement of corneal sensitivity. Most of these instruments are, however, too cumbersome to use with patients and have therefore only been used by a few researchers.

1.1.2 Corneal Touch Threshold (CTT) Variables.

CTT in Normal Corneas.

Several researchers have shown CTT to change with age. There is general agreement that the increase in CTT is small and steady up to approximately 40 years of age for the increase in CTT to accelerate thereafter (Boberg-Ans 1955; Jalavisto et al 1951; Millodot 1977). Millodot (1972) found CTT to undergo a diurnal variation with a decrease in CTT during the day. This was found to correlate with the diurnal variation seen in corneal thickness (Gerstmann 1972; Mandell & Fatt 1965; Mertz 1978). Furthermore, CTT varies across the cornea with the lowest values in the centre and the highest close to the superior limbus (Adler 1965; Cochet & Bonnet 1960; Draeger 1984; Millodot & Larson 1969, Rougier cited by Cochet & Bonnet 1960; Strughold 1953), and increases in CTT are seen in association with dark pigmented irides (Millodot 1975), the menstrual cycle (Millodot & Lamont 1974) and

pregnancy (Draeger 1984; Millodot 1977). Douthwaite & Kaye (1980) looked at corneal sensitivity and corneal thickness in a normal group and found a negative correlation. The fact that the filament can be seen approaching the cornea may influence CTT. By using infra-red light in the dark, Bonnet & Millodot (1966) reduced the apprehension and found the CTT to be higher. Furthermore, it seems that corneal sensitivity is a power function of the pressure applied to the cornea, meaning that a pressure of 20 mg/mm^2 applied to the cornea feels twice as intense as a pressure of 10 mg/mm^2 (Millodot 1968; 1969). He estimated the pain threshold to lie approximately 10 times above the touch threshold.

<u>CTT in Ocular and Systemic Diseases.</u>

Diseases involving the eye all have the same effect (if any) in reducing corneal sensitivity. Boberg-Ans (1955) investigated in detail sensitivity changes associated with corneal pathology such as perforating injuries, chemical injuries, corneal ulcers, various forms of keratitis and dystrophies. Glaucoma with intra ocular pressure above 30 mmHg and specially an acute attack will reduce corneal sensitivity (Boberg-Ans 1955, Cochet & Bonnet 1960, Schirmer et al 1961). No change was seen in corneal sensitivity due to the presence of a Hudson-Stahli line (Norn 1970), but exposure to ultraviolet light dramatically reduced corneal sensitivity (Millodot & Earlam 1984). When

this study was embarked upon no information was available in the literature about corneal sensitivity in keratoconus. Since then Millodot & Owens (1983) and Bleshoy (1986) have published results which show corneal sensitivity to be reduced in keratoconic eyes.

Corneal sensitivity reduction has been linked with systemic diseases including diabetes (Draeger et al 1985, Nielsen 1978, Nielsen & Lund 1979, Schwartz 1974), hypertension (Radzikhovsky 1974), myasthenia gravis (Nazarian et al 1985), trachoma (Cochet & Bonnet 1960) and herpetic ulcers in man (Draeger 1979, Norn 1970). In the herpetic rabbit model Metcalf (1982) demonstrated corneal nerves by acetylcholinesterase histochemistry and concluded that loss of sensitivity was not due to a destruction of the corneal nerve fibres. Corneal oedema may be responsible for the reduction in corneal sensitivity during the majority of these disease processes (Millodot 1974). No reports could be found of corneal sensitivity changes related to collagen diseases.

The use of timolol maleate has been shown to have no short term effect on corneal sensitivity (Kitazawa et al 1980), however when used for more than three months Van Buskirk (1979) demonstrated a reduction in corneal sensitivity in some patients.

Some cases of neurological disorders have also been associated with a reduction in corneal sensitivity Boberg-Ans 1955, Lewis et al 1982). Boberg-Ans (1955) reported cases involving intra-cranial tumours (glioma, astrocytoma, pituitary adenoma and meningioma).

The effect of surgery on CTT.

Schirmer et al (1961), using a modified version of the Cochet-Bonnet aesthesiometer with a double filament, found the sensitivity to be lower in the areas and sectors adjacent to the corneal scar after section for cataract extraction. These results were confirmed for cataract surgery (Draeger 1979, Lyne 1982), trabeculectomy, iridectomy (Lyne 1982), keratoplasty (Draeger 1979, Gullapalli et al 1985, Ruben et al 1979) and epikeratophakia (Koenig et al 1983).

The effect of contact lenses on CTT.

Knoll & Williams (1970) did not find any reduction in corneal sensitivity in soft lens wearers compared to non-lens wearers. This was partially supported by Millodot (1974,1976) who after 8 hours wear found the sensitivity to be similar to the initial value in the morning. However, after removal of the lenses the corneal touch threshold continued to decrease, indicating that the soft lenses did have some effect. Knoll & Williams did not take into account the diurnal variation in corneal touch threshold. Millodot

blamed corneal oedema for the change in corneal sensitivity. This view is supported by Bradley & Schoessler (1979) who found that thick soft lenses induced corneal oedema and a greater reduction in corneal sensitivity than thin soft lenses. Furthermore, Larke & Hirji (1979) found prolonged wear of soft contact lenses to reduce corneal sensitivity. They were, however, unable to determine whether the increase in corneal touch threshold was of mechanical or physiological origin.

The loss of sensitivity was attributed to an alteration in the corneal nerve function or neural output rather than to patient bias or a higher sensory mechanism (Beuerman & Rozsa 1985). These findings were consistant with those of an earlier study in which Tanelian & Beuerman (1980) used thermal stimulation to induce corneal irritation in a group of hard contact lens wearers. They monitored the recovery after removal of the lenses and came to the conclusion that the recovery from adaptation at the receptor level occurred spontaneously and rapidly after removal of the stimulus, whereas recovery of more complex centrally based responses may require many hours.

Hard contact lenses have been shown to reduce corneal sensitivity considerably (Boberg-Ans 1955; Cochet & Bonnet 1960; Dixon 1964; Hamano 1960; Schirmer 1963). Dixon (1964) commented that the reduction was of a magnitude which

allowed intra ocular pressure to be measured with the Schiøtz tonometer without the use of an anaesthetic. Furthermore, he found the reduction in sensitivity only to affect the area covered by the contact lens, the peripheral cornea remaining unaffected. More recent studies disagree with the comments made by Dixon. After 8 hours of hard contact lens wear, Millodot (1975) found sensitivity to be reduced in the periphery as well as in the centre of the cornea with a reduction in sensitivity of 116% and 94% respectively. The long term effect of wearing hard contact lenses is an exponential decrease in corneal sensitivity (Millodot 1978a,1978b) with little change in corneal thickness. Recovery happens overnight after a few years of lens wear, however, it may take several months to recover to baseline level from more than 10 years of lens wear.

In trying to explain the loss of corneal sensitivity Millodot et al (1979) used various types of hard lens materials. These lenses provided different oxygen tensions at the cornea but did not result in dissimilar reduction in sensitivity thus indicating the aetiology of the change in sensitivity as being mechanical. They did, however, not exclude that a reduction in acetylcholine and choline acetyltransferase could be responsible for the changes seen in corneal sensitivity. These components have been shown to be reduced during lid closure (Mindel et al 1978) after which corneal sensitivity is reduced without the influence

of mechanical stimulation.

Exposing the cornea to oxygen free atmosphere induced corneal oedema without a decrease in sensitivity (Polse 1978). This led him to the conclusion that sensory adaptation to contact lens wear is a phenomenon related mainly to mechanical stimulation and not to a metabolic disturbance. The work of Millodot & O'Leary (1979 & 1980) disagree with Polse's conclusion. Millodot & O'Leary reduced the oxygen tension at the surface of the cornea by closing the eyelid or by passing a gas with a reduced oxygen mixture in front of the cornea. They found a clear relationship between the loss of corneal sensitivity and the reduction in partial oxygen pressure at the corneal surface. The effect on corneal sensitivity was much greater than the small change seen in corneal swelling. These results imply a correlation between loss of corneal sensitivity and corneal swelling, and therefore support the hypothesis of loss of sensitivity having a physiological origin.

Accordingly there is general agreement that rapid loss of sensitivity is likely to be of local origin in the corneal nerve fibres and involve mechanical as well as physiological stimuli. Long term sensitivity changes may furthermore involve more complex centrally based components.

Response to Thermal Stimulation of the Cornea.

Since Von Frey (1894) claimed that pain was the only sensory modality of the cornea, several researchers have looked more closely at the role of thermal stimulation of the cornea. Nafe & Wagoner (1937) claim the cornea to be sensitive to touch, pressure and pain, but not to warmth, cold or heat. This is in disagreement with Kenshalo (1960) and Beuerman & Tanelian (1979) who showed that warm stimuli represent an adequate excitatory stimulus for the corneal receptors, however, the sensory experience is of a nociceptive nature and not one of temperature sensation. Using the rabbit as a model Tanelian & Beuerman (1984) found that 11% of neurons showed bimodality by responding to mechanical and warm stimuli. Giraldez et al (1979) and Belmonte et al (1981) used electrophysical techniques on cat to obtain information on corneal sensory receptors. The bimodal function of mechanoreceptive fibres also responding to thermal stimuli reported by Lele and Weddell (1959) was confirmed. The high threshold and range to warm stimuli in conjunction with their mechanical bimodality make them similar to cutaneous warm receptors except for their lower mechanical threshold. Kolstad (1970) found corneal sensitivity to be reduced in very low ambient temperatures.

1.1.3 Lid margin touch threstold (LTT).

Very little has been written about the sensitivity of the

eyelid margin, and the few reports available give conflicting information.

Strughold (1953) argued that the tarsal conjunctiva and the palpebral margin demonstrate touch and pressure as well as pain sensation whereas the cornea is capable only of pain sensation. Using Von Frey hairs, Strughold found that the thresholds of the tarsal conjunctiva and the palpebral margin corresponded to those of the pressure sense on the skin of the lower arm and were slightly higher than the thresholds on the outer skin of the eyelid. Testing the conjunctiva in the central tarsal area and near the lid margin on its conjunctival side, Dixon (1964) recorded the touch threshold at the lid margin to be approximately 13 fold higher than the mid-peripheral cornea. After adaptation to hard contact lenses, the touch threshold for the mid-peripheral cornea rose to the level of the lid margin prior to lens wear, and the lid margin threshold rose 4 times its level prior to lens wear. The conjunctiva near the lid margin remained considerably less sensitive compared to a mid-peripheral corneal area.

Using a modified version of the Cochet-Bonnet aesthesiometer, Lowther and Hill (1968) focussed their attention on the lower lid margin during the course of adaptation to hard contact lenses. They tested several points in the area between the Meibomian gland orifices and

the inner conjunctival limit of the margin, and found the lower lid margins of the pre-lens wearer equally or in some cases even more sensitive to touch stimuli than their respective corneal centres.

In more recent studies using the standard Cochet-Bonnet aesthesiometer, Norn (1973,1983) measured the touch threshold at the lid margin in a position behind the eyelashes and in front of the conjunctiva. The lid margin was found to be much less sensitive than the central cornea and approximately half as sensitive as the inferior and superior cornea near the limbus. He found no difference between the upper and the lower lid margins. Interestingly Norn produced corneal and conjunctival anaesthesia with 10% cocaine and Novesin (oxibuprocaini chloridum NFN) while the sensitivity of the lid margin remained unaffected. In a more recent study, Collins et al (1989) measured LTT at the centre of the upper lid margin. The LTT was higher compared to the central and peripheral CTT, however, they found the LTT to increase with the use of a topical anaesthetic contrary to the report by Norn. Some of the discrepencies among the results of different researchers must be due to differences in non-uniform instrumentation and technique. Strughold's use of Von Frey hairs is acknowledged to give less accurate measurements than, for example the sensitive setup used by Lowther and Hill.

1.2 CORNEAL INNERVATION

1.2.1 Corneal Nerve Supply.

The ophthalmic division of the trigeminal nerve is generally accepted to be responsible for the majority of nerves innervating the cornea. The ophthalmic nerve is a sensory nerve, however, sympathetic fibres join it. The nasociliary branch of the ophthalmic nerve branches before it penetrates the eyeball and gives rise to the long (containing sensory and sympathetic fibres) and the short ciliary nerves. Parasympathetic fibres join the ciliary ganglion via the oculomotor nerve and mix with sensory and sympathetic nerves in the short ciliary nerves before penetrating the posterior segment of the eyeball. The ciliary nerves enter the sclera and flatten in their course in the suprachoroidal space towards the anterior segment of the globe. At the level of the ciliary body the nerves enter the sclera to form a peri-limbal plexus just proximal to the limbus (Kelly 1985, Klyce & Beuerman 1988, Ruskell 1989, Warwick 1976).

Stromal innervation.

From the limbus 70 to 80 nerve bundles enter the corneal stroma radially at the middle and anterior level. The nerves usually lose their myelin sheath at the limbus and shortly after entering the cornea the perineurium and endoneurium terminates. Each nerve bundle contain many axons which are completely or partly enclosed by Schwann cell sheaths. The nerve bundles flatten, divide dichotomously and move towards

the central cornea and the anterior cornea to form a dense plexus close to Bowman's layer (Matsuda 1968, Zander & Weddell 1951). Stromal axons demonstrate small beadlike varicosities and some lose their Schwann cell investment to become terminal axons. One limbal nerve bundle contains nerve fibres which cover as much as two-thirds of the corneal area. Accordingly, there is a vast overlap of nerve fibres from the limbal nerves. Belmonte et al (1961) recorded the receptive fields from single nerve fibres in the cat and found the receptive fields to overlap considerably. Most authors agree that Descemet's membrane and the endothelium do not receive any innervation (Matsuda 1968, Maurice 1969, Tankarua et al 1969, Tripathi et al 1972, Warwick 1976, Zander & Weddell 1951), however, Wolter (1957) claimed to have observed nerves in the endothelium of rabbits.

There are no specialised nerve terminals in the cornea. Axon terminals, commonly referred to as free nerve endings, represent the sensory receptors and are most abundant in the epithelium. Occasionally axons make contact with keratocytes although still separated by their basement membrane, and it is not known whether this represents a form for termination (Matsuda 1968, Tripathi et al 1972).

<u>Epithelial innervation.</u>

Some stromal nerves penetrate Bowman's layer from the subepithelial plexus and in doing so lose their Schwann cell sheath. These naked axon bundles divide among the basal

epithelial cells to form leashes of axons which may run a considerable distance. Axon terminals are numerous in the basal epithelial cell layer but some axons from the basal epithelial cell plexus turn and ascend towards the more superficial layers of the epithelium (Rozsa et al 1982, Schimmelpfennig 1982, Tervo et al 1985). Other branches from the ciliary nerves pass directly from the conjunctival epithelium to the corneal epithelium at the basal epithelial cell level (Lim & Ruskell 1978). These nerves may be the main supply to the peripheral corneal epithelium whereas the supply to the central corneal epithelium predominantly arise from the penetrating stromal nerves. Trigeminal neurectomy has been shown to cause corneal changes especially of the epithelium. This suggests that the corneal sensory nerves have not only an afferent but also an efferent effect which is expressed as a trophic function. This may happen via peptidergic mediators such as substance P and calcitonin gene-related peptide (Bito 1984; Unger et al 1985), but the mechanism is not well understood (Ruskell 1989).

Autonomic innervation.

Although both sensory and autonomic nerve fibres are present in the ciliary nerves, the presence of autonomic nerves in the cornea is still open to debate. Corneal sympathetic innervation has been suggested from the point of view of physiology (Friedenwald & Buschke 1944; Mishima 1957; Sweeney et al 1985) and biochemistry (Klyce et al (1973). Matsuda (1968) suggested a classification of function based

on histological investigation. He suggested the presence of vesicles and mitochondria in the axon terminals of the epithelium to indicate a motor function. Retrograde and anterograde horseradish peroxidase tracing techniques have demonstrated that some corneal nerve fibres originate in the superior cervical ganglion in non-primate eyes (Marfurt 1988; Morgan et al 1987), and although Ehinger (1971) found sparse evidence of sympathetic motor fibres in primate eyes, more recent studies of Toivanen et al (1984;1987) and Ueda et al (1989) have demonstrated sympathetic fibres in adult human cornea. There is little evidence in support of parasymapthetic fibres being present in the human cornea (Ehinger 1964, 1966, 1971, Hutala et al 1975, Lim 1976, Matsuda 1968, Tervo et al 1978a, 1978b, 1978c, 1979). The function of an autonomic innervation can still only be speculated upon, but it has been suggested that the adrenergic terminals of adults govern metabolism of mitotic activity of epithelial cells (Butterfield & Neufeld 1977, Mishima 1957), pump activity of epithelial cells (Klyce & Crosson 1985), by decreasing the release of collagen and collagenase from keratocytes (Berman et al 1976, Chao & Wallenbach 1986) as well as metabolic activity of nerve sensitivity and regulation of some embryonal secretory activity of the corneal epithelium (Ehinger et al 1971, Tervo et al 1976).

1.2.2 Techniques to Study Corneal Innervation.

Staining techniques such as gold chloride, silver impregnation and acetylcholinesterase have been the most common methods used for studying the nerves in the cornea by light microscopy. These histochemical techniques give a non-specific overview of the total innervation as for example acetylcholinesterase (AChE) which has been demonstrated in cholinergic, adrenergic and sensory nerves (Koelle 1955). Experimental work in primates and non-primates with degeneration of nerves after selected lesions have assisted in identifying the sources and distribution of corneal innervation, however, only the resolving power of electron microscopy has provided information about the ultrastructure of corneal nerves (Primate: Lim & Ruskell 1978; Matsuda 1968; Schimmelpfennig 1982; Tervo et al 1983,1985,1987; Toivanen 1987 and in non-primate: Bee et al 1986,1988; Matsuda 1968; Marfurt et al 1989; Metcalf 1982; Rozsa et al 1983; Stone et al 1986; Wolter 1957 and many others).

Immunohistochemical staining for various peptides in corneal nerves have received much attention over the last decade. Substance-P has been positively identified in corneal nerves (Tervo et al 1982) and recently immunoreactivity in corneal nerve fibres have been demonstrated by antibodies to neuron specific enolase (NSE) calcitonin gene-related peptide (CGRP) and tyrosine hydroxylase (TH) (Udea et al 1989). However, despite this variety of investigative techniques

there is still no clear correlation between morphologic appearance and functional differentiations.

1.2.3 Nerve Visibility in Keratoconus.

Many investigators have commented on the increased visibility of corneal nerves in keratoconus. Thomas (1955) and Mensher (1974) did not believe that this was due to an increase in corneal nerves but thought that a change had occurred in the physical characteristics of the surrounding media in which the nerves are situated. Biochemical studies have shown that the types of collagen in normal corneas are present in the same proportion in the keratoconic corneas. It remains, however, to be investigated whether the ground substance differs in composition and refractive index. The altered shape and thinning of the cornea has been claimed to be responsible for the visible nerves (Vogt 1919). The change in corneal curvature is most marked in the centre of the cornea and very little change takes place in the periphery. Accordingly a change in corneal shape cannot account for the peripheral nerve fibres becoming more visible. The possibility of an anatomical change in the nerves in keratoconus has not been discussed in the literature.

1.2.4 Corneal Innervation in Other Diseases.

Corneal nerves have been observed to undergo changes in some diseases including multiple endocrine neoplasia (Tervo et al

1987; Valentines et al 1984) and leprosy (Klyce et al 1985). In these diseases the nerves increase in thickness due to degeneration of axons and Schwann cells. Psychophysical studies have revealed a decrease in corneal tactile sensitivity in diabetes, leprosy and other localised corneal disorders (Klyce & Beuerman 1985). The literature does not contain information on neural behaviour in collagen diseases.

1.3 THE DISEASE

1.3.1 Clinical Characteristics.

The diagnosis of keratoconus is based on a series of signs. These include central corneal thinning, central corneal steepening, distortion of the central corneal surface, Fleischer's ring (pigmentation of the epithelium), subepithelial fibrillary lines, prominent nerve fibres, striae in deep stroma and in more advanced cases, central scarring and hydrops (acute oedema caused by a rupture in Descemet's membrane). All these clinical signs are unlikely to be present in every case at the same time. As the disease progresses more of the signs become manifest. In addition the apex of the cone is usually situated inferiorly and temporally (Marchall-Courtois 1965, Tomlinson et al 1980, Wesley 1982).

1.3.2 Grading of the Disease.

Several authors have attempted to grade the disease according to its progression. Amsler (1938) was the first to apply a classification to the progression of the cone. He used 4 stages : I, II, III & IV, based on viewing the cornea and observing the distortion seen by the reflection from a placido disc. The mild stage (I) indicated a misalignment in the two half horizontal mire axes of the order of 1-3 degrees. The second stage (II) was at a misalignment of 4-8 degrees. For the last two stages (III & IV) the distortion was so great that it was impossible to measure the angle of deviation between the two half horizontal axes. In stage III corneal thinning and opacities are seen. For stage IV the mires are so distorted and appear small due to the steep curvature that it is difficult to recognise them as mires.

Hadeyama (see Poster et al 1968) classified the disease into 4 types on the basis of visual acuity. Applebaum (1936) used 7 distinct types of corneal alteration to include corneal thinning, endothelial reflex, striae, scarring, rupture of Descemet's membrane, increased nerve fibre visibility and appearance of Fleischer's ring. He comments that all signs may not be found in every case, and he does not attempt to quantify them as did Brooks et al (1984). Light- and electron microscopy provides detailed information of the histopathology, but they have no direct clinical value (Teng 1963).

The various grading systems in use (all authors) all rely on very few of the clinical signs of the disease, and for the purpose of this study a new system was produced to quantify clinical findings in addition to corneal thickness, curvature and distortion. For this purpose a severity index was designed which incorporated the most common diagnostic signs (Lobascher & Buckley 1982).

1.3.3 Histopathology.

There is general agreement in the literature that the pathology of keratoconus occurs first and most evident in the anterior layers of the cornea (Caffi 1966; Chi et al 1956; Gottinger et al 1970; Hervouet 1958; Iwamoto et al 1975; Kanai 1968; McPherson et al 1968; McTigue 1967; Pataa et al 1970; Pau 1967; Pouliquen et al 1968,1972; Salzmann 1907; Teng 1963). Anatomical studies have, however, given rise to various theories as to the site of the initial pathogenesis i.e. basal epithelial cells, Bowman's membrane and anterior stroma.

Corneal Epithelium.

The epithelium appears wavy and the thickness of the epithelial layer varies from thin to very thick (Kanai 1968; Pau 1967). Teng (1963) claimed that the pathologic process was first detected by necrosis of basal epithelial cells. He found early changes in the endoplasmic reticulum and the Golgi apparatus giving rise to inclusion bodies. Kanai

(1968) support this and furthermore found free ribosomes to be more numerous in the basal epithelial cells. The fine details of mitochondria appear less clear in the basal cells (Kanai 1968; Teng 1963).

Particles with dense bodies are seen in intra- and intercellular spaces of the basal epithelial cells and appear in the region of Fleischer's ring. They were often found in membrane-limited dense vesicles (Kanai 1968). Similarities between the primary lesions of the skin and the basal cells of the corneal epithelium have been reported (Sabiston 1966).

The basement membrane is involved early in the pathogenesis and appears irregular and interrupted with breaks through which processes of basal epithelial cells invade the stroma (Chi 1956; Kanai 1968). Pataa et al (1970) identified osmiophilic deposits between the basement membrane and the epithelium, and Bowman's membrane may be missing, ruptured, displaced, thickened or even doubled and may become replaced by stromal collagen fibres (Chi 1956; Kanai 1968; Pau 1967).

Small rounded or oval particles, of approximately 200 um in diameter, in the subepithelial basement-like layer have been described (Iwamoto 1975). They consisted of a central core and an envelope and seemed to arise from the basal epithelial cells. They could not be located in normal or

other pathologic corneas like Fuch's dystrophy and late interstitial keratitis. However, they have been seen in peripheral corneal lesions of marginal degeneration and in Bowen's disease of intraepithelial epithelioma. They may be a product of epithelial cell degeneration, however, the possibility that they may be a virus or an organism of similar category cannot be excluded.

Corneal Stroma.

In keratoconus the stroma becomes abnormally thin with the lamellae being grossly disorientated and irregular (Gottinger 1970; Kanai 1968; Pau 1967; Pouliquen 1970). Thinning of individual collagen lamellae had been thought as the cause of thinning of the stroma (Jakus 1962), however, Pouliquen et al (1970;1984) more recently argued that the collagen fibres and lamellae remain normal in size and collagen lamellae decrease in number. Keratocytes have been observed to undergo degenerative processes. Six different types were described by Pouliquen (1968;1972) according to their appearance and corresponding physiological state. The number of keratocytes has been observed to increase in the anterior part of the stroma and they no longer appeared parallel to the basement membrane (Pataa et al 1970). They furthermore noticed a light osmiophilic zone between adjacent keratocytes where no collagen fibres were present. This clear or electron-lucid area was occasionally also noted in association with keratocytes which seemed to be

compressed in a localised zone.

Granules or microfibrillary material has been noticed, spread over different layers of the stroma. This material appears in pools in contact with keratocytes and fills large cavities of the cytoplasm. It may also be seen to penetrate lamellae as well as being located between lamellae, and may originate from interrupted fibrillogenesis which consequently leads to collagen fibril precursors (Gottinger et al 1970; Pouliquen et al (1968). McPherson et al (1968) found amyloid deposits in some keratoconic specimens. The authors believe that the amyloid deposits along lamellar fibres may indicate chemical change in the corneal lamellar fibres to explain the thinning, atrophy and general distortion of the corneal lamellae in keratoconus.

Depending on the stage of the disease every layer of the cornea becomes involved. Descemet's membrane may be normal or thinned or even replaced by connective tissue (Pau 1967). The most significant changes take place when Descemet's membrane undergoes healing after hydrops. The rupture and detachment of Descemet's membrane associated with hydrops result in extensive ledges occurring. New endothelium resurfaces the entire exposed area and the endothelium regenerate considerable amount of new basement membrane (Stone et al 1976).

Corneal nerves.

Although several authors have illustrated epithelial nerves in keratoconic corneas and commented on their clinical visibility, very few have paid detailed attention to them. Kanai (1968) illustrate epithelial nerve groups containing 2 to 9 axons containing vesicles. Teng (1963) found signs of degeneration in epithelial nerves and claim "One thing is fairly certain: the ÆepithelialÅ degeneration in the disease process of keratoconus is not due to nerve degeneration." However, Pataa et al (1970) noticed that nerves were sometimes seen near breaks in Bowman's membrane and put forward the suggestion that nerve involvement needed further investigation.

1.3.4 Aetiology

Ocular Rigidity.

There are some reports that keratoconus develops in people with low corneal tensile strength (Davies et al 1975, Werb 1972). This has led to several inconclusive studies on ocular rigidity. Although there is some disagreement with regard to ocular rigidity in keratoconus, most reports point towards a reduction in ocular rigidity largely being caused by the reduced corneal rigidity. The mechanical strength of the keratoconic cornea being reduced not only because of a reduced volume but also because of a decrease in attachment and increase in sliding of collagen structues (Andreassen et

al 1980; Brooks et al 1984; Edmund 1988;1989; Foster et al 1978: Poole 1973).

Down's- & Ehlers-Danlos Syndrome.

Keratoconus in association with Down's- and Ehlers-Danlos syndromes have been reported extensively (Boger et al 1981; Cullen & Butler 1963; Hyams et al 1969; Kuming & Joffe 1977; Madison 1968; Rados 1948; Robertson 1975) and may suggest genetic control. In his review of keratoconus, Krachmer (1984) quote a study by Hallerman & Wilson who reviewed a large group of keratoconus cases and found a frequency of inheritance of at least 7%. However, Krachmer conclude that the whole process may be genetically determined as well as being influenced by unspecific external factors, and a genetic control still remains unproven and may only be regarded as one aetiological possibility. High maternal age has been linked with keratoconus (Woodward 1981) although Millodot & Owens (1983) could not find any significant correlation in their study.

Atopy.

Since Gonzales (1920) first described a connection between vernal catarrh and keratoconus many investigators have examined the ralationship between keratoconus and atopic diseases (Davies 1976; Gasset et al 1978; Gonzales 1920; Karseras 1976; Rahi 1977; Ridley 1956; Sabiston 1966; Spencer 1959). IgE levels may be raised in keratoconus

(Davies et al 1976, Rahi et al 1977, Kemp et al 1982), and in a large controlled study by Rahi et al (1977) the incidence of atopy was 35% in the keratoconic group compared to 12% in the control group. Although Lowell et al (1970) found no difference in atopy, most investigators agree that the incidence of atopy among keratoconic patients is higher (Copeman 1965; Davies et al 1976; Galin & Berger 1958; Gasset et al 1978; Sabiston 1966; Spencer et al 1959). The incidence of keratoconus among patients with atopic dermatitis, however, is very low (Brunsting et al 1955, Roth et al 1964).

Eye rubbing was first mentioned by Ridley (1956) in association with keratoconus and Gasset et al (1978) supported this in a more recent study. Furthermore hard contact lens wear has been linked to keratoconus although firm evidence is lacking (Brady 1972; Brightbill et al 1979; Gasset et al 1978; Hartstein et al 1965;1968;1970; Nauheim 1969;1985; Steahly 1978).

Other Associations.

Hormonal control has been suggested due to the disease frequently becoming manifest around puberty (Appelbaum 1936, Copeman 1965), whereas there seems to be some disagreement about the involvement of HLA antigens (Davies et al 1976; Gasset 1977; Krachmer 1984; McKinney 1984; Wachtmeister 1982).

Keratoconus may be the result of the lysis of fibrin or involve impeded elaboration of fibrin (Millin et al (1986), but Klintworth et al (1982) did not believe that the breaks in Bowman's membrane was caused by enzymatic degradation. Marginal pellucid degeneration of the cornea has been claimed to be an excentrically placed form of keratoconus (Pouliquen et al 1980). Mitral valve prolapse (Beardsley et al 1982), retinal aplasia, macular coloboma & cataract (Leighton et al 1973), retrolental fibroplasia, retinitis pigmentosa and glaucoma (Lorfel et al 1976) have also been mentioned in association with keratoconus.

1.4 PURPOSE OF STUDY

This study set out to answer questions in two main areas: First to investigate the tactile sensitivity in keratoconic and normal eyes, and second to obtain information on the ultrastructure of corneal nerves in keratoconus.

As keratoconus primarily manifests itself in the central portion of the cornea it was of interest to test corneal touch threshold (CTT) in various corneal positions. Furthermore, lid margin touch threshold (LTT) was undertaken in order establish the aetiology of clinical reports of discomfort with contact lens wear. It would be of interest to correlate any change in corneal sensitivity with the

stage of progression of the disease.

Many researchers have commented on the increase in nerve fibre visibility in keratoconic corneas. As no satisfactory explanations have been offered in the literature, the possibility of any neurohyperplasia of epithelial and stromal nerves in keratoconus was studied by light- and electron microscopy. Morphologic as well as quantitative measurements of stromal and epithelial innervation was undertaken for this purpose.

The experimental design was not set out to investigate corneal reinnervation. However, a specimen unexpectedly became available which allowed long term reinnervation of a keratoconic cornea to be studied. The result from this helped the interpretation of the histological findings in keratoconus.

CHAPTER 2

MATERIAL

2.1 CORNEAL SENSITIVITY

Fifty-one caucasians suffering from keratoconus were examined. One was eliminated from the study due to long term contact lens wear. All had been diagnosed by an experienced ophthalmologist to suffer from keratoconus, but were otherwise in good general and mental health. All were male aged between 15 and 39 (mean age 24.4, SD ±6.0). The group of eyes examined consisted of 26 without contact lens wear (mean age 24.1±5.6) and 24 with contact lens wear (mean age 24.6±6.8). The type of contact lenses used were PMMA (54%), gas-permeable rigid (44%) and scleral lenses (2%). Not surprisingly, hydrogel contact lenses were not among the types of lenses used. The wearing time was 10.6±4.4 hours (range 1-16 hrs) and the length of wear was 16.1±16.0 months (range 1-60 months). The scleral lens was only worn for 1 hour every other day. Most patients removed their contact lenses for more than 48 hours prior to the experiment (mean 62.7±35.0).

The control group consisted of twenty-one caucasians who were of similar age range 18-34 (mean 24.4±5.0). One

patient (two eyes) who had corneal epithelial pathology and another who had corneal scarring in one eye were excluded from the study. The remaining thirty-nine eyes were free from any ocular abnormality. Thirty-two eyes did not wear a contact lens, 6 eyes wore daily-wear hydrogel lenses (2-36 months) and one eye wore a gas-permeable rigid lens (less than 2 months). None of the contact lens wearers had worn their lenses for more than 3 years. Most were in the adaptation period and removed the lenses for a continuous period of at least 48 hours (mean 74.3±29.1) prior to the experiment. (Further details are given in Appendix 2).

2.2 CORNEAL INNERVATION

Eleven keratoconic and thirteen normal corneas were examined. All material was human and only one cornea was obtained per human.

The keratoconic material was obtained from eleven patients undergoing routine penetrating keratoplasty. This limited the material to the central 7 or 8 mm corneal disc without peripheral or limbal tissue. All corneas were removed for restoration of vision, and accordingly the tissues used represent various late stages of the disease. The patients were mainly male and their age ranged from 17 to 37 years.

On five keratoconic patients (K1,K3,K4,K5,K6) topographical corneal aesthesiometry was performed prior to penetrating keratoplasty and subsequent electron microscopic examination. Four corneas (K2,K7,KP1,KP2) were used for whole-mount staining of corneal nerves.

One keratoconic cornea (K11) was used to study reinnervation. Two circular trephines had been used 10 years prior to penetrating keratoplasty producing non-penetrating lesions of 5 and 10 mm diameter and desensitising the central cone. A separate keratoconic cornea (K12) without previous surgery but with identical fixation methods was used as a control for this part of the study.

Thirteen control corneas were obtained from eyes which were removed mainly due to malignant melanomas. Most material consisted of half corneas or segments thereof, whole corneas rarely being available. The patients were of either sex and their age ranged from 1 to 59 years. Seven of the control corneas were fixed in formal saline and six were fixed in glutaraldehyde. (Further details are given in Appendix 1).

The operations were carried out by professor M Ruben, Mr R J Buckley, Mr J A Dart at Moorfields Eye Hospital, Dr J U Prause at the Institute for Eye Pathology, Copenhagen University, Mr B Lee at the Glasgow Eye Infirmary and surgeons at Columbus Ohio.

2.3 CORNEAL SENSITIVITY

2.3.1 Experimental Routine.

Patients attending for routine contact lens fitting in the Keratoconus Clinic at the Contact Lens Department, Moorfields Eye Hospital, were asked to participate in the study. These patients, who were referred either from external General Practitioners and hospital departments or from other clinics at Moorfields Eye Hospital, represent different stages of the disease. Patients were approached at random and asked to volunteer if they fulfilled the following criteria:

1. Male.
2. Caucasian.
3. Under 40 years of age.
4. A maximum of 4 years contact lens wear.
5. Willing to attend for measurements not having worn contact lenses for 48 hours.
6. Good general and mental health.
7. Free from ocular pathology other than keratoconus.

The experimental routine was as follows:

1. General and ocular history of patients.
2. Biomicroscopy examination.
3. Central keratometry.
4. Central pachometry.
5. Topographical corneal aesthesiometry.

6. Lid-margin aesthesiometry.

7. Biomicroscopy examination.

The keratoconus patients were divided into a contact lens wearing (KC) and a non-contact lens wearing group (KS). The control group (C) was similarly divided into contact lens wearing (CC) and non-contact lens wearing groups (CS).

General and Ocular History of Patients.

Routine questions on general health were made for symptoms relating to: systemic disorders, mental health, headaches and allergies. A record was made of the onset of ocular symptoms, any active treatment, first visual correction and type, first diagnosis of keratoconus and maternal age.

Biomicroscopy Examination.

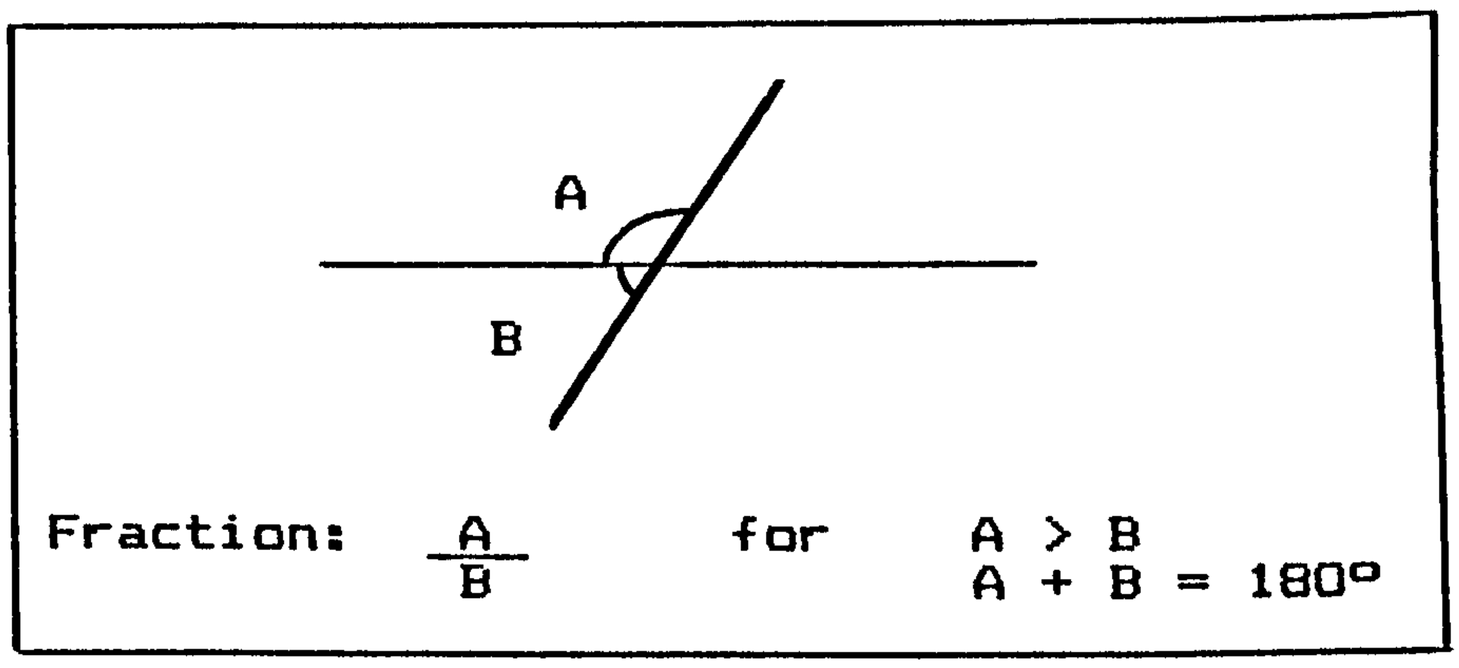
Using a Haag-Streit biomicroscope, general examination of the ocular adnexa and cornea was performed with special attention to: corneal scarring, corneal striae, nerve fibre visibility and Fleischer's ring.

2.3.2 Central Keratometry.

Corneal curvature was measured along the visual axis using an American Optical one position variable doubling keratometer. The measurement range of this instrument was 5.60 to 9.60 mm and had to be extended by using an auxiliary lens as previously described by Mandell (1981). It was

recalibrated against steel balls of known radii (Appendix 6). The appearance of the mire image was graded according to irregularity from clear to very distorted (1 to 5) as seen in Appendix 3. Furthermore, the misalignment of the principle meridians was quantified by calculating the fraction of the largest angle divided by the smallest angle between principle meridians (Fig 2.1).

Figure 2.1 Angles Between Principle Meridians.



Quantitative measure for misalignment of principle meridians.

2.3.3 Central Pachometry.

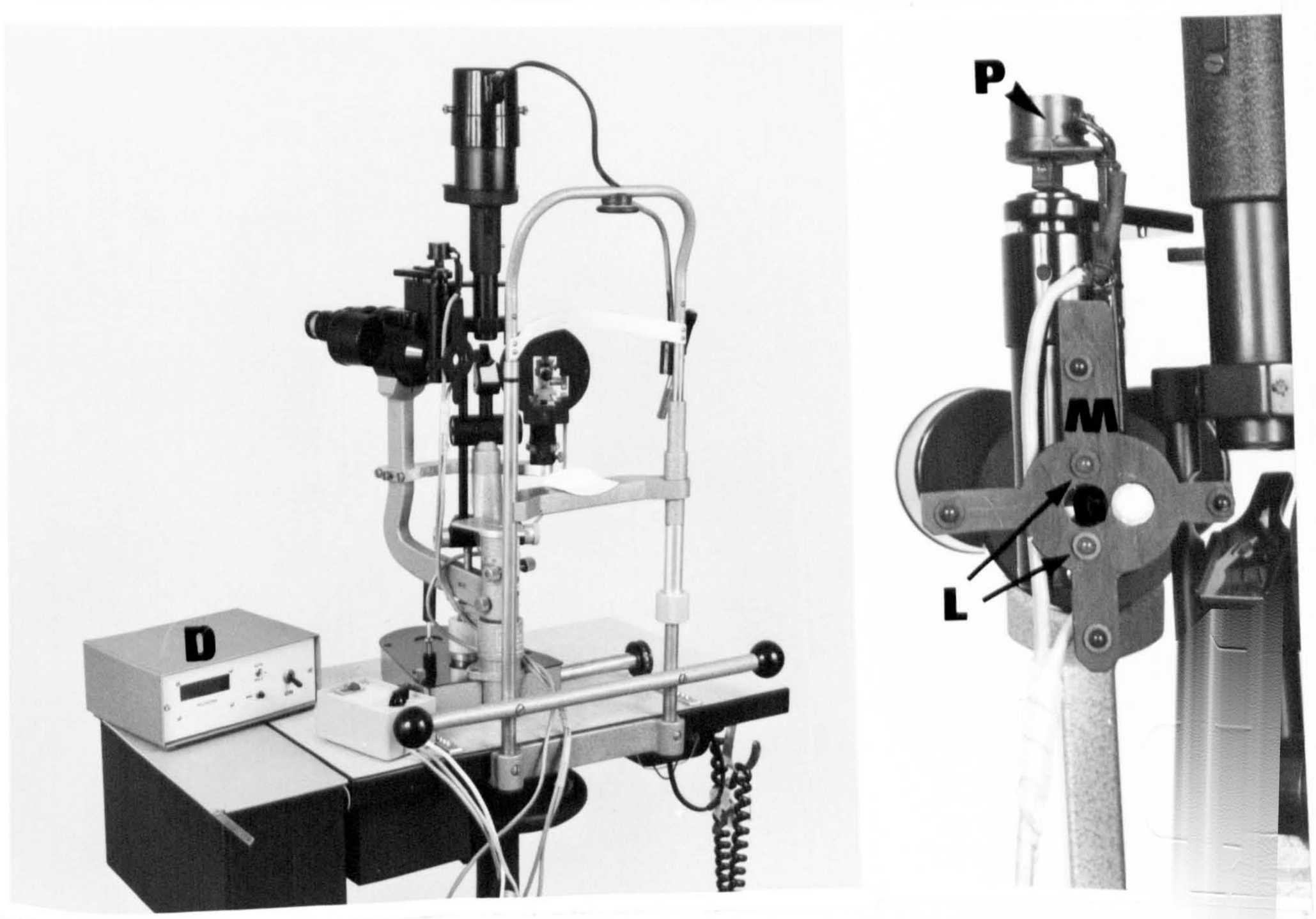
Corneal thickness was recorded using a Haag-Streit pachometer mounted on a Haag-Streit biomicroscope. For the standard method, the patient fixates the slit-beam from the slitlamp, and the investigator observes the corneal section through two glass plates in the pachometer. The latter is mounted in front of the observation system of the slitlamp and a beamsplitter situated in the eyepiece of the microscope divides the corneal section into an upper and a lower half. The lower glass plate is fixed and by turning the top of the pachometer, the upper glass plate is rotated and the upper corneal section displaced accordingly.

The posterior surface of the upper corneal section is aligned with the anterior surface of the lower corneal section thus displacing the the upper image by the precise width of the corneal section. This displacement is the apparent corneal thickness which is converted into real thickness by a scale on the pachometer.

For this study, the pachometer was modified by attaching a linear potentiometer to the vertical axis of the rotating glass plate (Fig 2.2).

Figure 2.2 Electronic Pachometer

Two LED's (L) mounted on a black perspex plate (M) were situated above, below and immediately in front of the glass plates (G). The rotating cylinder of the top glass plate was extended to accomodate the potentiometer (P) which was connected to a separate low voltage power supply and a digital read-out (D).



A low voltage direct current was passed through the potentiometer and connected to a digital output (Woodward 1980). On rotation of the glass plate during measurement, the current passing through the potentiometer would be altered. The potentiometer digital read-out was calibrated against PMMA contact lenses of known refractive index (1.490), three different uniform thicknesses and three different front optic radii.

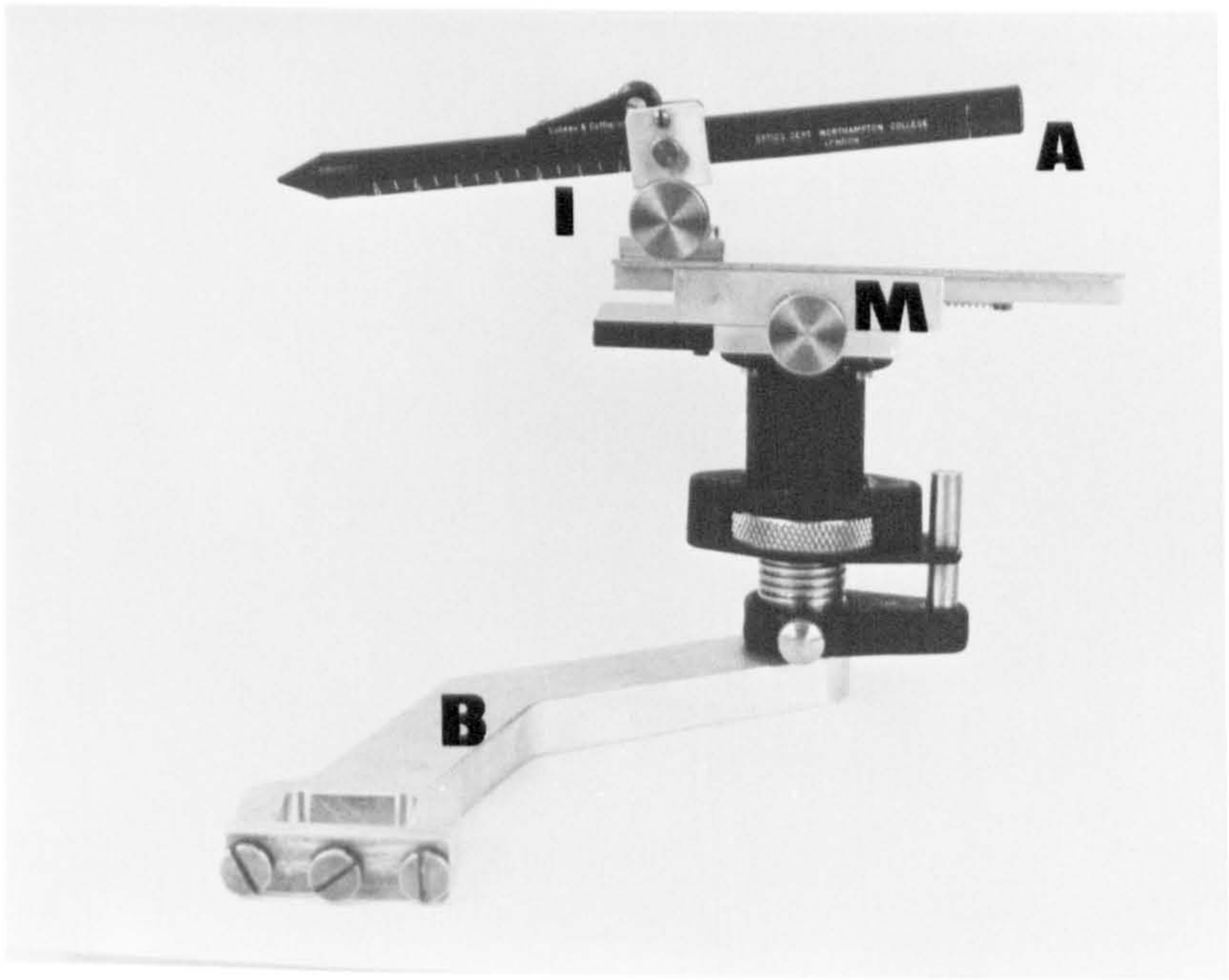
With one eye occluded, the patient observed the midpoint between two small light emitting diodes (LED). These were situated immediately in front and just above and below the pachometer glass plates, hence ensuring that the pachometer was perpendicular to the corneal surface (Donaldson 1966). Twelve readings were taken, the highest and lowest reading discarded and the mean and standard deviation calculated from the remaining ten. The mean was converted into real corneal thickness using the calibration used for the PMMA contact lenses taking into account the difference in refractive index from PMMA (1.490) to cornea (1.376) as indicated in Appendix 7.

2.3.4 Topographical Corneal Aesthesiometry.

A standard Cochet-Bonnet aesthesiometer with a 0.08 mm filament was used to provide a pressure range from 2 to 90 mg/mm^2. An attachment was designed to mount the instrument

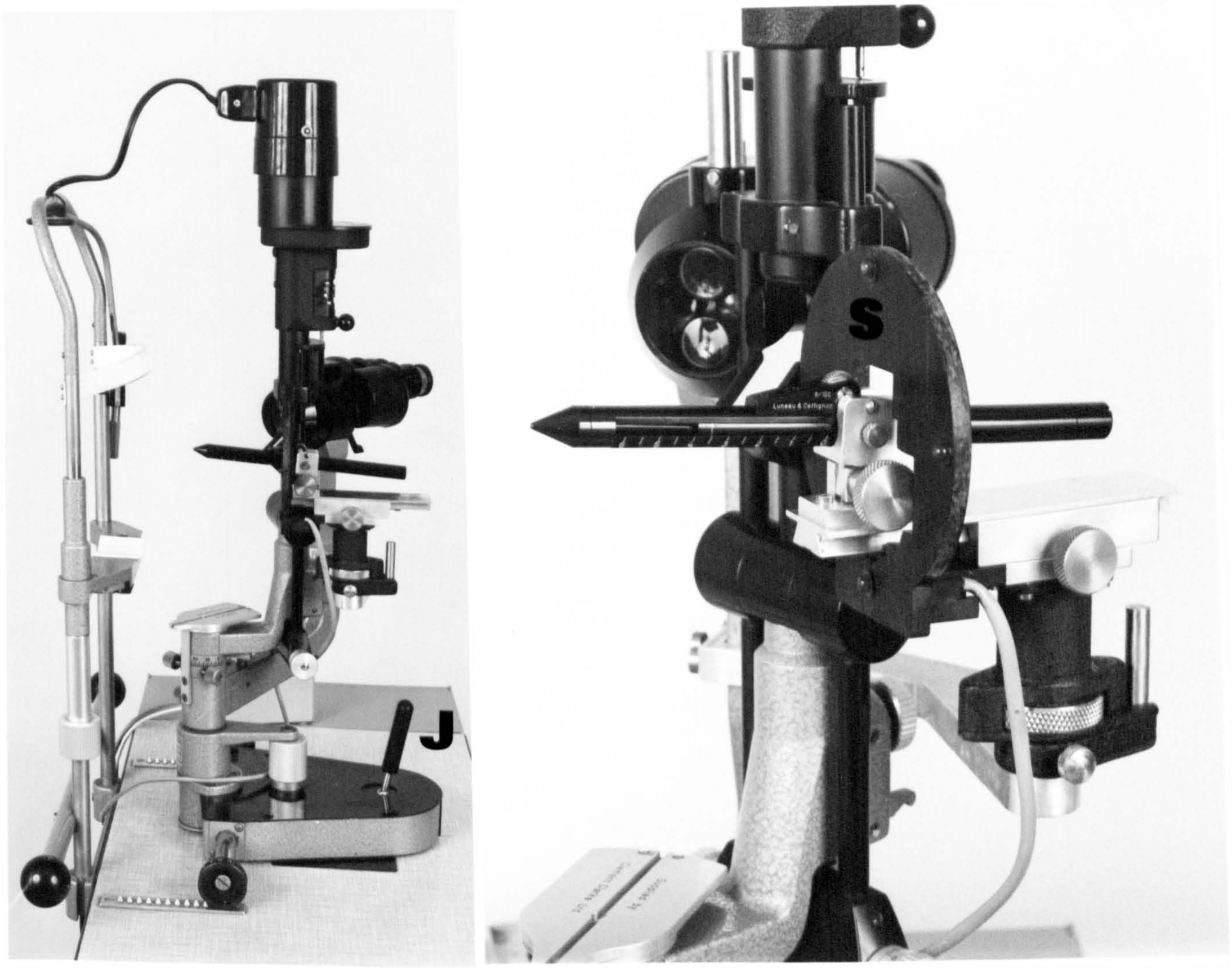
on a Haag-Streit biomicroscope (Figs 2.3 & 2.4).

Figure 2.3 Aesthesiometer Attachment



The Cochet-Bonnet aesthesiometer (A) was mounted on an attachment (M) which allowed movements in the X,Y,Z directions. The inclination of the aesthesiometer was also adjustable (I). The attachment was mounted to the support of the slit lamp observation syatem by a metal bar (B).

Figure 2.4 Aesthesiometer Attachment



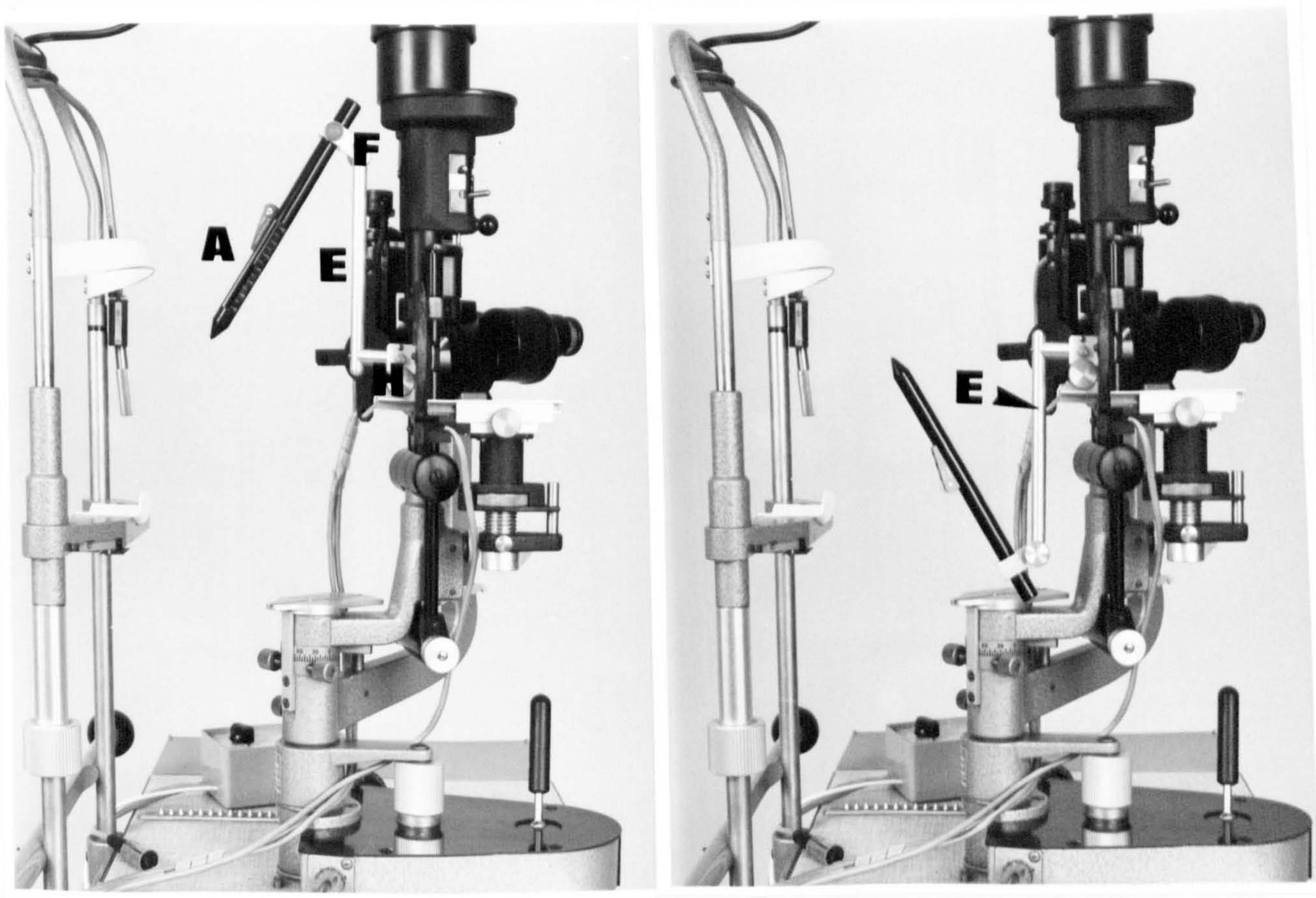
A small vertical black screen (S) partially obscured the aesthesiometer from the patient's view. The joy stick (J) of the slit lamp facilitated a smooth approach of the aesthesiometer to the cornea while the tip of the filament was observed through the eyepiece of the microscope.

The angle and speed of approach to the cornea were controlled by viewing the filament through the biomicroscope and using the joy stick. The measurement was carried out as recommended by Bonnet & Millodot (1966) starting with the filament extended to full length and stimulating the cornea six times. If the patient did not respond to the stimulus by tapping the table, the filament length was shortened by 0.5 cm and the cornea stimulated a further six times. The corneal touch threshold (CTT) was recorded as the filament length at which the patient would respond to 50% of the stimuli. For each run of six corneal stimuli, one dummy stimulus was added to check patient reliability ie the filament was moved towards the cornea without making contact. All patients were trained in the procedure before testing commenced. The whole experiment was then repeated for one central and four peripheral points approximately 1-1.5 mm in from the limbus at the three, six, nine and twelve o'clock positions. When appropriate the fellow eye was also measured.

Eyelid-margin sensitivity was recorded for the mid-point position on the upper and lower lid. The aesthesiometer mounting was changed to enable the filament to make contact normally to the lid-margin (Figs 2.5). Normal blinking action of the lid made it necessary to prevent the filament being pushed on to the cornea. A thin (0.10 mm) high water content plano therapeutic lens (Duragel 75%) with a back

optic radius of 8.70 mm and an overall size of 15.00 mm was placed on the cornea. The diameter was large to ensure that the contact lens edge was situated behind the upper and lower lid at the position where the touch threshold was measured. The lens was allowed to settle for a minimum of 15 minutes or until the patient was unaware of the lens. On normal or induced blink from the stimulus, the lens would protect the cornea. Some patients with deep set eyes or poor adaptation to the therapeutic lens had to be eliminated from this part of the study.

Figure 2.5 Aesthesiometer Attachment



An extension (E) was inserted into the aesthesiometer holder (H) and the aesthesiometer (A) moved to the adjustable end (F) of the metal extension. This made the angle of the filament approximately normal to the lower lid margin. By turning the extension (E) in the aesthesiometer holder (H) the upper lid margin could be approached.

The ambient humidity and temperature were recorded at the time of each session, and were 58.3% (SD ±8.0) and 21.7° C (SD ±1.8°) respectively. The cornea was examined with fluorescein for punctate staining at the end of all tests.

2.4 TISSUE FIXATION & PREPARATION FOR HISTOLOGY

The material was fixed by two different methods. Nine keratoconic and six control corneas were fixed within 20 minutes of removal from the eye in a 5% glutaraldehyde solution buffered with sodium cacodylate or phosphate to pH 7.4 and stored at 4° C. Two corneas had the 12 o'clock position marked before removal from the eye. The second method involved fixation in 10% formal saline and was used for seven control corneas. In addition, two keratoconic corneas were treated differently and will be described later under reinnervation.

Radial segments were prepared, washed in buffered sucrose overnight, postfixed in 1% osmium tetroxide for approximately 1 hour and dehydrated in changes of 50%, 70%, 90% and absolute alcohol for 1.5 to 2 hours. The segments were cleared in xylene for 20 minutes, transferred to a 1 to 1 mixture of xylene and Araldite for a further period of 20 minutes and agitated in Araldite overnight. The tissues were embedded in Araldite-filled gelatine capsules and incubated

at 60° C for 48 hours. The gelatine capsules were briefly removed from the oven after approximately 6 hours in order to orientate tissues in a desired position.

Sections of approximately 0.5 to 1 microns thickness were cut with glass knives on a Reichart-Jung Ultracut E microtome, transferred to glass slides then heated to remove wrinkles and dried at 60° C. The Araldite was removed by a method described by Mayor et al (1961) and the sections stained for 30 to 90 seconds in a mixture of equal parts of 2.5% sodium carbonate and 1% toluidine blue (Trump et al, 1961). They were rinsed, dried and mounted with DPX and a cover-slip before being examined by light microscopy.

Thin sections of 60 nm thickness were cut using glass or diamond knives and mounted on 200 mesh copper grids. Staining was undertaken in a saturated solution of uranyl acetate in a 1 to 1 mixture of distilled water and absolute alcohol for 15 to 20 minutes, rinsed in three changes of distilled water. Then immersed in 0.4% lead citrate (0.1N sodium hydroxide and carbonate free) for another 15 to 20 minutes, rinsed in three changes of distilled water, blotted and stored dry. The sections were examined using a Jeol Jem 100 B electron microscope.

All sections for light and electron microscopy were cut parallel to the limbal plane at two sample regions. These

were approximately 3.5 mm and 1 mm from the centre of the cornea and represent the mid-peripheral and central corneal areas respectively.

2.5 CORNEAL REINNERVATION

One central 8 mm corneal disc (K11) was obtained from a 29 year old male keratoconic patient undergoing corneal grafting. Ten years previously two circular non-penetrating corneal incisions had been performed 5 and 10 mm in diameter in order to desensitise the cornea.

On removal, the cornea was stored in isotonic Ringer solution for 30 min, transferred to 10% formal saline for 17 hours before being cut into radial segments and postfixed in 5% glutaraldehyde. The tissue was prepared and embedded as described above and stained for light and electron microscopy in the same manner.

Radial sections were cut for light microscopy to evaluate the depth of the circular incision. LM and EM sections were cut parallel to the limbal plane at three positions; close to the periphery, just peripheral to the incision and just central to the incision.

A separate keratoconic cornea (K12) of the same age and

without previous surgery was treated in the same manner and was used as control.

Qualitative and quantitative assessment of the reinnervation was undertaken at EM level.

2.6 WHOLE-MOUNT STAINING

Acetylcholinesterase (AChE) has been used by Laties & Jacobowitz (1964) and Robertson & Winkelmann (1970) to demonstrate corneal nerves in mammals. Using this whole-mount technique, several attempts were made on keratoconic material, and stromal and epithelial nerves were demonstrated. The technique, however, proved to give very variable results and was subsequently abandoned.

CHAPTER 3

RESULTS

3.1 CORNEAL SENSITIVITY

3.1.1 Biometric Data and Control of Variables.

The keratoconic group (K) was divided into a contact lens (KC) and a non-contact lens (KS) wearing group. Similarly the control group (C) was divided into a contact lens (CC) and a non-contact lens (CS) wearing group.

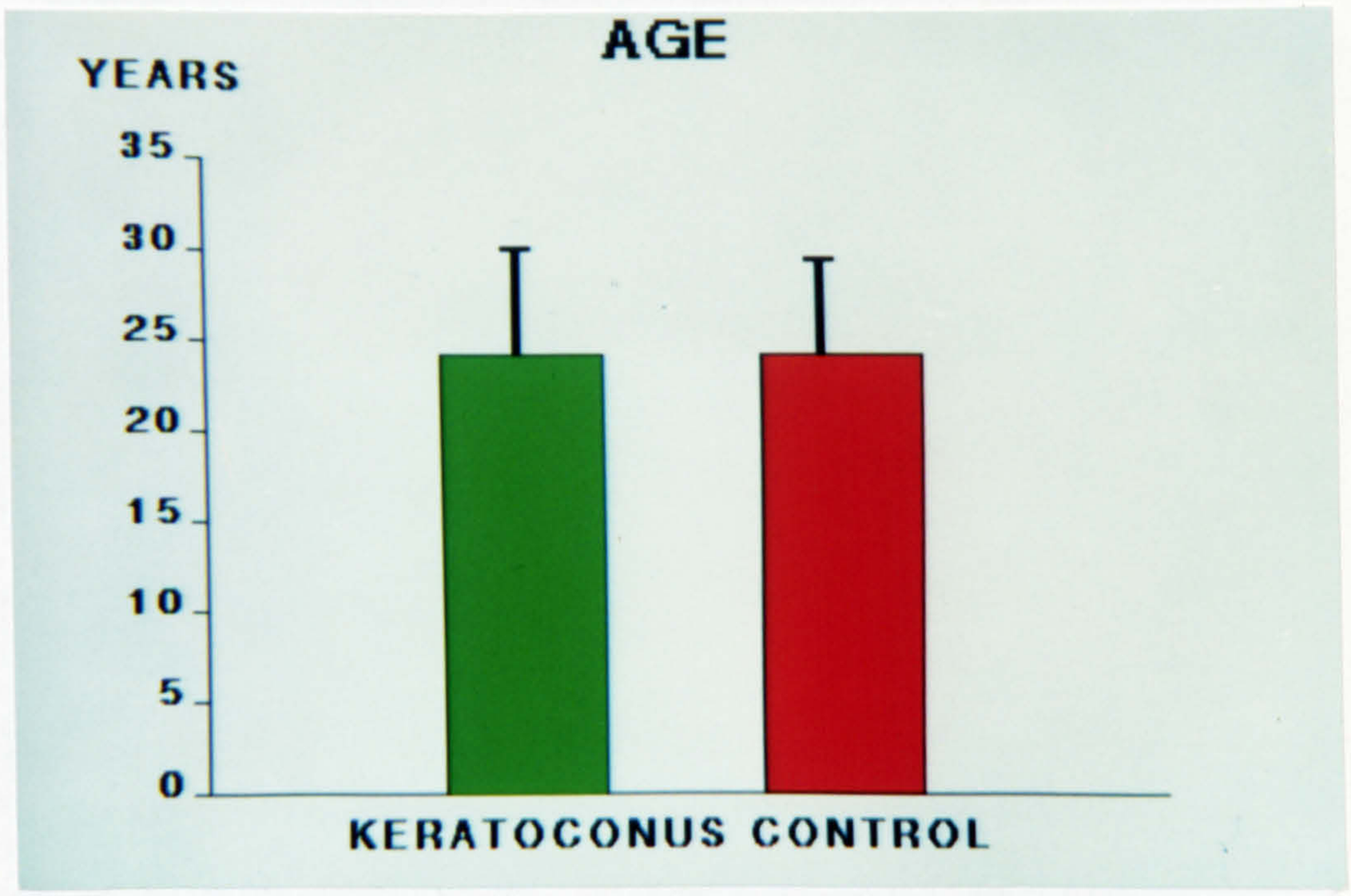
Age, Diagnostic Age & Maternal Age.

The keratoconic and control groups were matched for age (Fig. 3.1) with an overall mean of 24.4 years (one-way ANOVA P=0.95). The onset of keratoconus was taken to be the time when the patient was first referred and diagnosed, or when the history clearly revealed a significant reduction in visual acuity which could not be improved with a spectacle correction. The patient age at the time of diagnosis was 20.9 years (SD ±6.7) ranging from 15 to 38 years. This is in good agreement with that found by Millodot & Owens (1983) of 20.2 years (±7.1). The maternal age of 27.3 years (±5.5) in the whole keratoconic group was found to be lower than that of the control group 30.7 (±6.5) (Fig. 3.2).

Allergies.

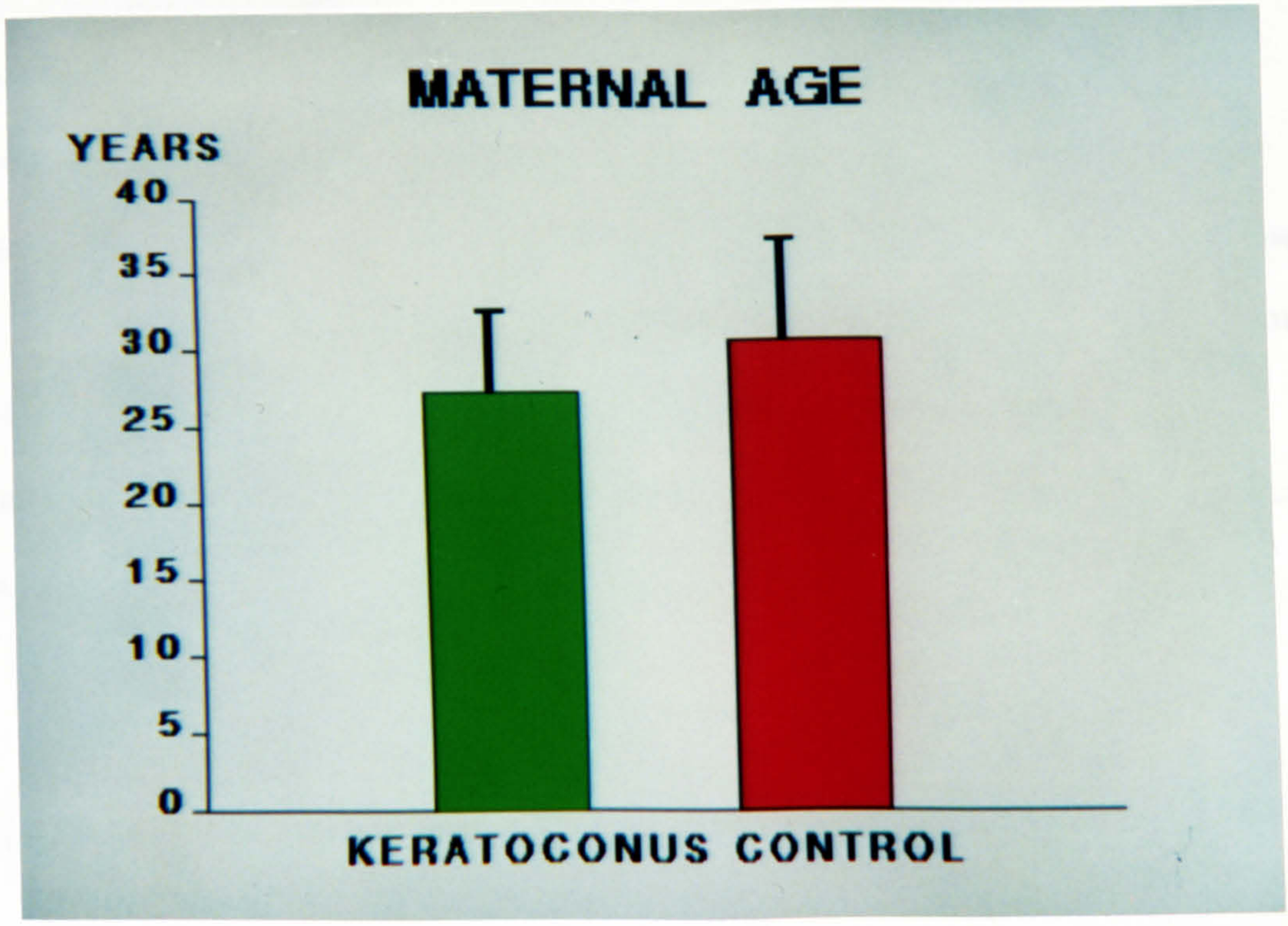
A large number of patients reported allergic reactions such as asthma, eczema and hayfever (Fig 3.3). The incidence of hayfever was more marked than asthma and eczema, however, a Wilcoxon rank-sum test for two groups revealed no difference between the keratoconic and control groups for any of the three conditions (asthma P=0.28; eczema P=0.50; hayfever P=0.08).

Figure 3.1



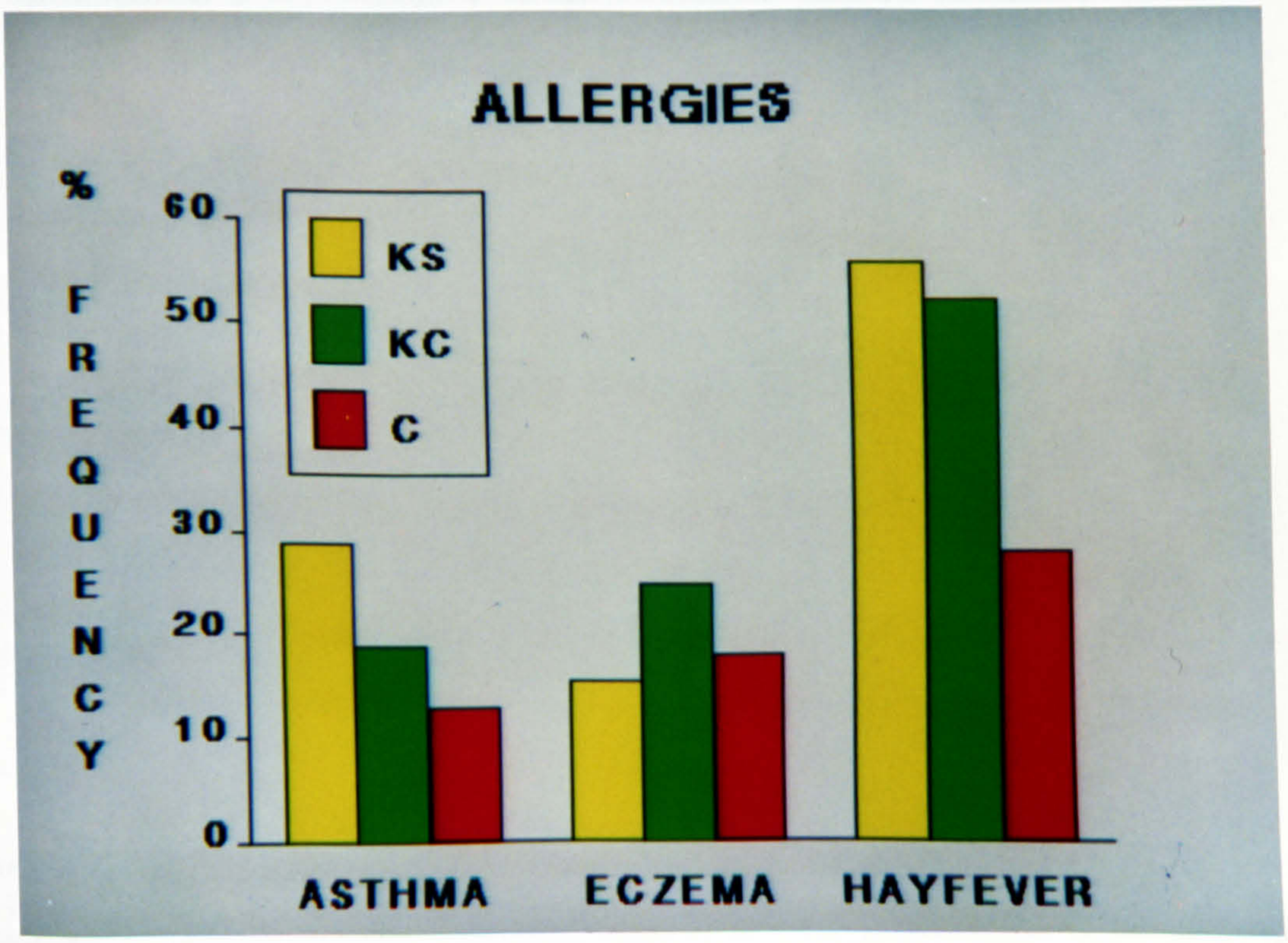
Mean age for keratoconic (n=50) and control (n=20) groups.

Figure 3.2



Mean maternal age for keratoconic (n=50) and control (n=19) groups. One control patient was adopted without knowledge of maternal age.

Figure 3.3

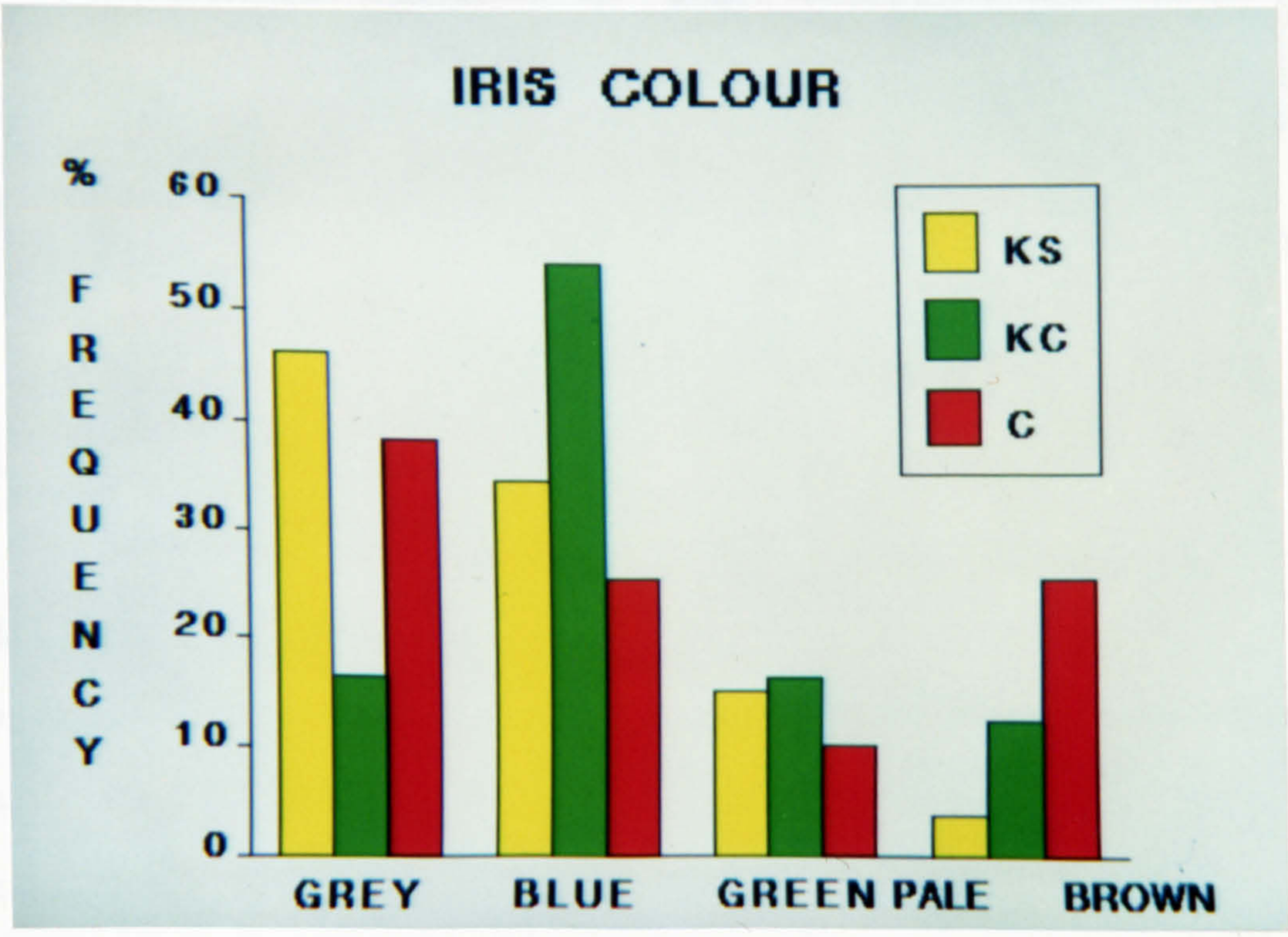


Frequency of asthma, eczema and hayfever for keratoconic non-contact lens wearing (KS: n=26), keratoconic contact lens wearing (KC: n=24) and control (C: n=20) groups.

Iris Pigmentation.

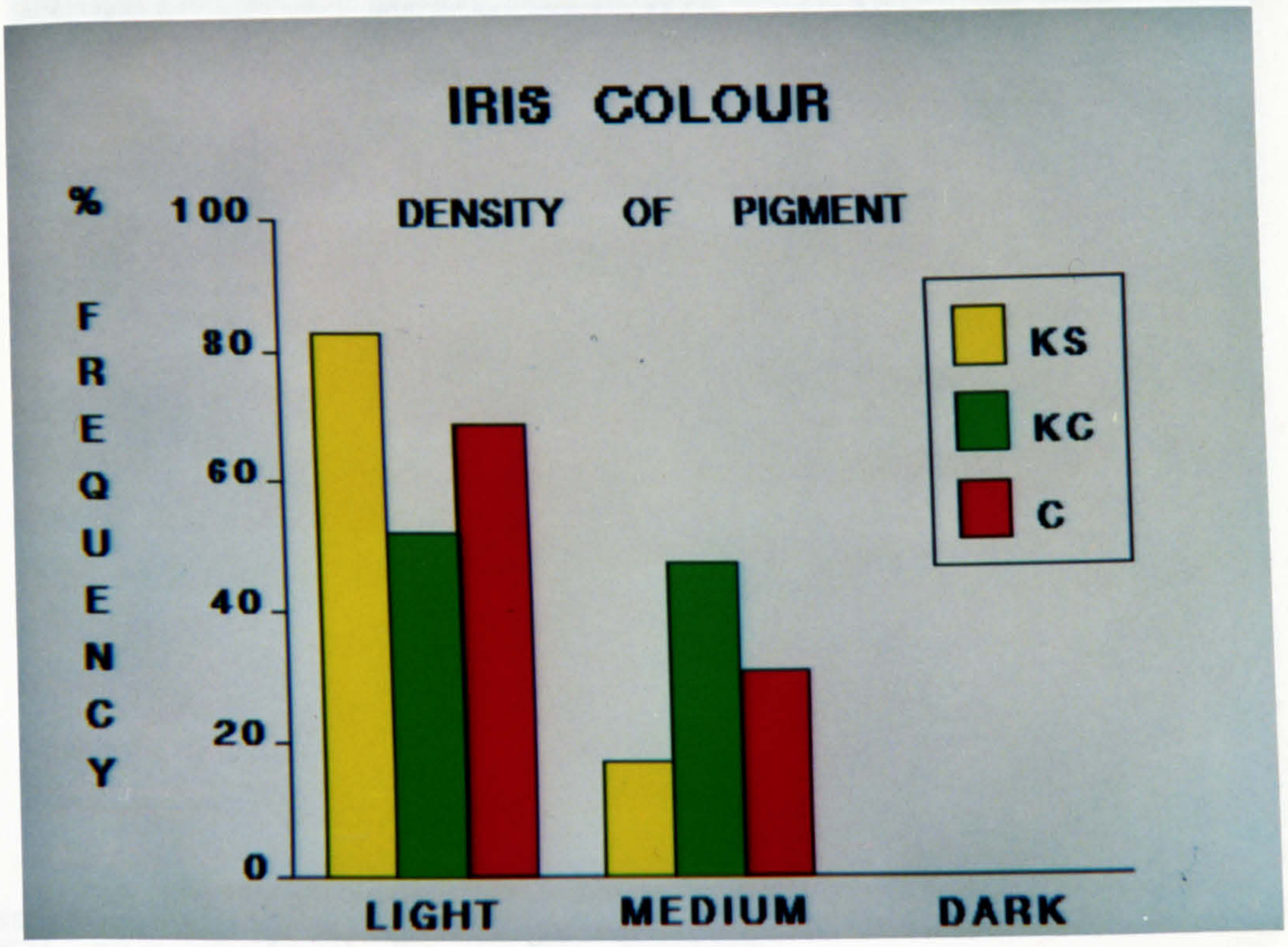
Iris colour (Fig. 3.4) and density (Fig. 3.5) of pigmentation was graded for all eyes (Appendix 2). All patients being caucasians, dark brown pigmented eyes were not encountered and a Wilcoxon ranked sum analysis showed no difference in the colour (P=0.28) and density of pigmentation (P=0.46) between the keratoconic and control groups.

Figure 3.4



Frequency of grey, blue, green and pale brown eyes for keratoconic (KS: n=52, KC: n=48) and control (C: n=39) groups.

Figure 3.5



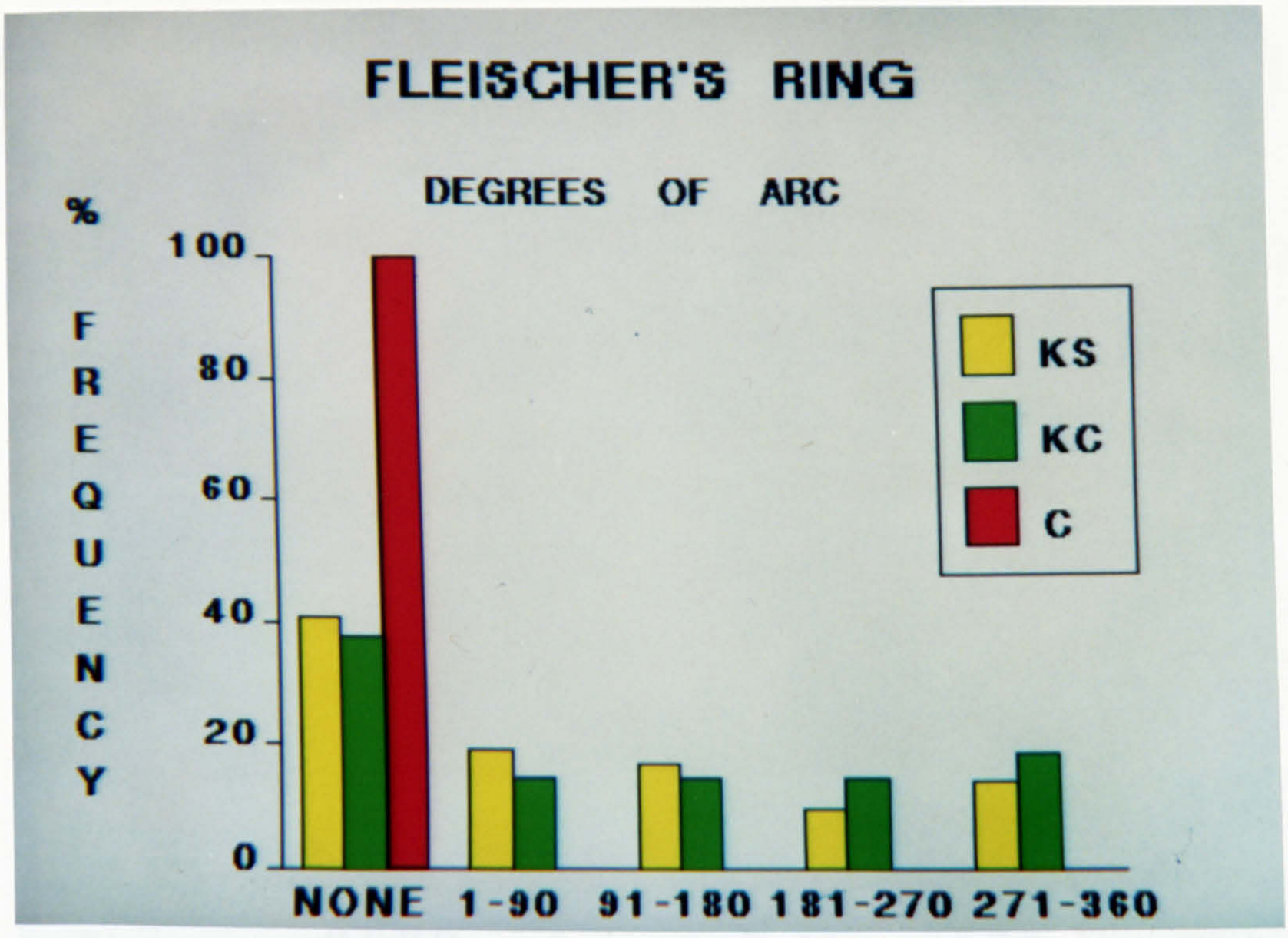
Frequency of light, medium and densely pigmented eyes for keratoconic (KS: n=52, KC: n=48) and control (C: n=39) groups.

3.1.2 Clinical Data.

Fleischer's Ring.

The presence of a Fleischer's ring was noted, recorded and ranked in 90 degrees of arc intervals (Fig. 3.6) to facilitate statistical analysis. A one-way ANOVA Kruskal-Wallis test for three groups indicated a highly significant difference between the groups ($P<0.001$). Multiple comparison revealed no difference between the keratoconic groups (with and without contact lenses) but as expected a significant difference to the control ($P<0.001$).

Figure 3.6

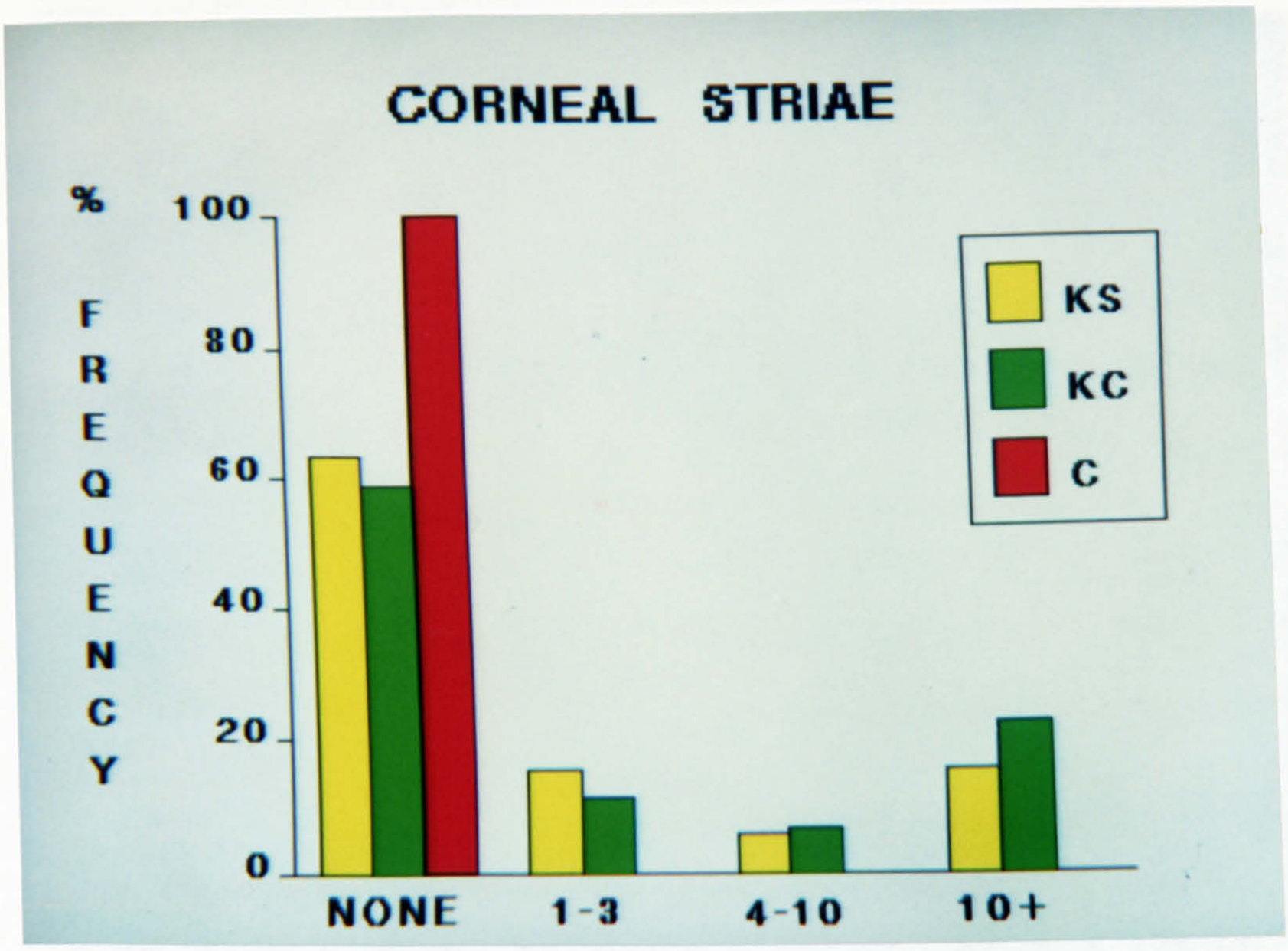


Frequency of Fleischer's ring for keratoconic (KS: n=48, KC: n=42) and control C: n=39) groups.

Corneal Striae & Scarring.

Striae in the deep corneal stroma are a common clinical sign in keratoconus. They were graded according to the number of lines seen (Fig. 3.7) and none were observed in the control corneas (Kruskal-Wallis: $P<0.001$). A multiple comparison showed no statistical difference between the two keratoconic groups ($P>0.50$).

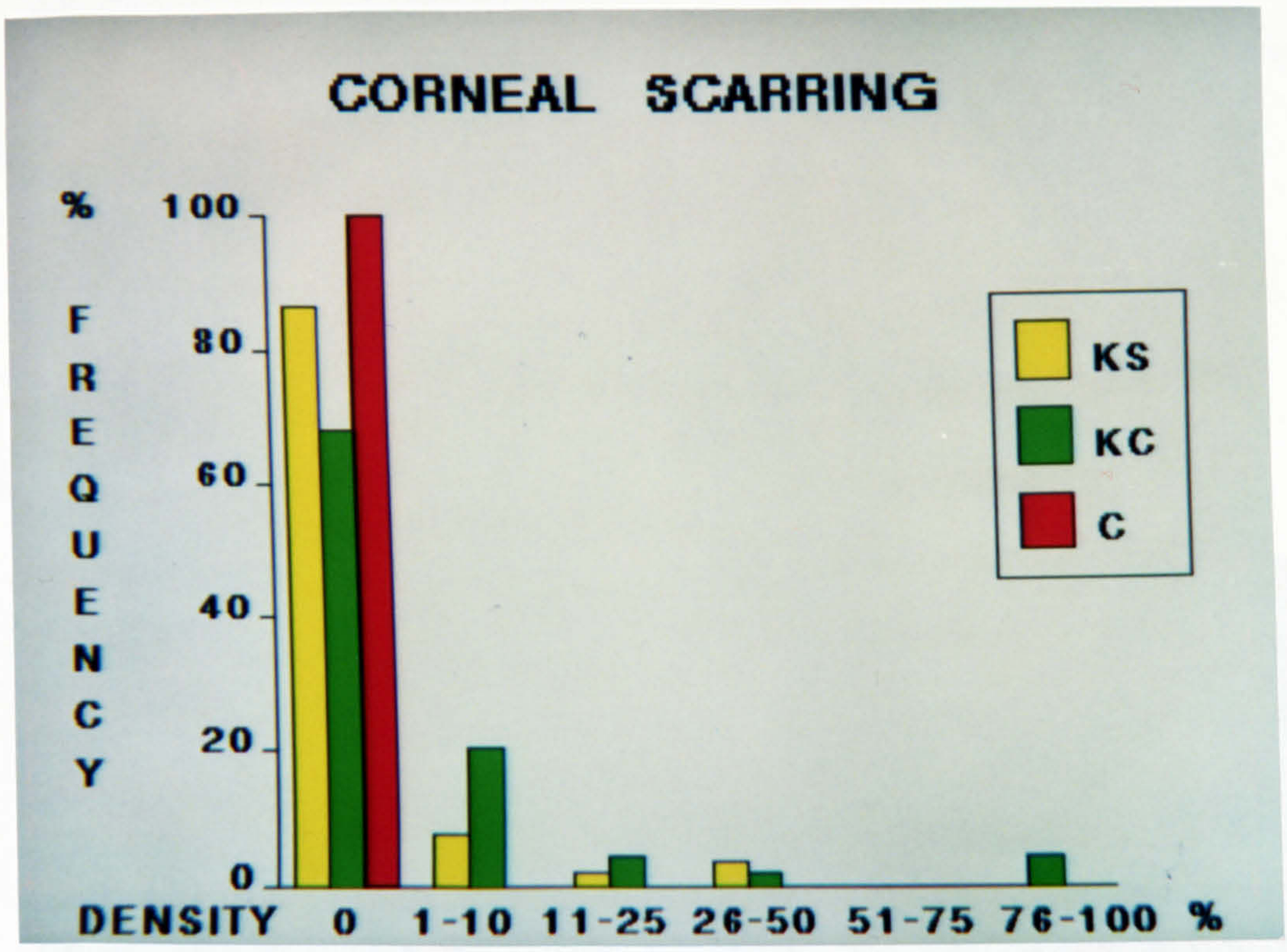
Figure 3.7



Frequency of corneal striae for keratoconic (KS: n=52, KC: n=44) and control (C: n=39) groups. Grade 0: no striae, grade 1: 1-3 striae, grade 2: 4-10 and grade 4: more than 10.

Corneal scarring was absent in the control group and never exceeded a central corneal area of 3 mm in the keratoconic corneas. The scarring was graded relative to corneal stromal thickness; 0% indicating no scarring and 76-100% indicating scarring being observed up to full stromal thickness. Two keratoconic corneas showed scarring involving more that 75% of the corneal thickness (Fig. 3.8).

Figure 3.8



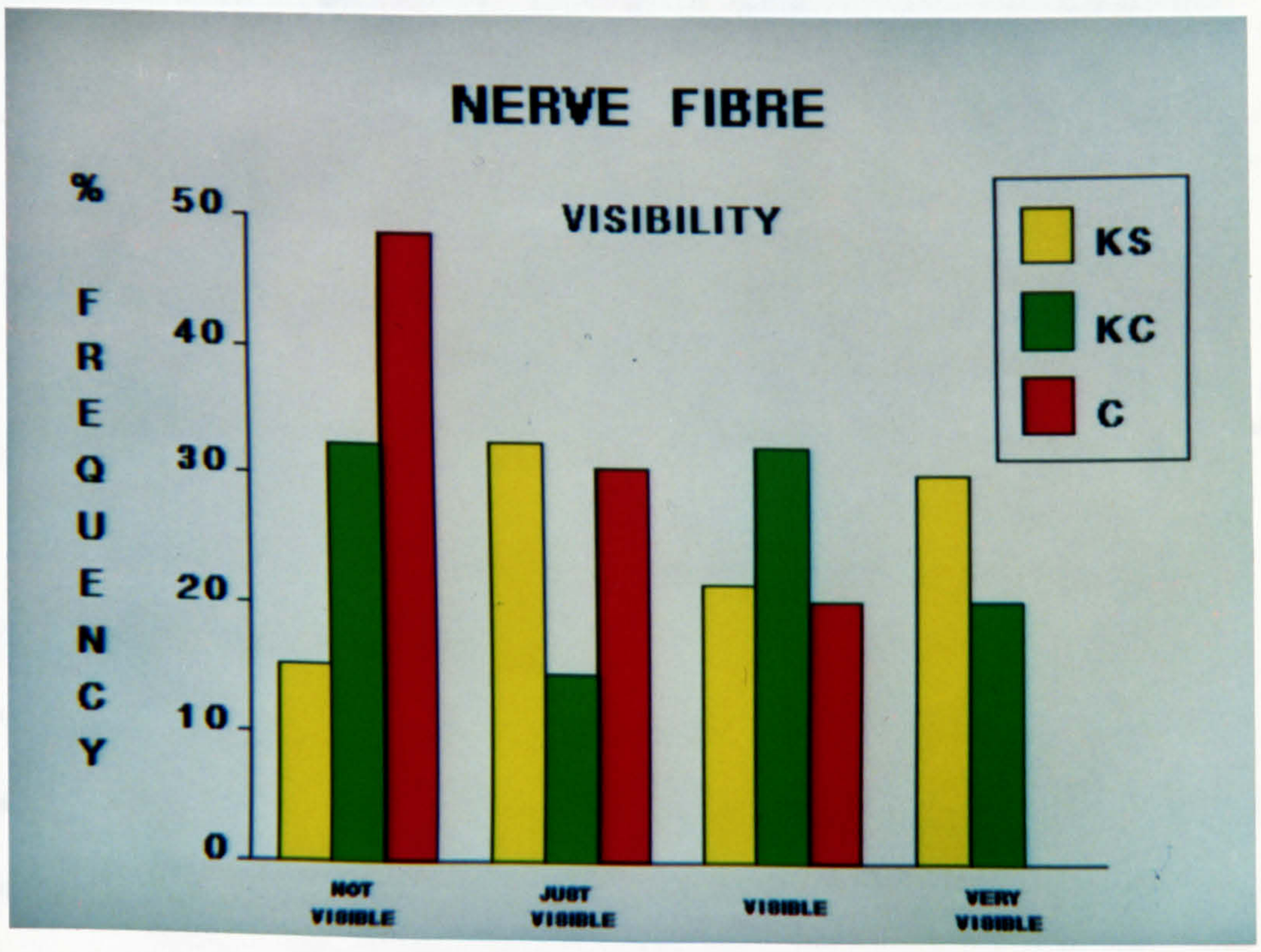
Frequency of corneal scarring for keratoconic (KS: n=52, KC: n=44) and control (C: n=39) groups. Density on the abcissa represents the % corneal thickness where scarring is present.

Nerve Fibre Visibility.

Nerve fibre visibility was recorded with regard to clarity (Fig. 3.9) and corneal location (Fig. 3.10). The Kruskal-Wallis ranked test was highly significant for both observations. Multiple comparison tests, however, showed only the keratoconic group without contact lens wear to be significantly different with regard to nerve clarity ($P<0.005$). Nerves were observed more frequently in the mid-peripheral and central corneal zones for both the keratoconic contact lens wearing group ($P<0.05$) and the

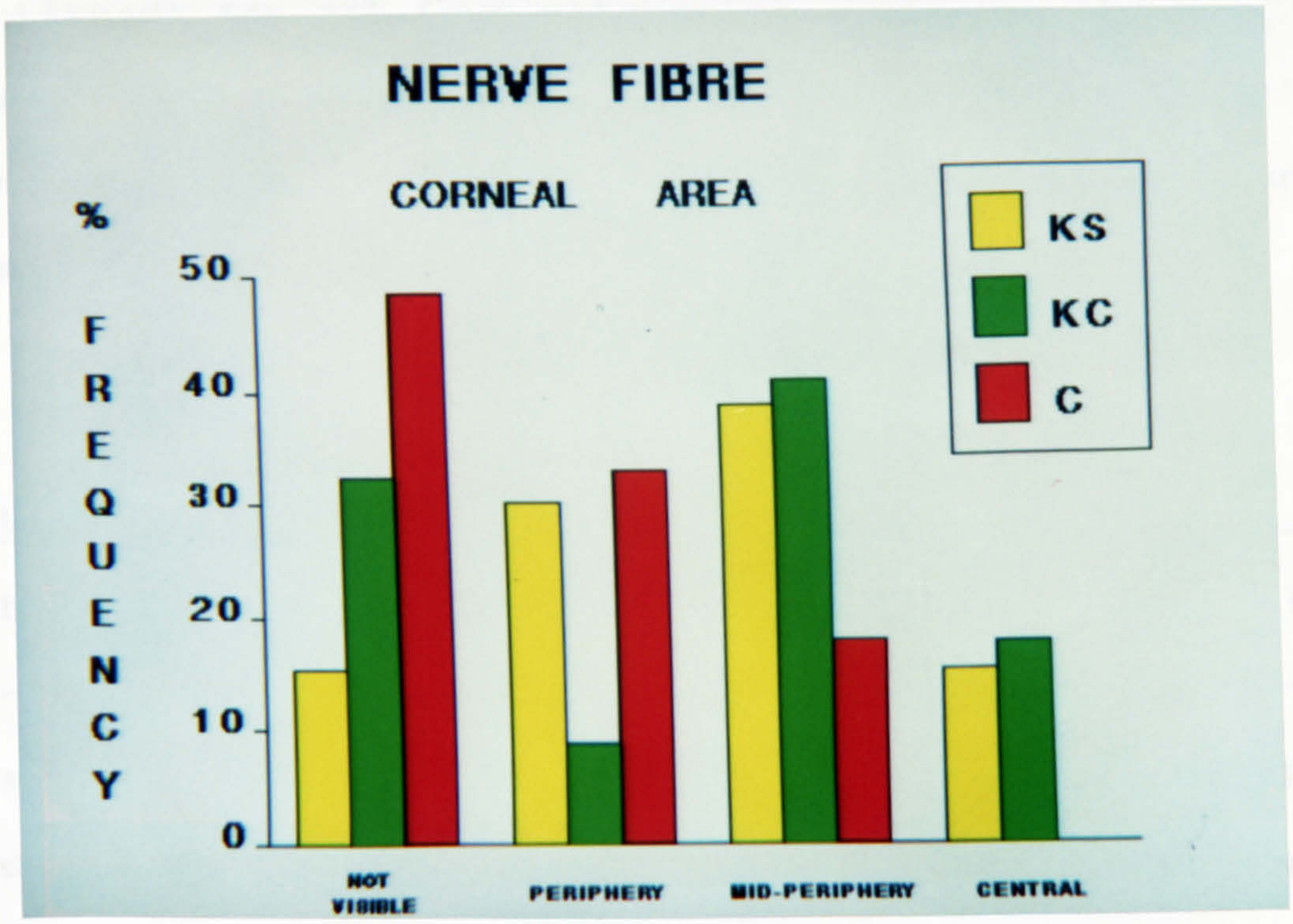
non-contact lens wearing group (P<0.005). The contact lens wearing group had slightly less visible corneal nerves that the non-contact lens wearing group although this was not of statistical significance.

Figure 3.9



Frequency of corneal nerve fibre visibility for keratoconic (KS: n=46, KC: n=34) and control (C: n=39) groups.

Figure 3.10



Frequency of corneal nerve fibre visibility with reference to corneal position. Keratoconic (KS: n=46, KC:n=34) and control (C: n=39) groups.

Central Keratometry.

In keratoconus, the range of mean corneal curvature (K-mean = ($K_{FLATTEST}$ + $K_{STEEPEST}$) /2) was large and skewed towards the steeper curves (Fig 3.11) resulting in a markedly steeper group mean (Fig 3.12). The curvature difference (K-diff) between principle meridians ($K_{FLATTEST}$ - $K_{STEEPEST}$) showed higher corneal toricity (Fig 3.13) as well as corneal distortion (K-dist) (Fig 3.14) for keratoconus. The principle meridians in keratoconic corneas were significantly misaligned (K-angle) compared to the control group (Fig 3.15).

The mean values for K-mean, K-diff, K-dist and K-angle are

given in Table 3.1, and the differences are highly significant for all four categories. However, the two keratoconic groups (without and with contact lens wear) were found to be similar on multiple comparison for all four categories.

Table 3.1 Central Keratometry

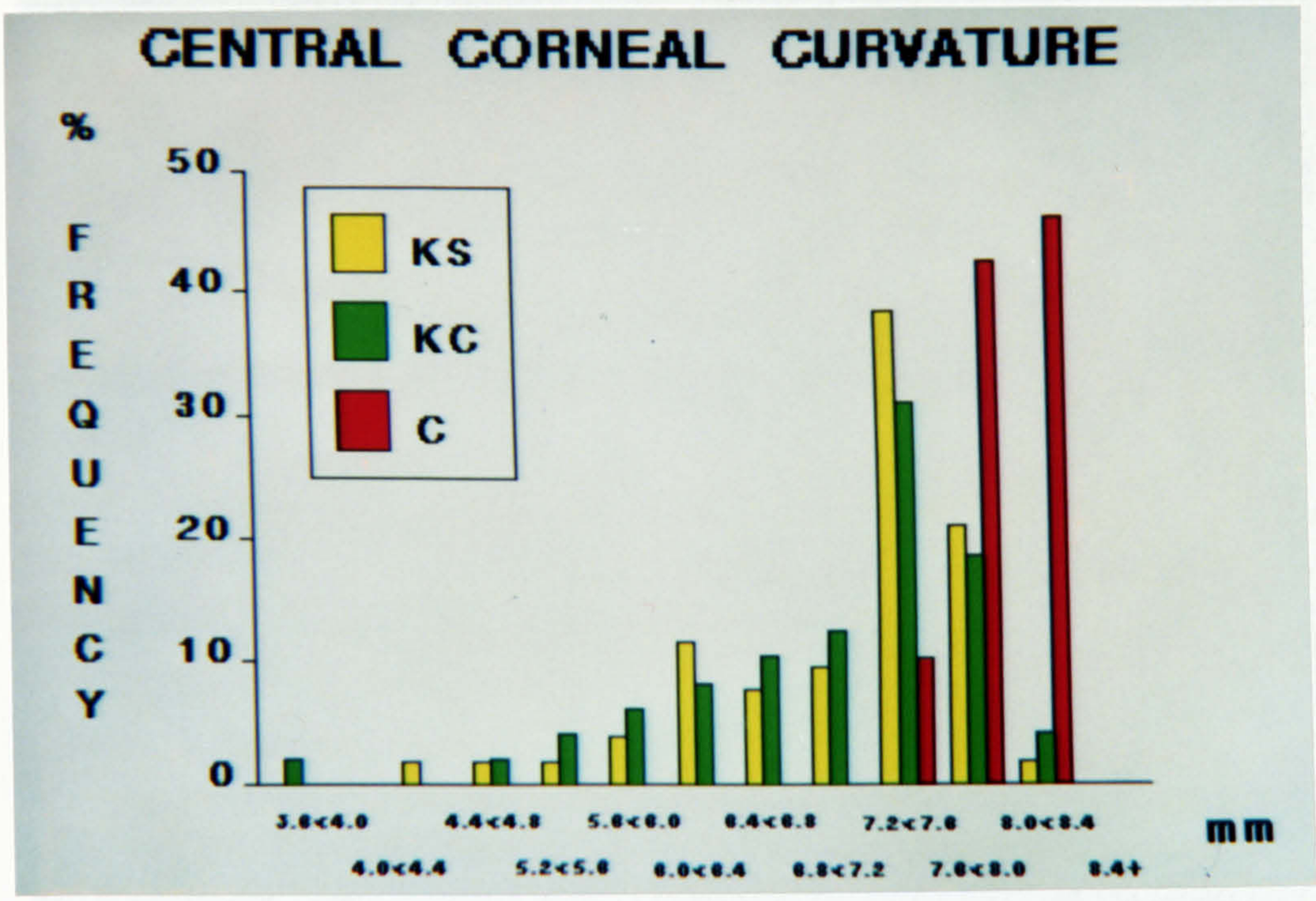
Category	KS	KC	C	Significance
K-mean	7.05±0.76	6.96±0.88	7.93±0.26	P<0.001 *
K-diff.	0.74±0.57	0.61±0.48	0.14±0.10	P<0.001 *
K-dist.	81.81±32.55	94.58±27.87	24.00±0.00	P<0.001 @
K-angle	73.21±33.67	90.34±37.53	39.00±0.00	P<0.001 @

* - One-way ANOVA

@ - Kruskal-Wallis one-way ANOVA ranked test

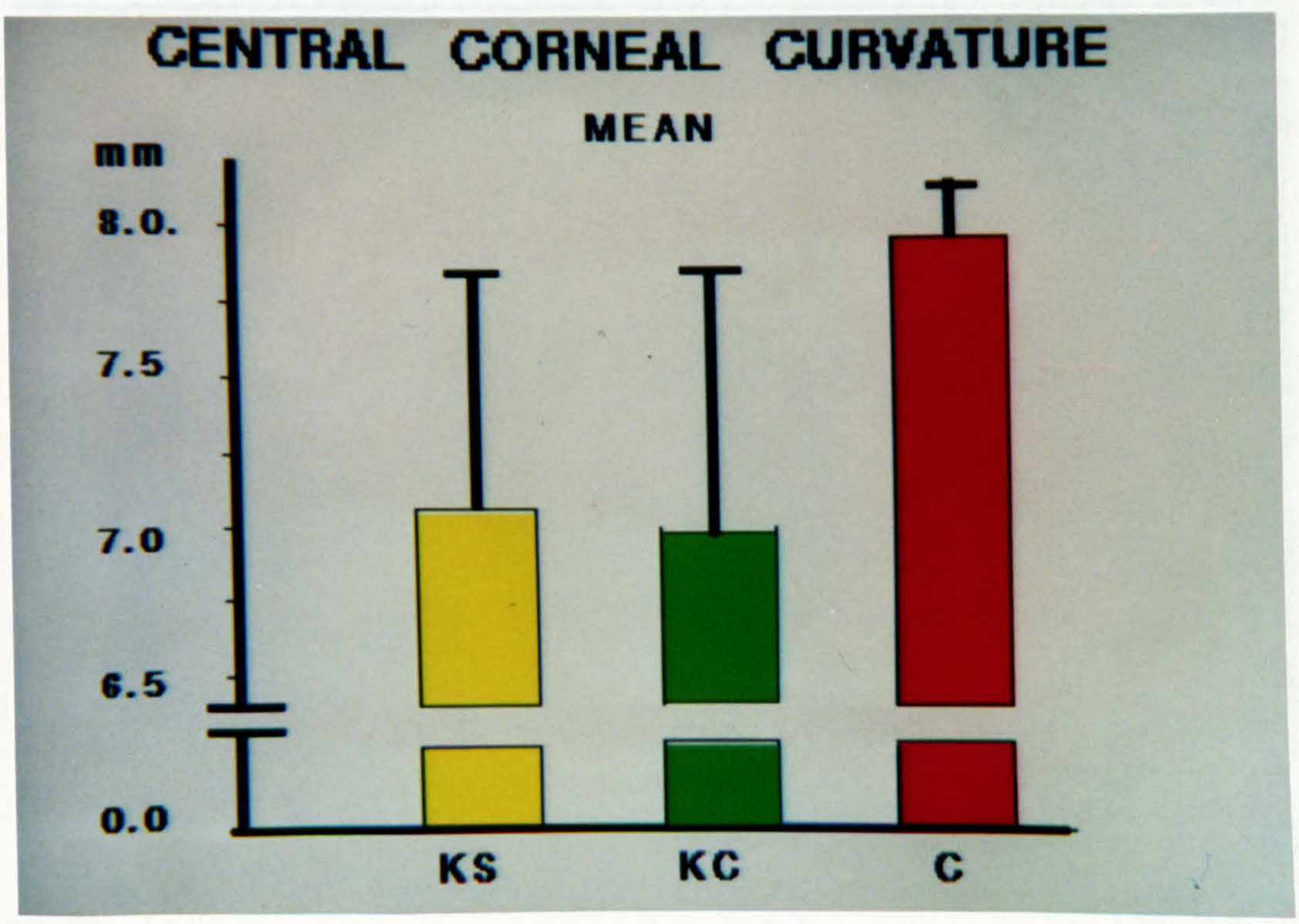
KS & KC are not statistically different on multiple comparison testing.

Figure 3.11



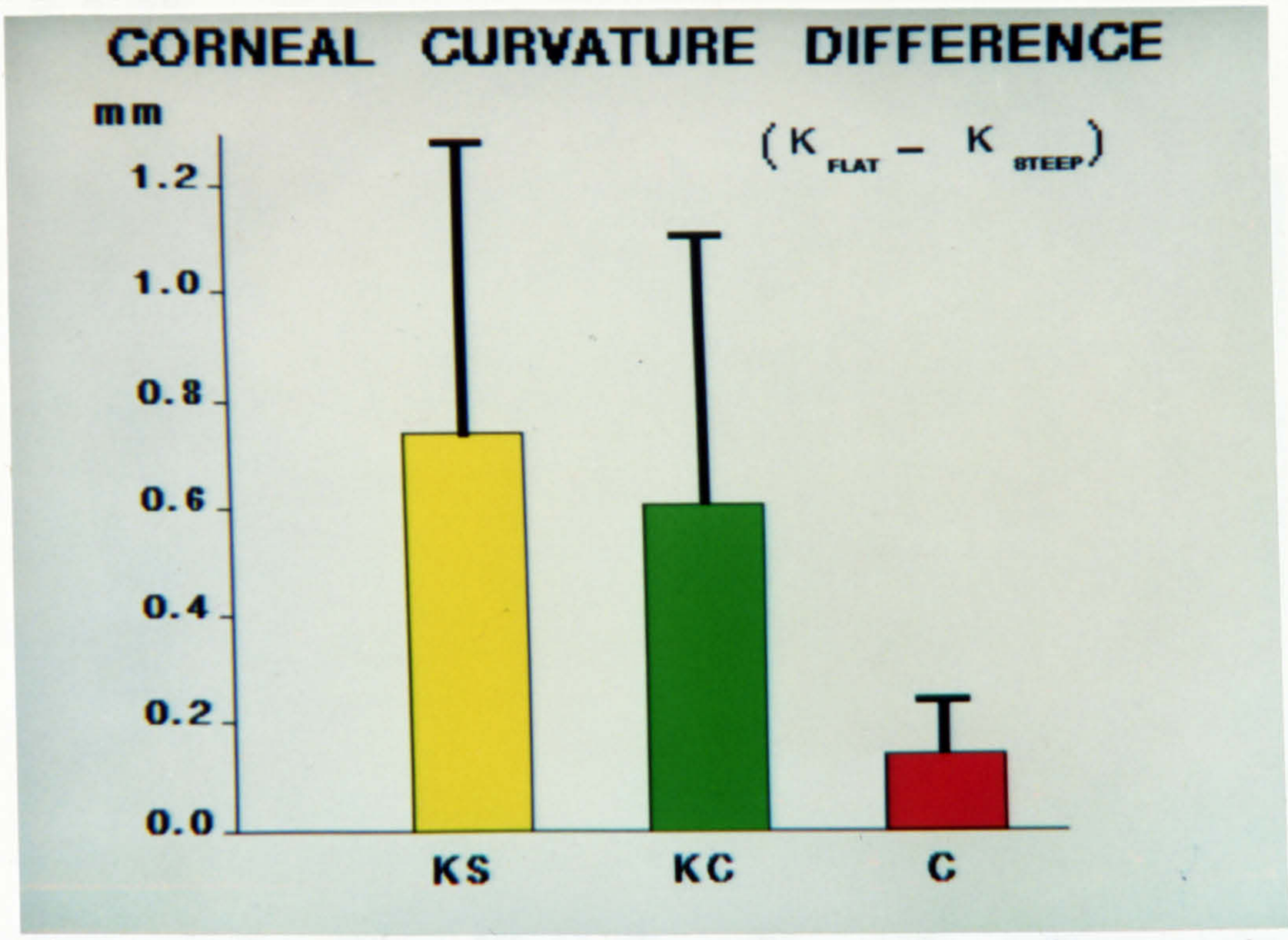
Frequency distribution of mean corneal curvature for keratoconic (KS: n=52, KC: n=48) and control (C: n=39) groups.

Figure 3.12



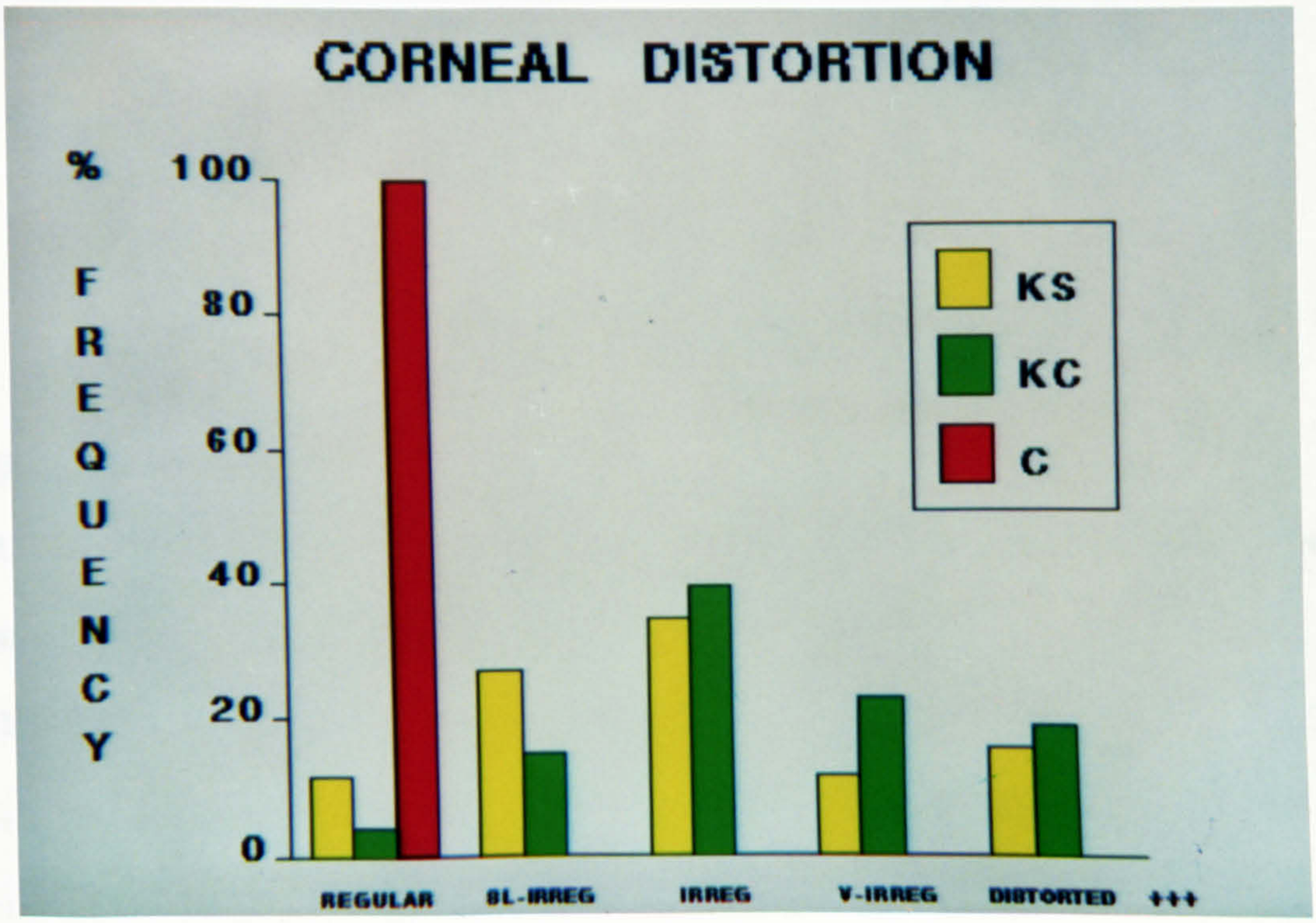
Mean central corneal curvature for keratoconic (KS: n=52, KC: n=48) and control (C: n=39) groups.

Figure 3.13



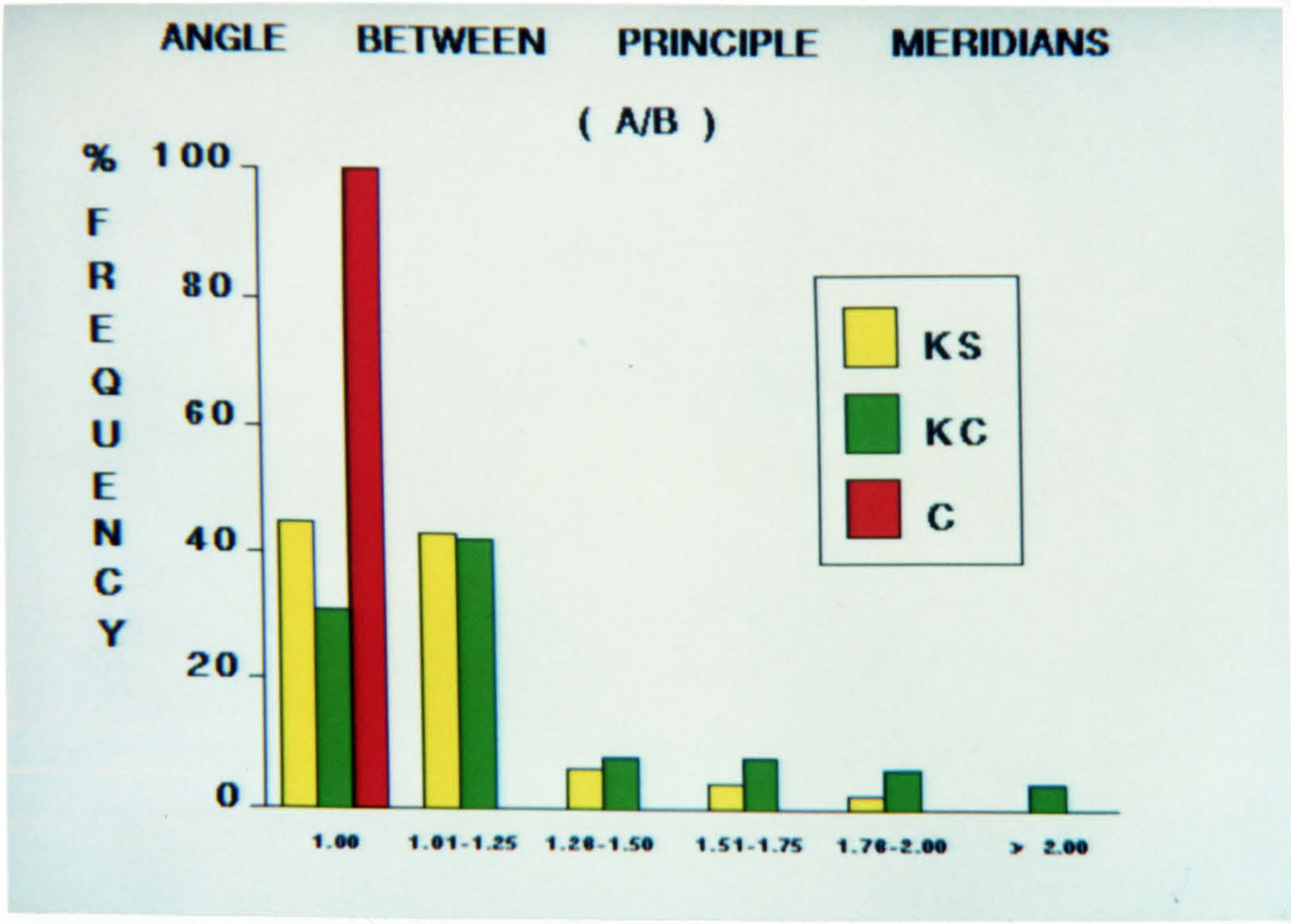
Curvature difference at principle meridians for keratoconic (KS: n=52, KC: n=48) and control (C: n=39) groups.

Figure 3.14



Distortion of mires in central keratometry for keratoconic non-contact lens wearing (KS: n=52, KC: n=48) and control (C: n=39) groups.

Figure 3.15



Frequency distribution of angles between principle meridians (fraction A/B) for keratoconic (KS: n=51, KC: n=48) and control (C: n=39) groups.

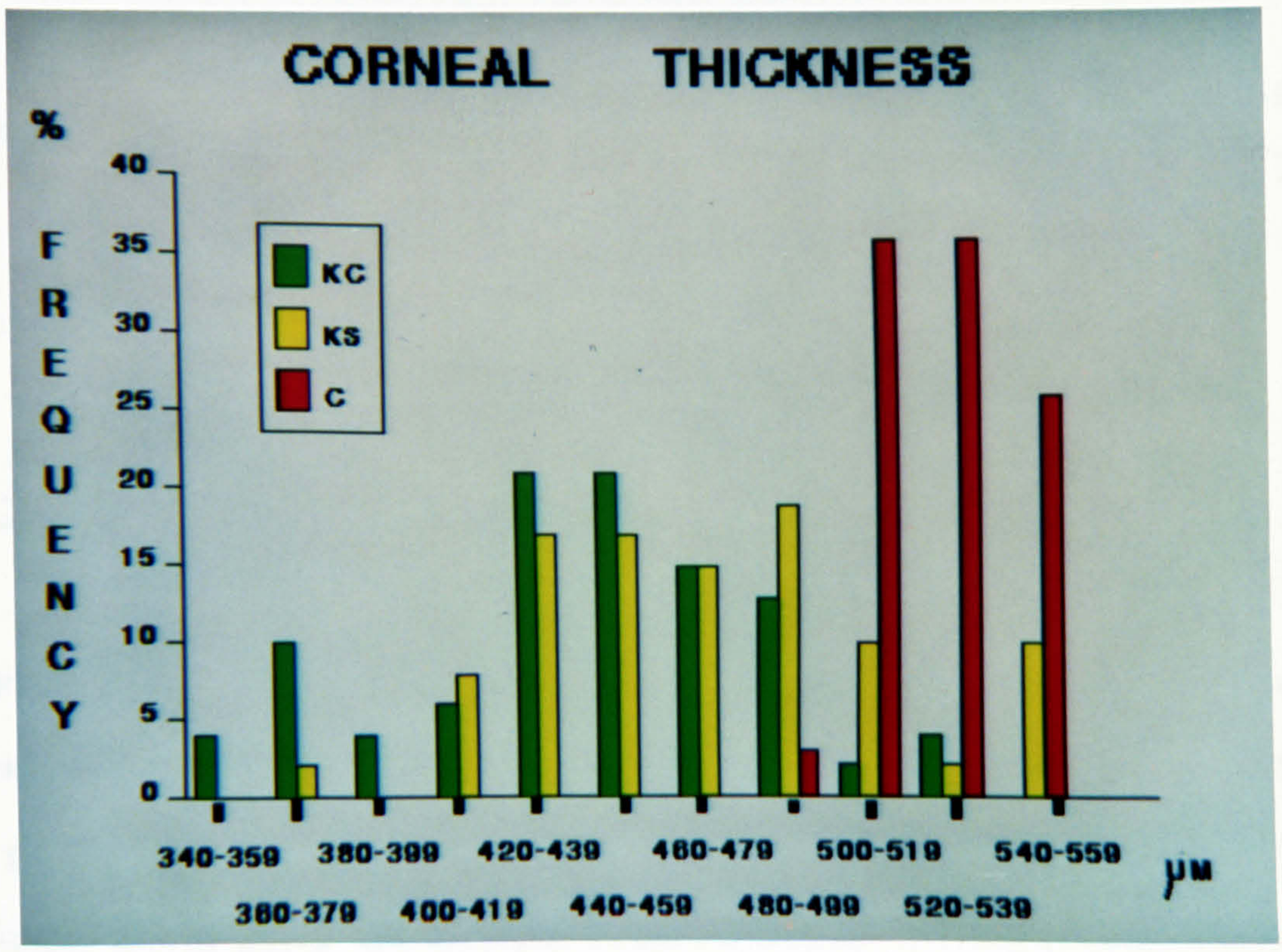
Corneal Thickness.

Central corneal thinning in keratoconus has been extensively investigated. This study is in agreement with previous published work and the results can be seen in Table 3.2 and Fig. 3.16 and 3.17. All three groups are different at $P<0.001$.

Table 3.2 Mean Corneal Thickness

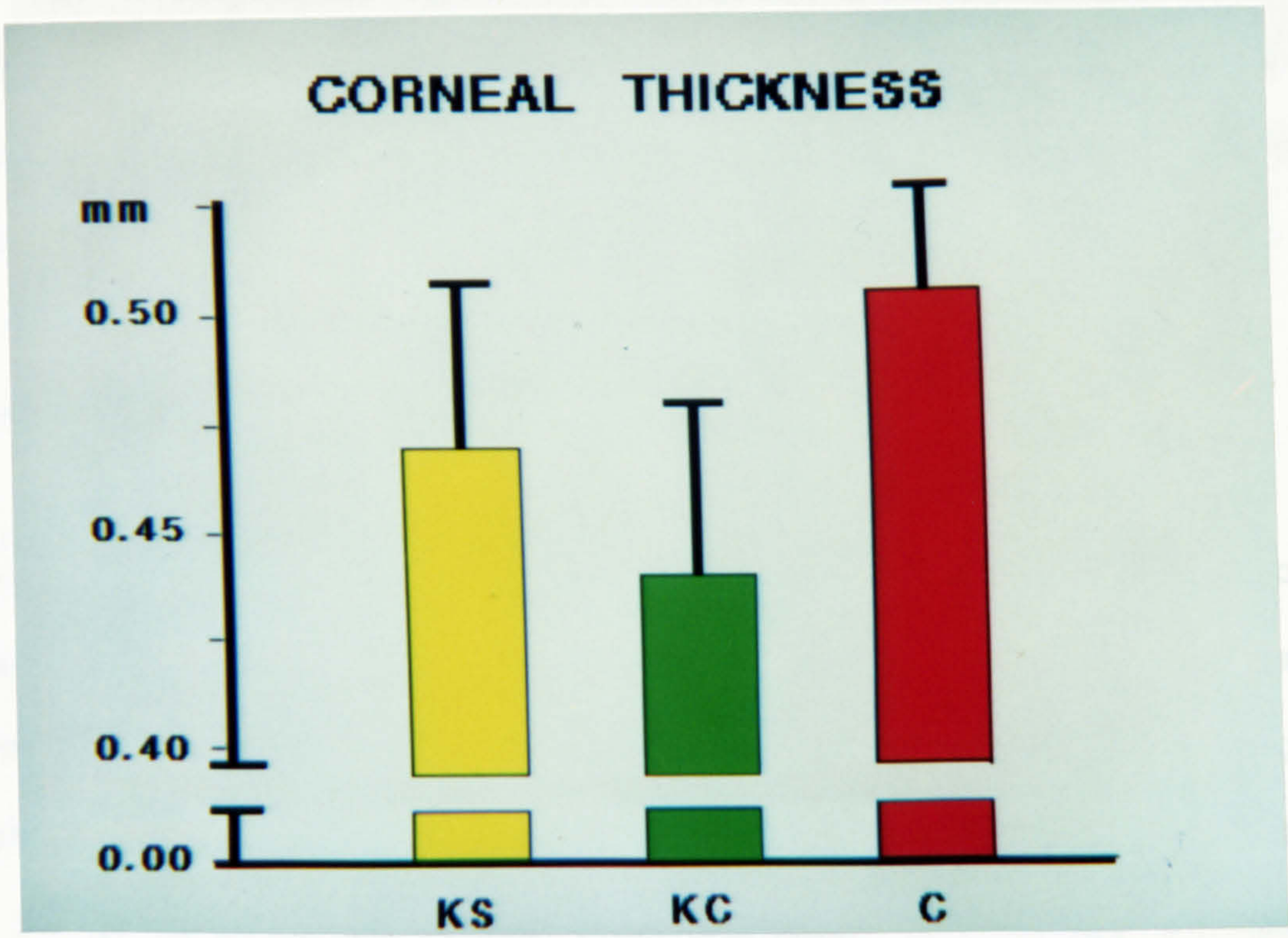
	KS	KC	C	One-way ANOVA
Corneal thickness (mm)	0.468 ±0.041	0.439 ±0.044	0.525 ±0.016	P<0.001

Figure 3.16



Frequency distribution of central corneal thickness for keratoconic (KS: n=51, KC: n=48) and control (C: n=39) groups.

Figure 3.17



Mean central corneal thickness for keratoconic (KS: n=51, KC: n=48) and control (C: n=39) groups.

3.1.3 Severity of Keratoconus.

Diagnosis of the early and descriptions of the later stages of keratoconus have been dealt with by a number of authors. The photokeratoscopic image has been used to classify the disease according to distortion (Amsler 1938), and location of the apex and base of the cone (Poster et al 1968). Hall (1963) claimed the Placido disc to be a satisfactory tool and Hadeyama (1963) based his classification on visual

acuity. Von der Heydt (1930) and Applebaum (1936) used corneal changes as seen by the slitlamp for their classifications and Teng (1963), using electronmicroscopy, claimed the death of corneal epithelial cells to be the most important single factor in keratoconus. Unfortunately, none of these classifications are universally accepted or quantifiable.

Many clinical signs may be observed in keratoconus such as Fleischer's ring, corneal striae, visible nerve fibres, corneal scarring and hydrops. Unfortunately, these signs are rather unreliable on their own in diagnosing the disease and it is generally accepted that corneal thinning, steepening and surface distortion are the important diagnostic factors. For this reason, a severity index was designed to try to incorporate the latter factors into some quantifiable value which would relate to the stage of the disease.

Severity index :

$$S = \sqrt{[(10-K) + \Delta K] \times D} \times A/B \times (1/CT^3 - 10)$$

where :

K = Mean radius (mm)

$\Delta K = K_{FLATTEST} - K_{STEEPEST}$

D = Keratometry distortion code :

D1 = 1.00 (clear image, no distortion)

D2 = 1.50 (slightly irregular image)

D3 = 2.25 (irregular image, not at right angles)

D4 = 3.38 (very irregular image, reading possible)

D5 = 5.06 (very distorted, reading not reliable)

A = Largest angle between the two principle meridians *

B = Smallest angle between the two principle meridians *

CT = Corneal thickness (mm)

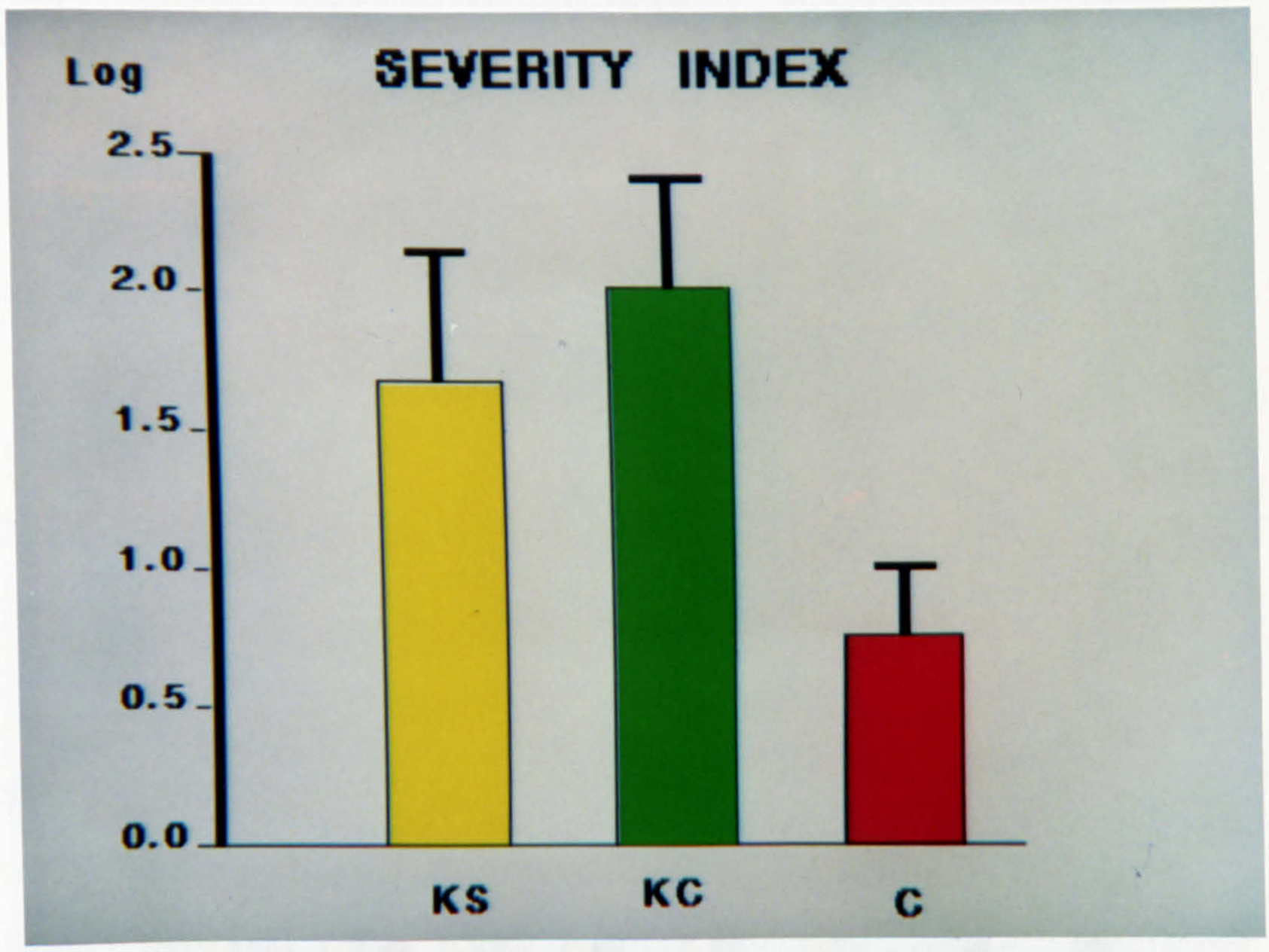
* See Figure 2.1

The above severity index (S) was designed to give near equal weighting to each of the diagnostic signs, and will be used to correlate corneal threshold data later. To convert the data into a normally distributed population the logarithm was taken of each value (Fig 3.18 & Table 3.3)

Table 3.3 Severity index

	KS	KC	C	ANOVA
Mean Log(S)	1.66 ±0.51	2.01 ±0.42	0.77 ±0.25	P<0.001

Figure 3.18

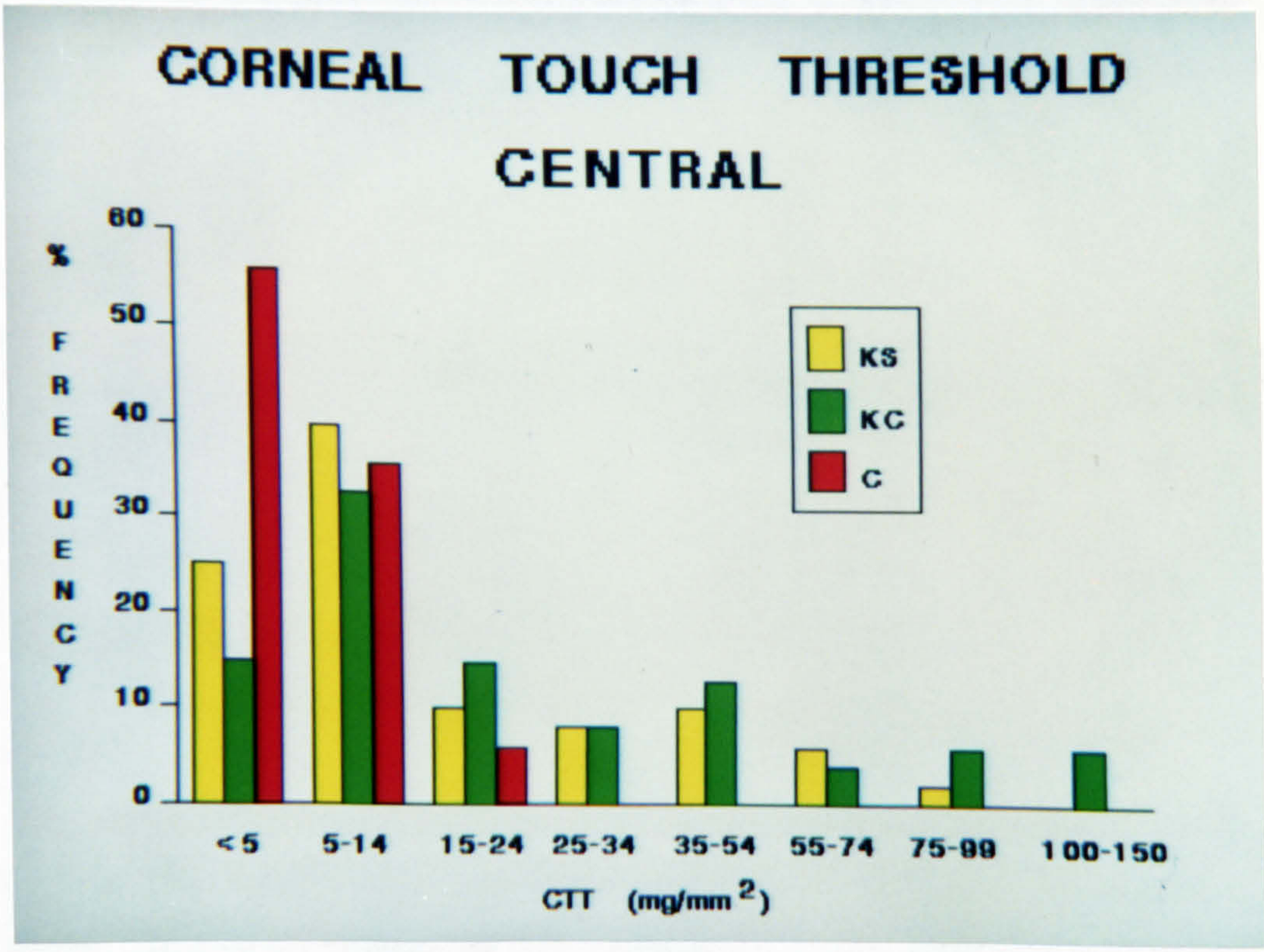


Mean log values for severity index incorporating corneal thickness, central keratometry, difference between principle meridians, mire distortion and angle between principle meridians. Keratoconic (KS: n=51, KC: n=48) and control (C: n=39) groups.

3.1.4 Corneal Touch Threshold (CTT).

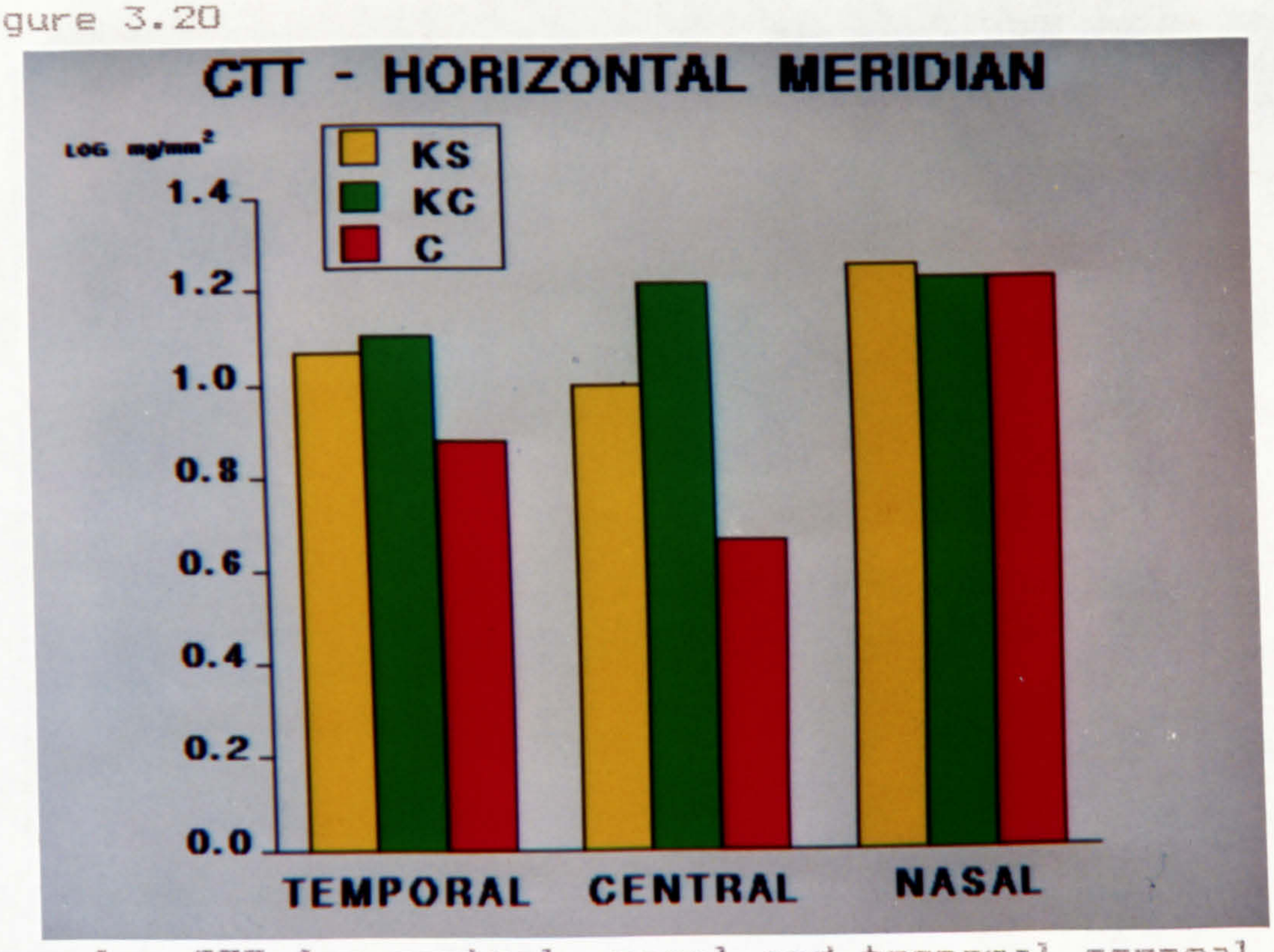
The corneal touch threshold was measured for 5 corneal positions as described previously (chapter 2). The frequency distribution is positively skewed centrally (Fig 3.18) and peripherally (Appendix 8) in the three experimental groups. The logarithm was taken of the CTT values and used for statistical analysis (Fig. 3.20 & 3.21 and Table 3.4).

Figure 3.19



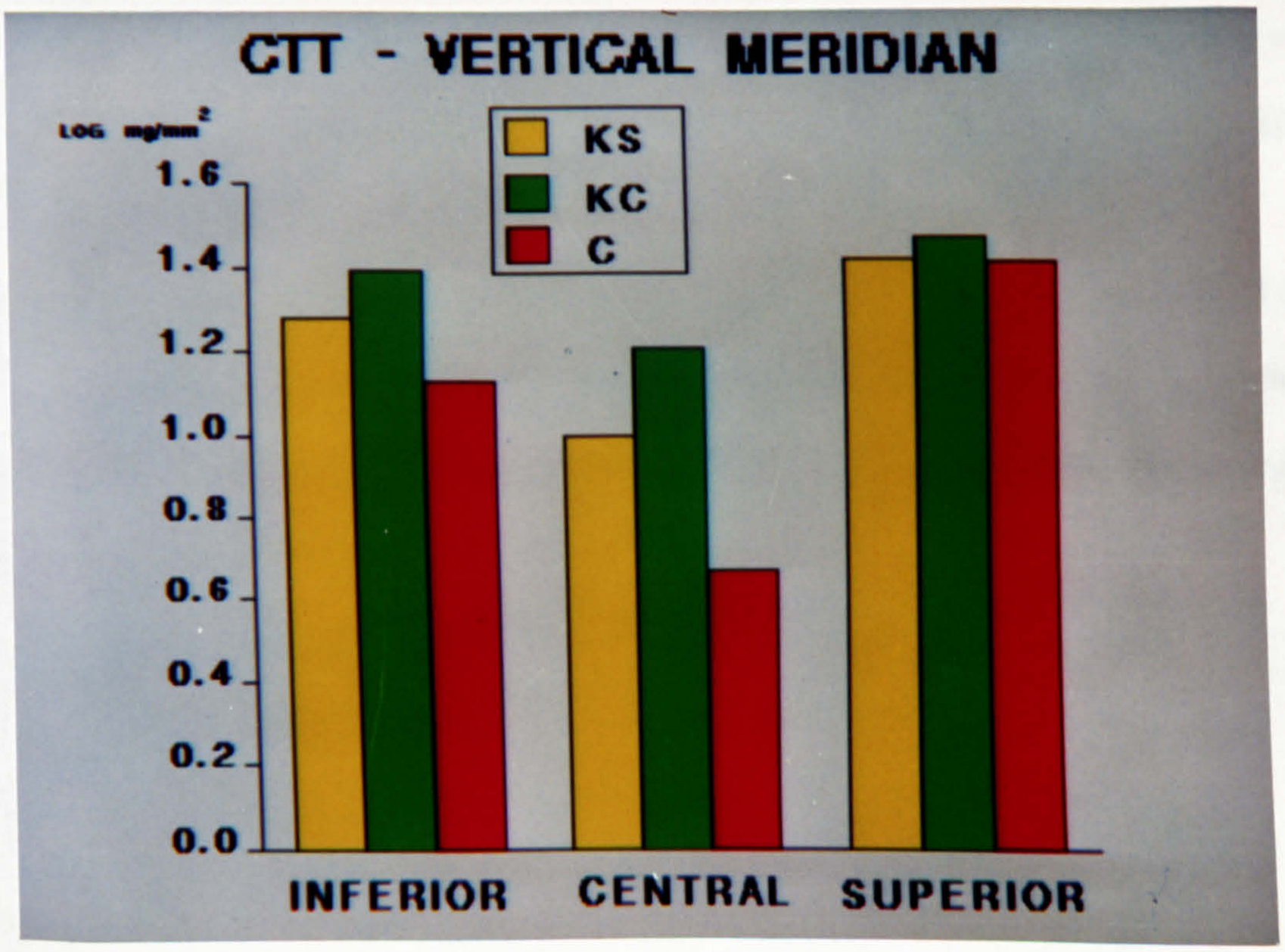
Frequency distribution of central CTT FOR keratoconic (KS: n=52, KC: n=48) and control (n=39) groups.

Figure 3.20



Mean log CTT for central, nasal and temporal corneal positions.

Figure 3.21



Mean log CTT for central, inferior and superior corneal positions.

Table 3.4 Mean Corneal Touch Thresholds (log-CTT).

Test group	Corneal position				
	Cen	Nasal	Temp	Inf	Sup
CS	0.646	1.283	0.885	1.136	1.412
CC	0.755	1.013	0.883	1.094	1.407
KS	0.997	1.248	1.071	1.279	1.416
KC	1.214	1.219	1.097	1.389	1.470

All values are in mg/mm²

3.1.5 Statistical Analysis of CTT.

An unbalanced analysis of variance was used due to the unequal numbers of eyes in each experimental group. The level of significant difference between groups or corneal positions was calculated for $P<0.05$ (Table 3.5 to 3.10).

CTT data for all five corneal positions were pooled for each experimental group. For this part of the study the control group was divided into two groups, one without and one with contact lens wear. Table 3.5 shows that there is no difference between the two control groups and that the two keratoconic groups are similar. However, keratoconic and control groups are significantly different ($P<0.05$).

Table 3.5 Mean log(CTT) for all Corneal Positions in Each Experimental Group.

CS	CC	KS	KC
1.073	1.030	1.200	1.280
_____	_____		
		_____	_____

Mean log(CTT) for each experimental group ($P<0.05$). Measurements are in mg/mm^2. The line indicate values which are statistically similar at the 5% level.

Data from all experimental groups were pooled and the significance of corneal position tested (Table 3.6). There appears to be no statistical difference in log(CTT) between the central and temporal corneal areas. The other peripheral areas all show a higher log(CTT). There is no difference between nasal and inferior regions with the superior cornea having the highest threshold.

Table 3.6 Pooled Data from All Patients.

Corneal position				
Cen	Tem	Nas	Inf	Sup
0.980	1.026	1.230	1.274	1.434

Mean log(CTT) for all patients at five corneal positions ($P<0.05$). Measurements are in mg/mm^2.

Data from different corneal positions were tested for significant variations for each experimental group separately (Table 3.7, 3.8, 3.9 & 3.10).

Table 3.7 Control Group Without Contact Lenses.

Corneal position				
Cen	Tem	Inf	Nas	Sup
0.646	0.885	1.136	1.283	1.412

Mean log(CTT) at five corneal positions ($P<0.05$). Measurements are in mg/mm^2.

Table 3.8 Control Group With Contact Lens Wear.

Corneal position				
Cen	Tem	Nas	Inf	Sup
0.755	0.883	1.013	1.094	1.407

Mean log(CTT) at five corneal positions (P<0.05).

Measurements are in mg/mm².

Table 3.9 Keratoconus without contact lens wear.

Corneal position				
Cen	Tem	Nas	Inf	Sup
0.997	1.071	1.248	1.279	1.416

Mean log(CTT) at five corneal positions (P<0.05).

Measurements are in mg/mm².

Table 3.10 Keratoconus With Contact Lens Wear.

Corneal position				
Tem	Cen	Nas	Inf	Sup
1.097	1.214	1.219	1.389	1.470

Mean log(CTT) at five corneal positions (P<0.05).

Measurements are in mg/mm².

It was of interest to see if the log(CTT) of each separate corneal position (ie; central, nasal, temporal, inferior & superior) was different between the experimental groups. This is shown for mean values of log(CTT) in Table 3.11. For keratoconus and control (with or without CL) all peripheral corneal areas show nearly equal levels of log(CTT). The only variation was for the control group without contact lens wear which showed a significant lower central log(CTT). The control group with contact lens wear was too small to show any significant variations. Accordingly it was the central corneal area in keratoconus which was affected and had a higher touch threshold (P<0.05).

Table 3.11 Comparison of Corneal Touch Thresholds log(CTT) For The Same Corneal Position in Each Experimental Group.

Corneal position	Control no CL	Control CL	Keratoconus no CL	Keratoconus CL
Central	0.646	0.755	0.997	1.214
Nasal	1.283	1.013	1.248	1.219
Temporal	0.885	0.883	1.071	1.097
Inferior	1.136	1.094	1.279	1.389
Superior	1.412	1.407	1.416	1.470

The measurements are in mg/mm². The significance of the results is indicated ($P<0.05$).

Repeatability of CTT measurements.

Three normal volunteers, who did not take part in the main study, agreed to have repeated measurements carried out on 3 separate occasions. One central and three peripheral corneal areas were stimulated in the same manner as in the experimental groups. All volunteers were normal male caucasians and were below 30 years of age. The results showed good repeatability for the central area of 5 % variation and slightly more variable for the peripheral areas of 20 % variation.

Correlations with central CTT.

Central CTT correlated inversely with central corneal curvature and central corneal thickness. Central corneal curvature was the most significant single factor to correlate with central CTT (Table 3.12) and indicates that CTT increases (sensitivity reduces) as the cornea steepens. The same trend was present for central corneal thinning and CTT although to a lesser degree. Corneal surface irregularity as measured by mire image distortion correlated positively with central CTT as did corneal scarring. Central CTT did not show any dependance on the duration of the disease nor the visibility of the corneal nerve fibres. No correlation was found between central CTT and iris colour and density of iris pigmentation. By excluding dark pigmented eyes from the study, this result was anticipated.

Table 3.12 Correlations of Central CTT Within the Keratoconic Group.

Correlating Variables		r	CV	*
log CTT	~ time since diagnosis	-0.168	±0.238	NS
-	~ mean central curvature	-0.406	±0.169	S
-	~ curvature difference	0.046	±0.169	NS
-	~ keratometry angle	0.104	±0.166	NS
-	~ mire distortion	0.369	±0.166	S
-	~ central corneal thickness	-0.204	±0.166	S
-	~ corneal scarring	0.195	±0.169	S
-	~ corneal nerve visibility	0.023	±0.185	NS
-	~ log severity index	0.204	±0.185	S
-	~ iris colour & density	0.028	±0.166	NS

r : correlation coefficient; + = positive correlation.

\- = negative correlation.

CV : critical value at 5%

* Significant (S) or non-significant (NS).

3.2 LID MARGIN TOUCH THRESHOLD (LTT)

For this part of the study the control group consisted of non-contact lens wearers only. The limitations of the technique (see Chapter 2) made the contact lens wearing control group too small to be significant (Table 3.13). The raw data is tabulated in Appendix 2 and the mean logarithmic values used in Table 3.14.

Table 3.13 Number of Eyelids Tested for LTT in Each Experimental Group.

Experimental group	Number of upper lids	Number of lower lids
KS	37	40
KC	30	28
CS	26	28

Table 3.14 Touch Thresholds for Upper and Lower Lid Margins (log LTT) in Three Experimental Groups.

	log LTT		
	upper lid	lower lid	M-C
KS	1.31±0.49	1.34±0.49	NS
KC	1.40±0.34	1.21±0.32	NS
CS	1.19±0.34	1.06±0.43	NS
one-way ANOVA	P>0.10	P>0.10	

M-C: Multiple comparison

NS : No significant difference.

Analysis of variance (ANOVA) in Table 3.14 show that the LTT is statistically comparable between and within the experimental groups for upper and lower lid margins. LTT correlations with allergy, diagnosis of keratoconus, contact lens wear and central CTT were tested and no correlation was found.

3.3 CTT ON KERATOCONIC CORNEAS USED SUBSEQUENTLY FOR HISTOLOGICAL STUDY.

Topographical corneal aesthesiometry was undertaken on 5 keratoconic corneas (K1, K3, K4, K5 & K6) prior to penetrating keratoplasty. The corneas were examined at EM level and a quantitative assessment made of the neural content (see Chapter 5). The corneal touch thresholds (CTT) and neural contents for these five corneas are summarized in Table 3.15 (Appendix 5 may be consulted for full clinical data).

Table 3.15 Corneal touch threshold and innervation of central and mid-peripheral corneal areas.

SPECIMEN	CORNEAL AREA	CTT mg/mm²	STROMA axons/mm	EPITHELIUM axons/mm
K1	central	8	3	140
	superior	8	58	103
K3 *	central	90	75	37
	superior	150	80	80
	temporal	14	72	22
K4 *	central	12	21	116
	temporal	12	15	108
	superior	30	22	54
K5	central	8	64	81
	temporal	5	196	72
	inferior	5	140	80
	nasal	5	143	40
	superior	N/R	162	209
K6 *	central	150	623	N/R
	nasal	90	150	12
	superior	55	31	32
	temporal	40	235	38
	inferior	30	26	34

* With central corneal scarring.

The logarithm was taken for all CTT measurements as previously and the mean values are tabulated in Table 3.16 together with corneal thickness and axon density.

Table 3.16 Mean Values for All Specimens.

	Mean	SD
Log CTT (mg/mm²)	1.33	± 0.52
Axons / mm (Stroma)	117.6	± 143.3
Axons / mm (Epith)	74.0	± 50.4
Corneal thickness (mm)	0.433	± 0.120

Corneal touch threshold (log CTT) correlates with epithelial axon density although it is only just present at the 5% level of confidence (Fig 3.22). There is no correlation between stromal axon density and corneal touch threshold (log CTT) (Fig 3.23). The reduction in sensitivity (high log CTT) correlates well with the density of corneal scarring. Clinically this was also very apparent when performing the aesthesiometry on these patients. The CTT would be considerably higher in the scarred area and become relatively low immediately next to the scarred area, except in one case K4. The statistical analysis indicate a log CTT dependance on corneal thickness, however, the corneal thickness data is not a normally distributed sample and

should be interpreted with caution. Furthermore, log CTT does not appear to be related to central corneal steepening or corneal nerve visibility. The correlations with log CTT are summarised in Table 3.17.

Table 3.17 Central Log CTT Correlation.

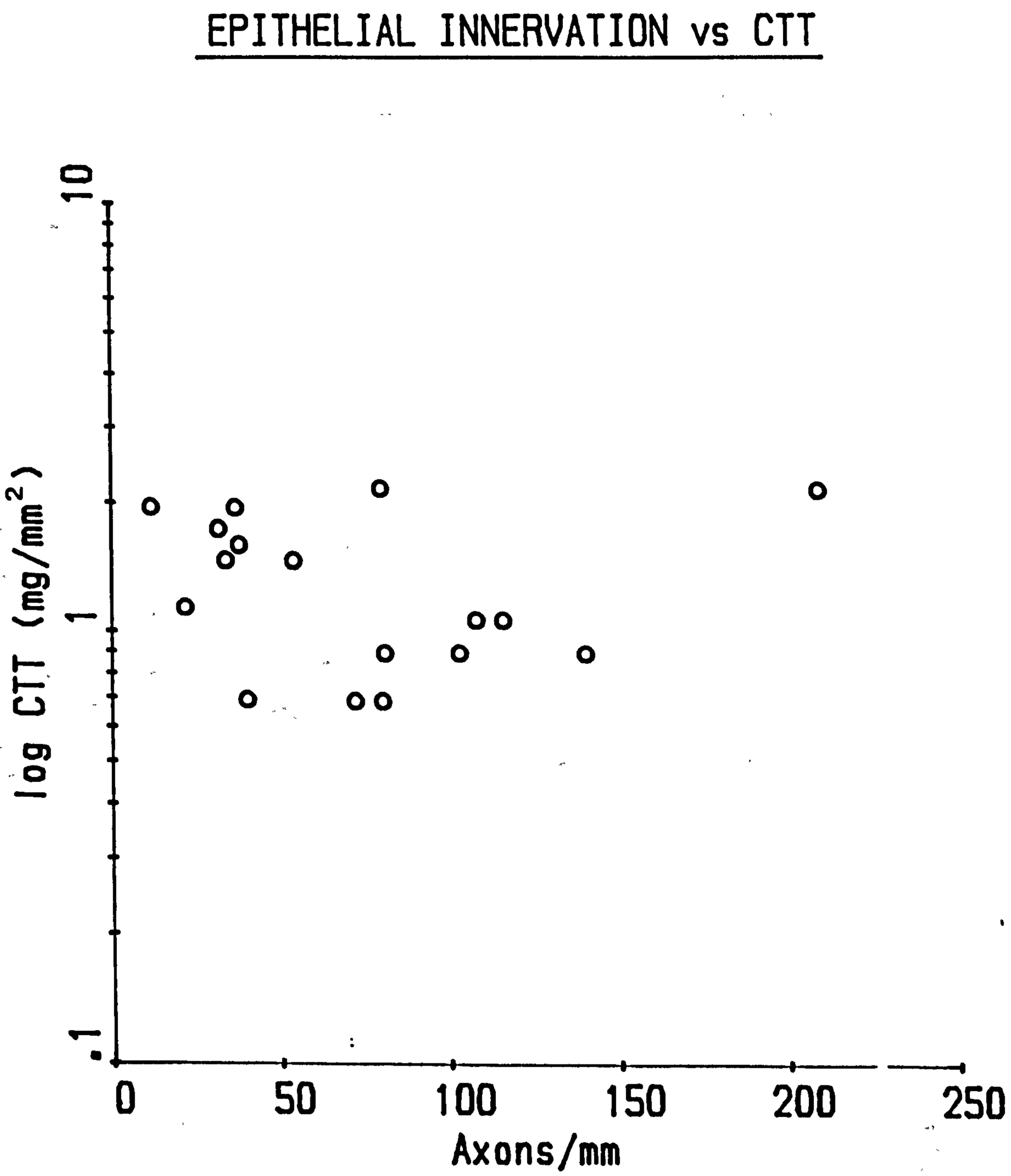
Correlation factors			r	*
log CTT	~	stromal axons	-0.040	NS
log CTT	~	epithelial axons	0.479	S
log CTT	~	corneal scarring	0.779	S
log CTT	~	corneal thickness	0.569	@ (S)
log CTT	~	mean curvature	-0.406	NS

r = Correlation Coefficient

* = Critical value at 5% level of significance = ±0.427

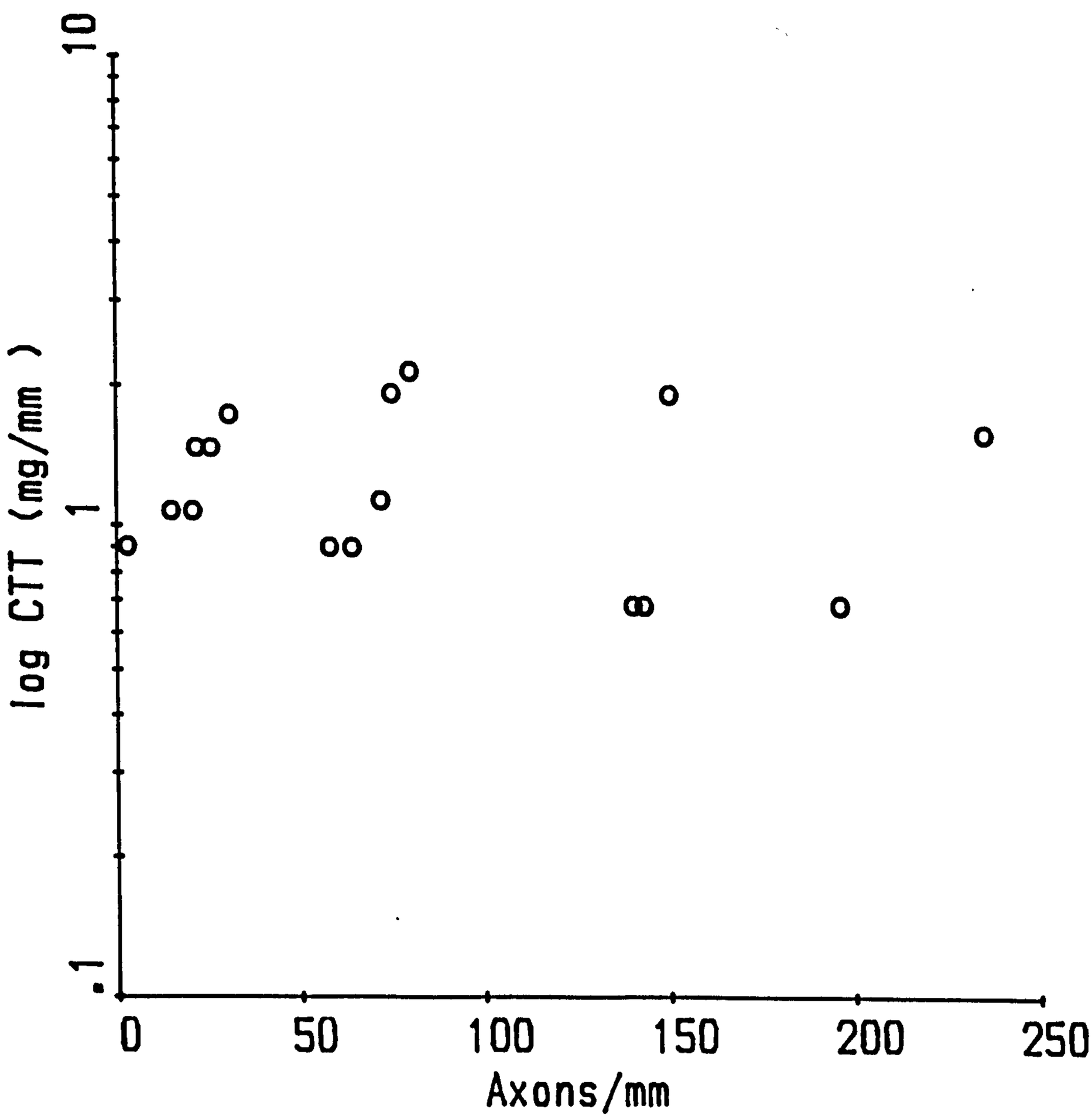
@ : Corneal thickness is not normally distributed.

Figure 3.22



Scatterplot of Log CTT and Epithelial Innervation (axons/mm). There is a Correlation at the 5% Level of Significance.

Figure 3.23



Scatterplot of Log CTT and Stromal Innervation (axons/mm).
There is No Correlation Between the Two Measurements.

CHAPTER 4

ULTRASTRUCTURE OF CORNEAL NERVES

4.1 Fixation Techniques

Two fixation methods were used as described in Chapter 2. In summary the keratoconic material was treated with glutaraldehyde and most of the control material was treated with formol saline. The fixation method of control material was beyond our control. In this chapter formol saline should be assumed as fixative for control material if nothing else is stated.

4.2 Stromal Nerve Morphology

Light microscopy was used to supplement electronmicroscopy but was found to be inadequate for the identification of all stromal nerve bundles and epithelial axons. The keratoconic material originated from discs of 7-8 mm diameter and accordingly a comparative investigation was performed for the mid-peripheral and central corneal areas.

4.2.1 Nerve position

All nerve bundles were located in the anterior two thirds of the cornea. This was true for keratoconus, desensitised keratoconus and control material. Early impressions were that some areas of the keratoconic material showed nerve

bundles infrequently whereas nerve bundles were seen very frequently in other areas, thus suggesting local aggregation. Such variation in nerve bundle distribution was not immediately apparent in the control material, and the nerve bundle position was recorded for 4 keratoconic and 4 control specimens. Although some variation was to be expected according to where the sampling was undertaken, the overall picture was similar for both tissue types.

Mid-peripheral nerve bundles had a slight oval and regular appearance in the control corneas. The keratoconic nerve bundles were disorganised and irregular, taking up the shape of the adjacent irregular collagen lamellae (Figure 4.1). The nerve bundles divided (Figure 4.2) as they moved towards the central cornea to become flatter in appearance (Figure 4.3). This was true for keratoconus and control material except where the stroma was irregular leading to irregular nerve bundles in keratoconus. Keratocytes were seen in close proximity to the nerve bundles (Figure 4.4), often sending processes to invade the nerve bundle (Figure 4.5 & 4.6) and occasionally substituted for perineurium (Figure 4.7).

4.2.2 Nerve content

The Schwann cell cytoplasm decreased towards the centre of the cornea and varied in appearence from electron-dense to electron-lucid irrespective of tissue origin (Figures 4.8; 4.9; 4.10 & 4.11). The electron density of different Schwann cell processes frequently varied within the same nerve

bundle (Figure 4.8). This variation in electron density was seen in keratoconic and control material and was not secondary to variations in fixation methods.

The Schwann cell cytoplasm contained microtubules (Figure 4.10), rough endoplasmic reticulum (Figure 4.11) and mitochondria which often were seen to be smaller than those of the axonplasm (Figure 4.8; 4.9 & 4.10), although occasionally they appeared larger (Figure 4.11).

A basal lamina usually completely surrounded all nerve bundles. It varied in electron density and thickness (Figure 4.8, 4.9 & 4.11) and occasionally it was absent in places (Figure 4.12). A basal lamina-like material was very frequently scattered round the nerve bundles (Figure 4.13). This material was found to be a useful clue in locating small nerve bundles.

The axoplasm seemed identical for the keratoconic and control tissues with regard to its content of mitochondria, neurofilaments, microtubules and vesicles. In the control material it was frequently difficult to observe the cristae of the mitochondria (Figure 4.5 & 4.9). Furthermore, the axoplasm often appeared electron lucid with patches of granular material (Figure 4.9). When glutaraldehyde was used for fixing control material the mitochondria appeared normal with cristae.

Axon enlargements (varicosities) were common and usually contained a number of mitochondria but not always vesicles (Figures 4.7 & 4.14). Vesicles were usually located in a varicosity and were 3-4 times the diameter of microtubules with either electron dense or lucid centres (Figure 4.8; 4.10; 4.11 & 4.15). Single vesicles could be identified in axons which did not appear to be sectioned through a varicosity (Figure 4.8 & 4.10).

4.2.3 Penetration of Bowman's layer

Processes from epithelial cells as well as connective tissue cells were obseved to penetrate Bowman's layer. The epithelial basement membrane became thicker at the site of penetration (Figure 4.16). Neural elements could not be identified positively in any of the material in Bowman's layer, however, semi-serial sections revealed nerves containing axonal organelles in the anterior stroma and epithelium immediately adjacent to the area where Bowman's layer was penetrated.

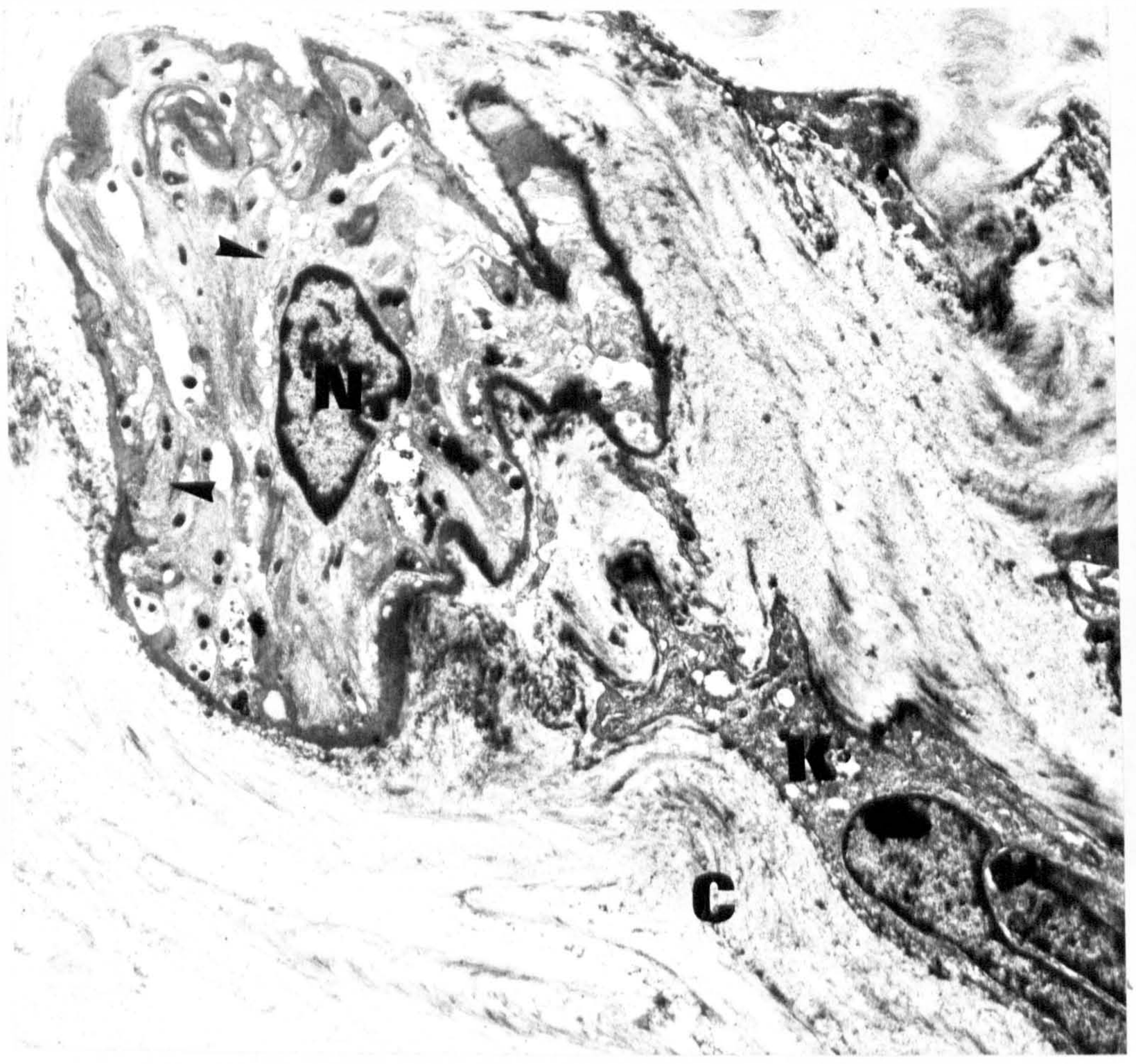


Figure 4.1

Mid-peripheral stromal nerve bundle showing irregular profile. The adjacent collagen lamellae (C) and keratocyte process (K) also appear irregular. A Schwann cell nucleus (N) is seen in the centre of the nerve bundle which contains numerous axons (arrowhead). (Keratoconus : 5,400 X)

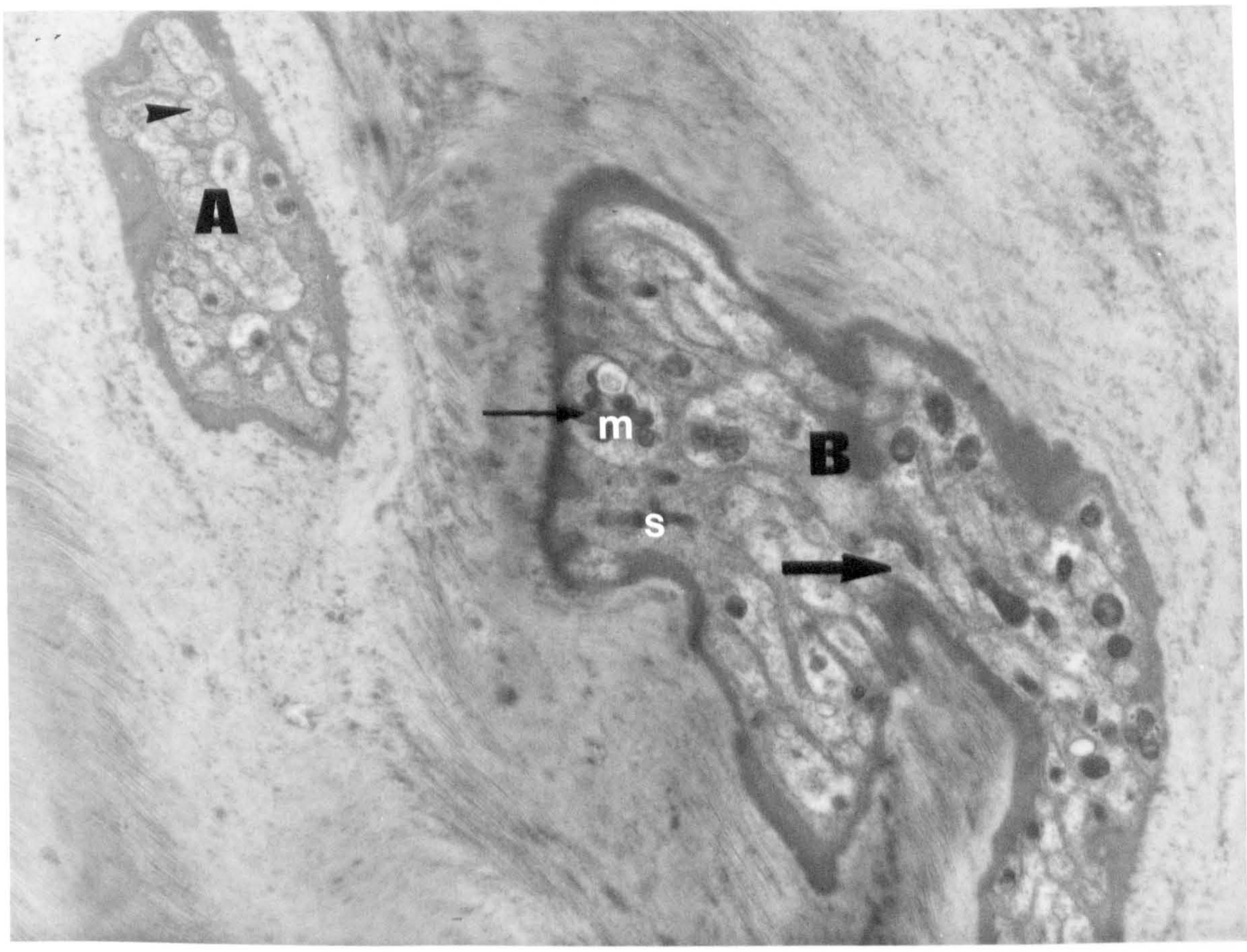


Figure 4.2

Two mid-peripheral stromal nerve bundles having separated from the same nerve a short distance away. Bundle A show axons (arrowhead) cut almost in perfect transverse section, whereas bundle B show axons (arrow) cut obliquely indicating that bundle B takes a different direction to bundle A. Mitochondria (m) are present in axons (long arrow) and Schwann cell processes (S). (Keratoconus : 10,400 X)

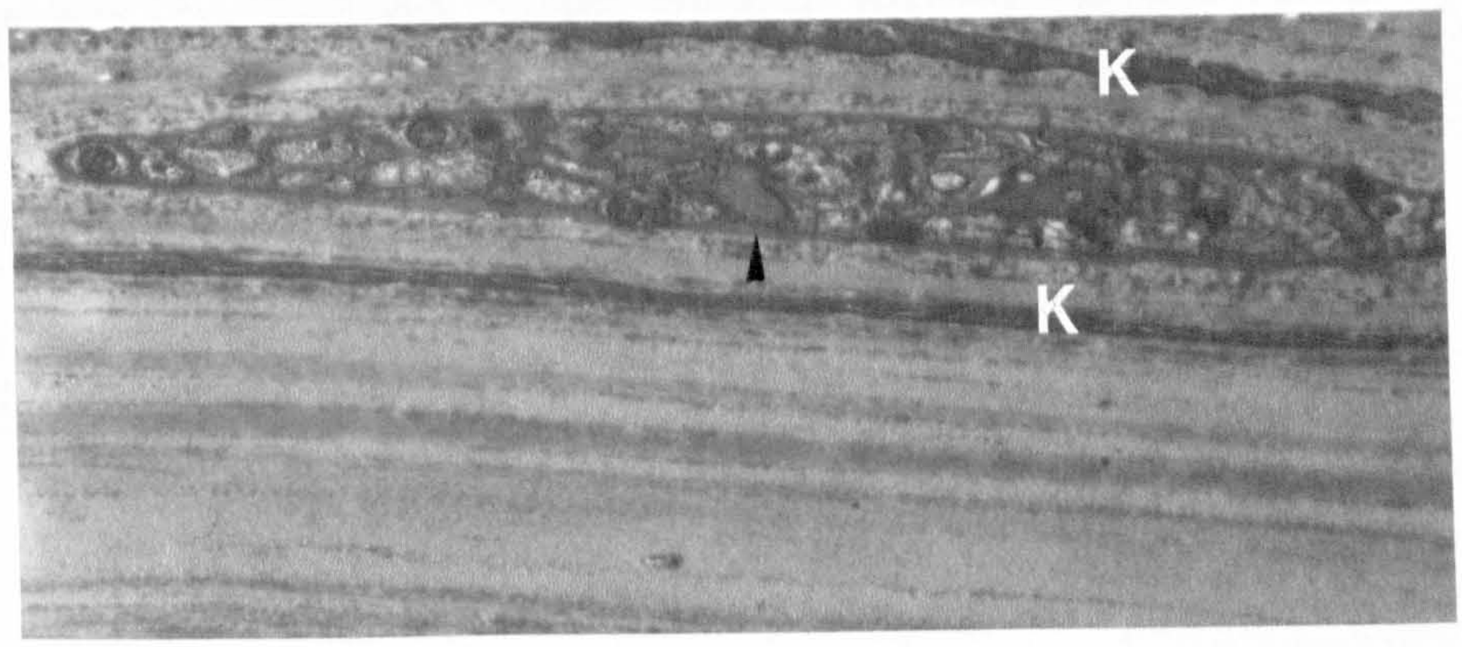


Figure 4.3

Stromal nerve bundle cut in transverse section and situated near the central part of the cornea. The nerve bundle appears to have flattend with the long axis parallel to the collagen lamellae. The basement membrane (arrowhead) is complete around the nerve bundle and has become thin. A keratocyte process (K) may be seen on either side of the nerve.

(Keratoconus : 13,300 X)

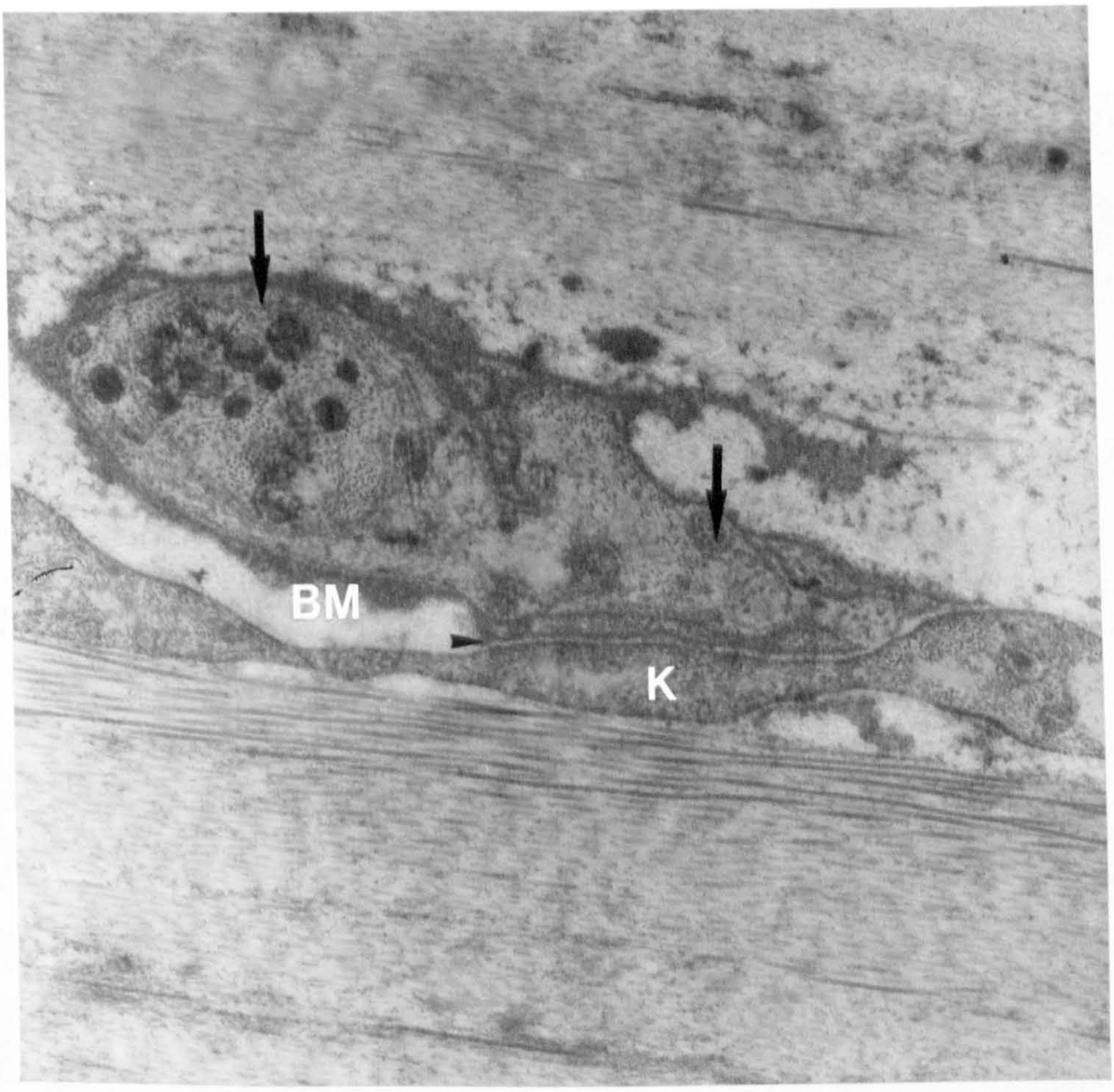


Figure 4.4

Keratocyte process (K) seen in close proximity of mid-peripheral stromal nerve bundle. The basement membrane (BM) of the nerve bundle is not in complete contact with the keratocyte process (arrowhead). Note the difference in axon size (arrow). (Control : 12.000 X)

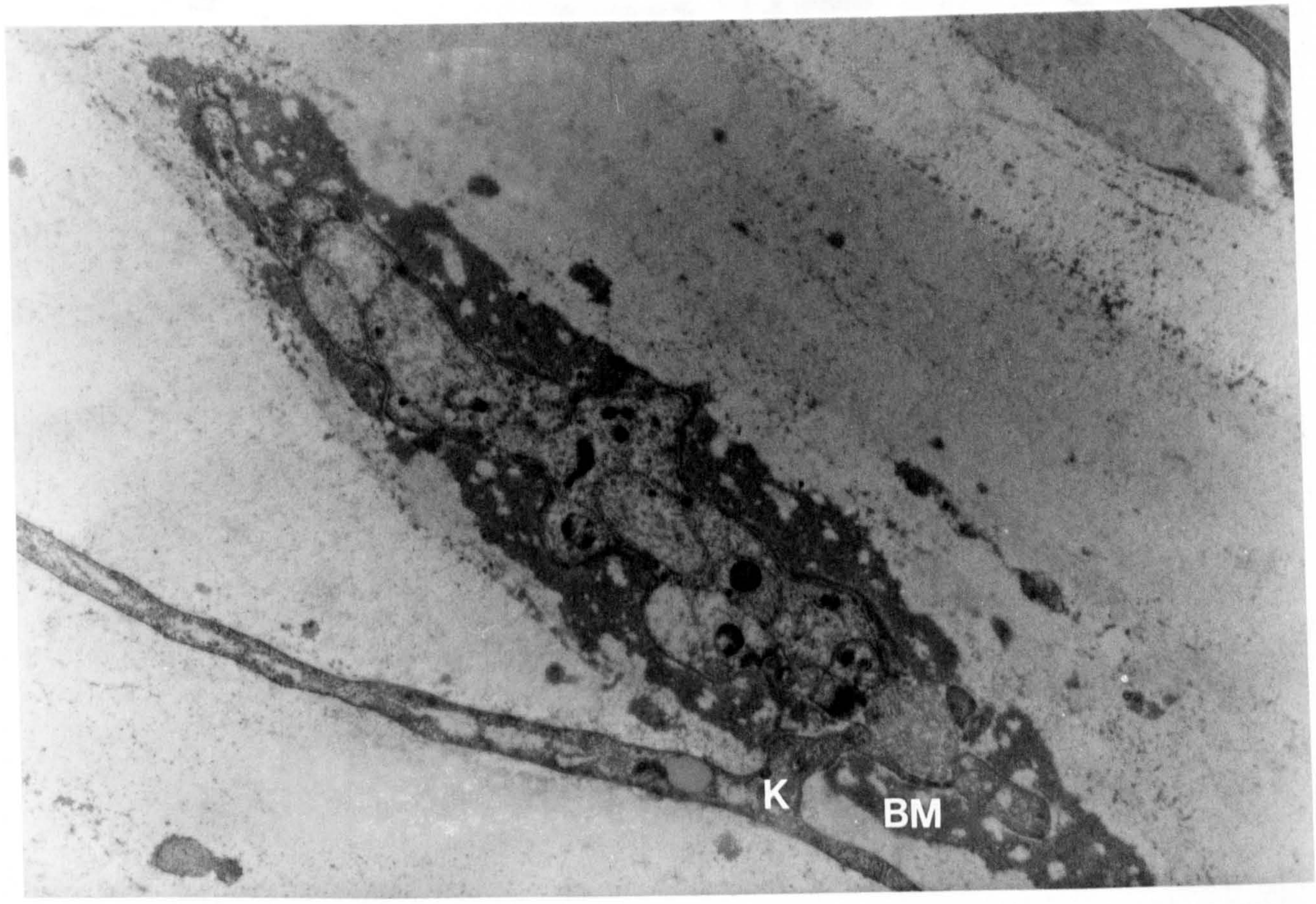


Figure 4.5

Keratocyte process (K) penetrating the thick basement membrane (BM) of a stromal nerve bundle. (Control : 12,500 X)

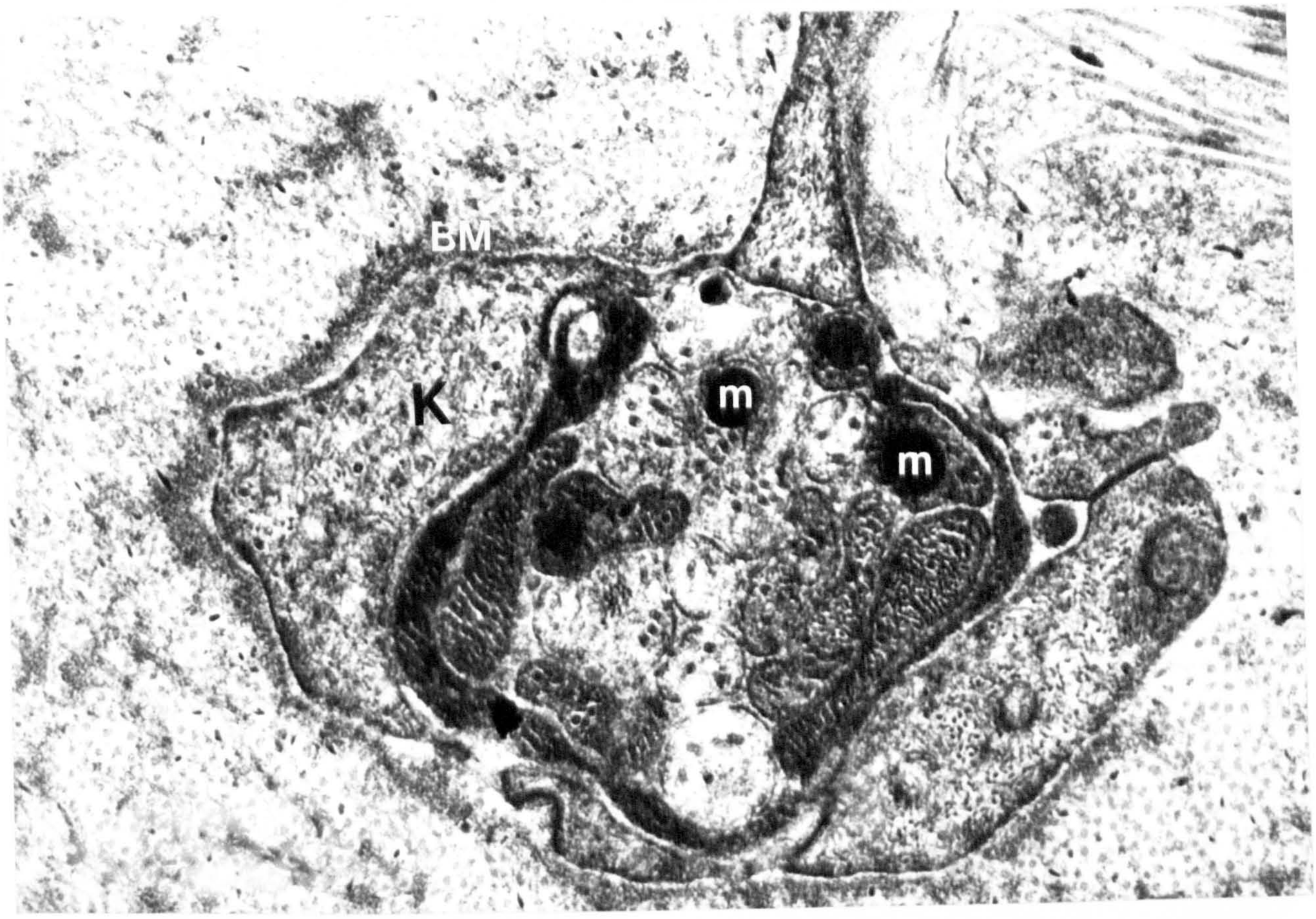


Figure 4.6

Keratocyte processes (K) have penetrated the space between the Schwann cell and the basement membrane (BM) of the nerve bundle. The keratocyte process can be distinguished from perineurium due to the lack of contact with the basement membrane. The section is from the central cornea. Large mitochondria (m) are seen in axons. (Keratoconus : 54,000 X)

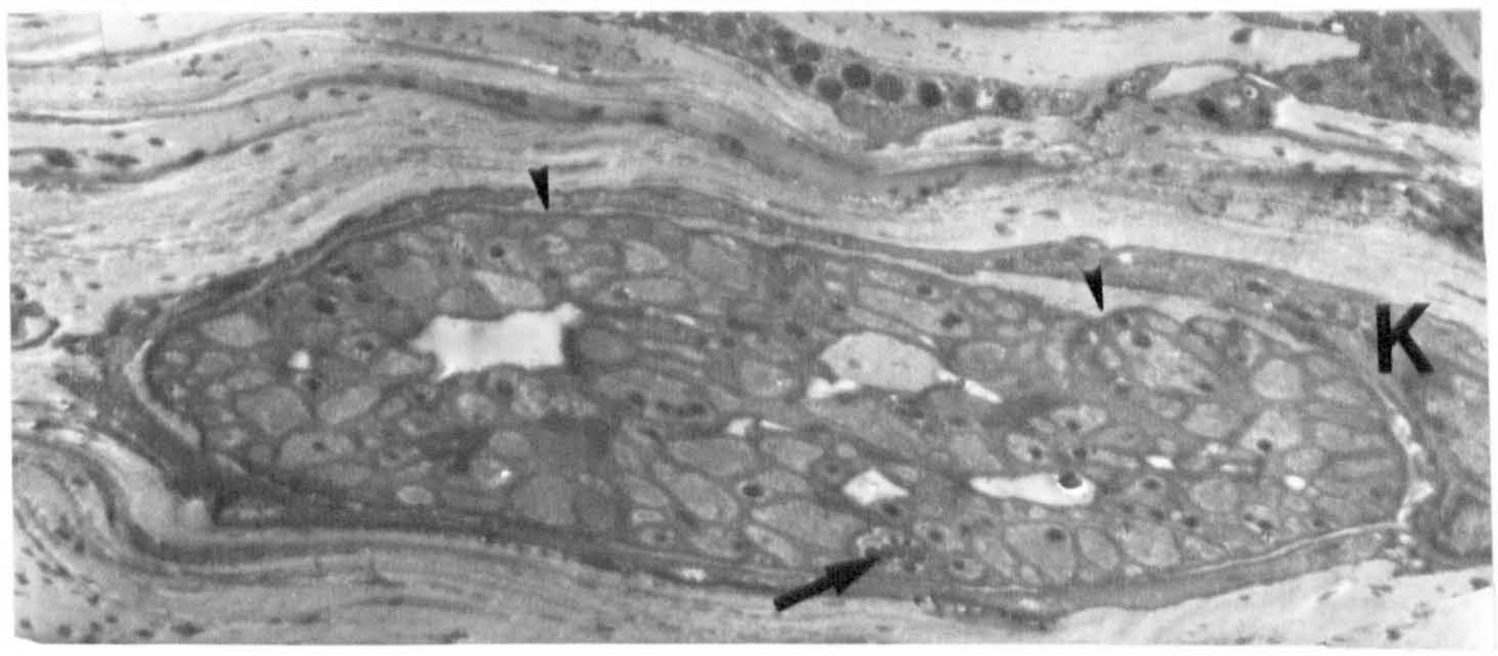


Figure 4.7

Stromal nerve bundle surrounded by what appears like perineurium. The section, however, is from the central cornea and the nerve bundle is completely enclosed by a keratocyte process (K). The basement membrane (arrowhead) has become very thin and the nerve contains more than 100 axons. A single varicosity is seen with a large number of mitochondria (arrow). (Keratoconus : 8,600 X)

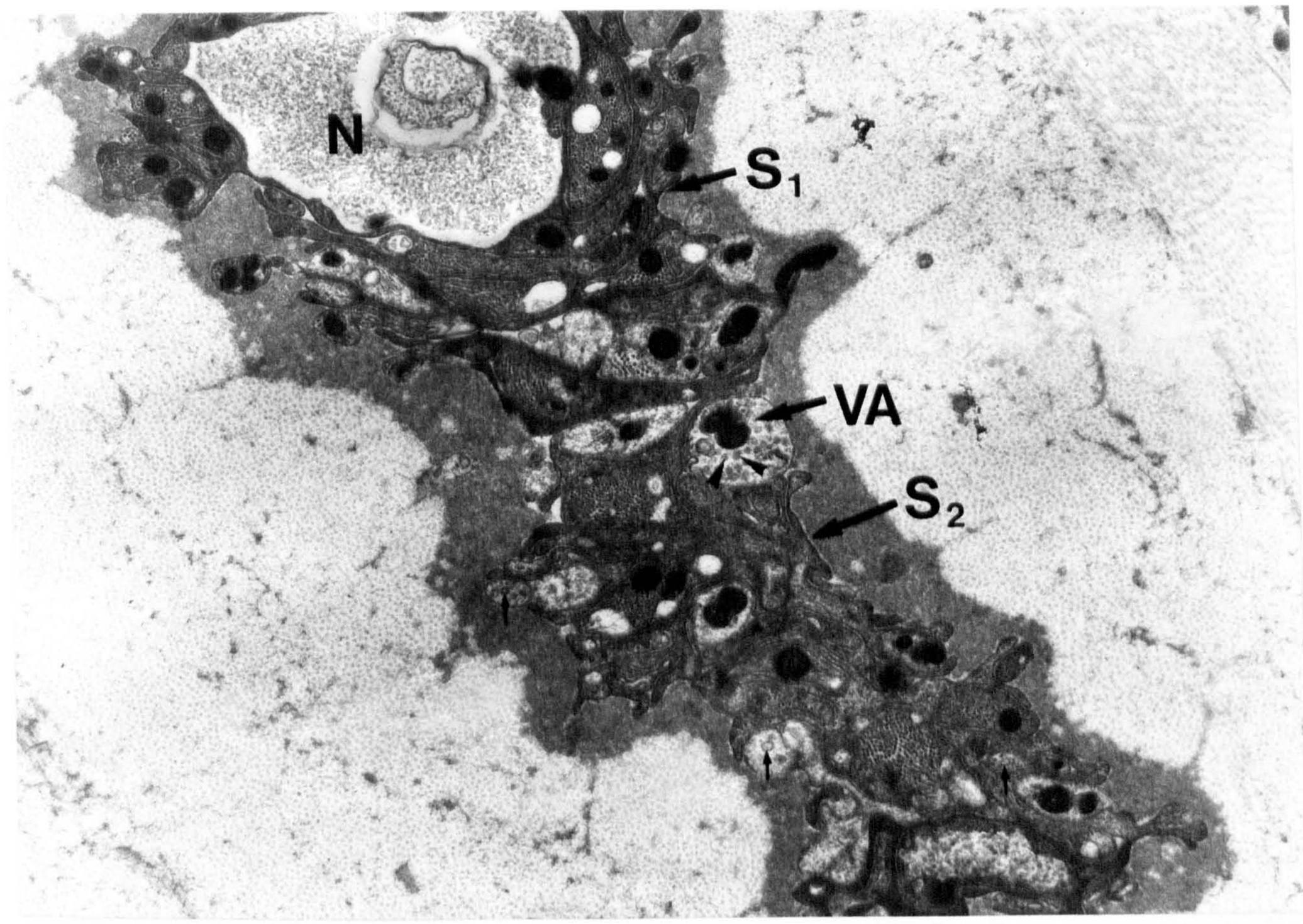


Figure 4.8

Transverse section of a mid-peripheral corneal nerve. Note the variation in electrondensity of the Schwann cell processes of two different Schwann cells (S1 & S2). The pale structure (N) is assumed to be a Schwann cell nucleus in poor condition. An axon varicosity (VA) is seen near the centre of the nerve bundle containing mitochondria and dense cored vesicles (arrowheads). Other axons show clear centred vesicles (short arrows). The basement membrane is thick and electron dense. (Keratoconus : 16,600 X)

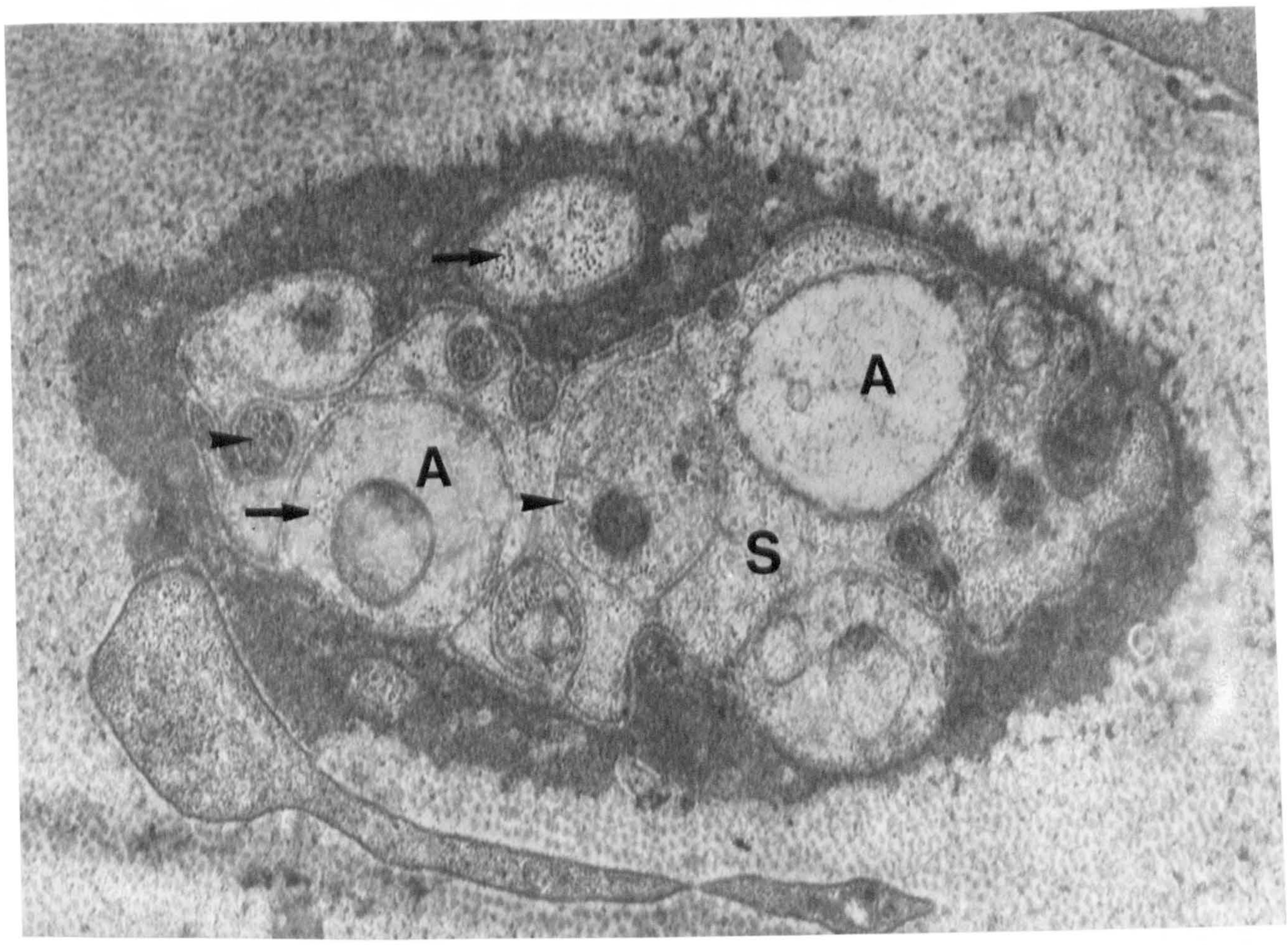


FIGURE 4.9

Transverse section of a mid-peripheral corneal nerve. The electrondensity of the Schwann cell cytoplasm (S) and the axons (A) was similar. Neurofilaments (arrows) and microtubules (arrowheads) are seen in the axons. Mitochondria are of similar size in axons and Schwann cytoplasm. The basement membrane is thick and electron dense.
(Control : 32,300 X)

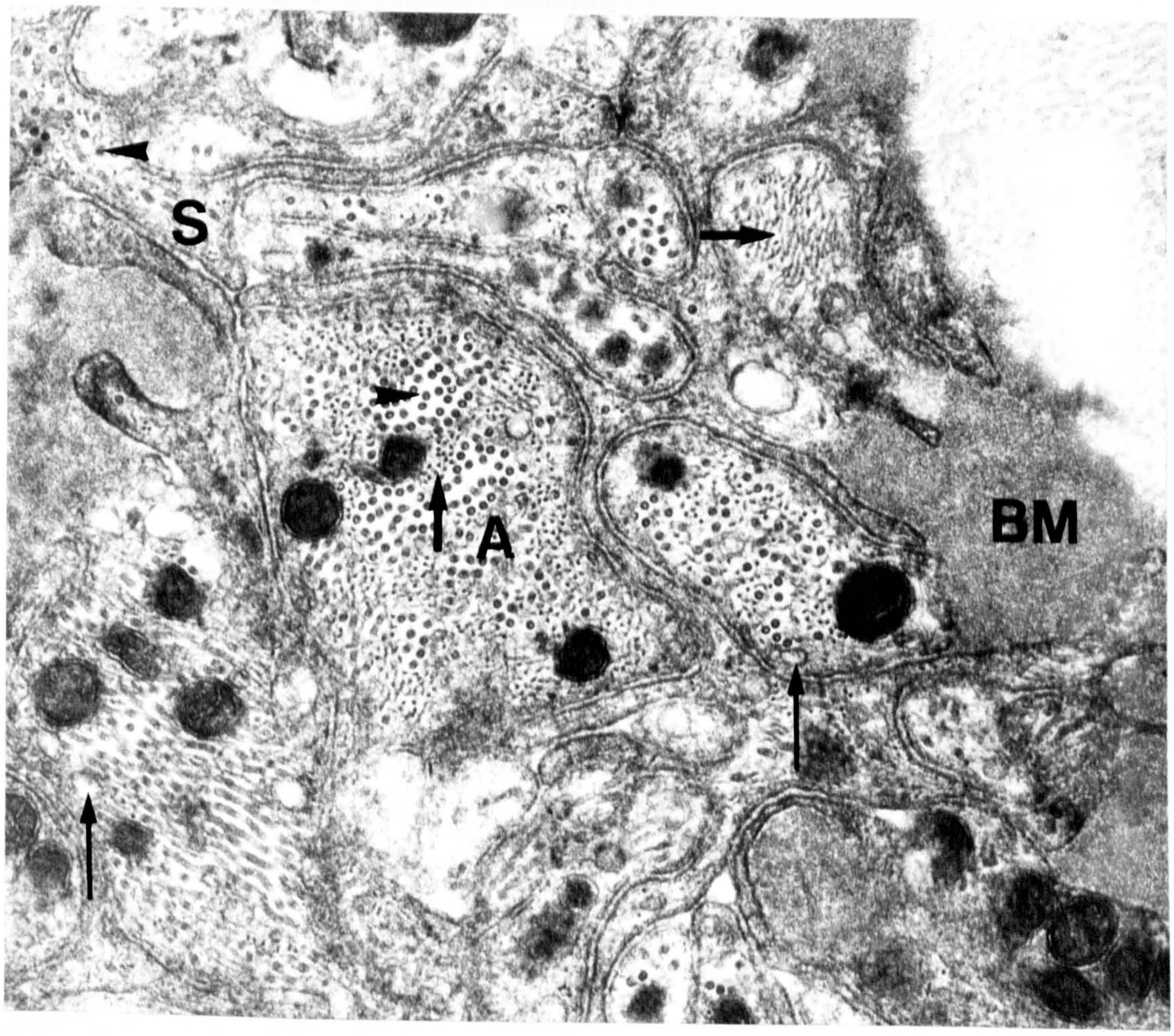


FIGURE 4.10

Transverse section of a mid-peripheral corneal nerve. The electrondensity of the Schwann cell cytoplasm (S) and the axons (A) was similar. Neurofilaments (thick arrows) are seen in the axons and microtubules (arrowheads) are present in axons and Schwann cell processes. Some axons contain vesicles (thin arrows). The basement membrane (BM) appears electron dense. (Keratoconus : 50,000 X)

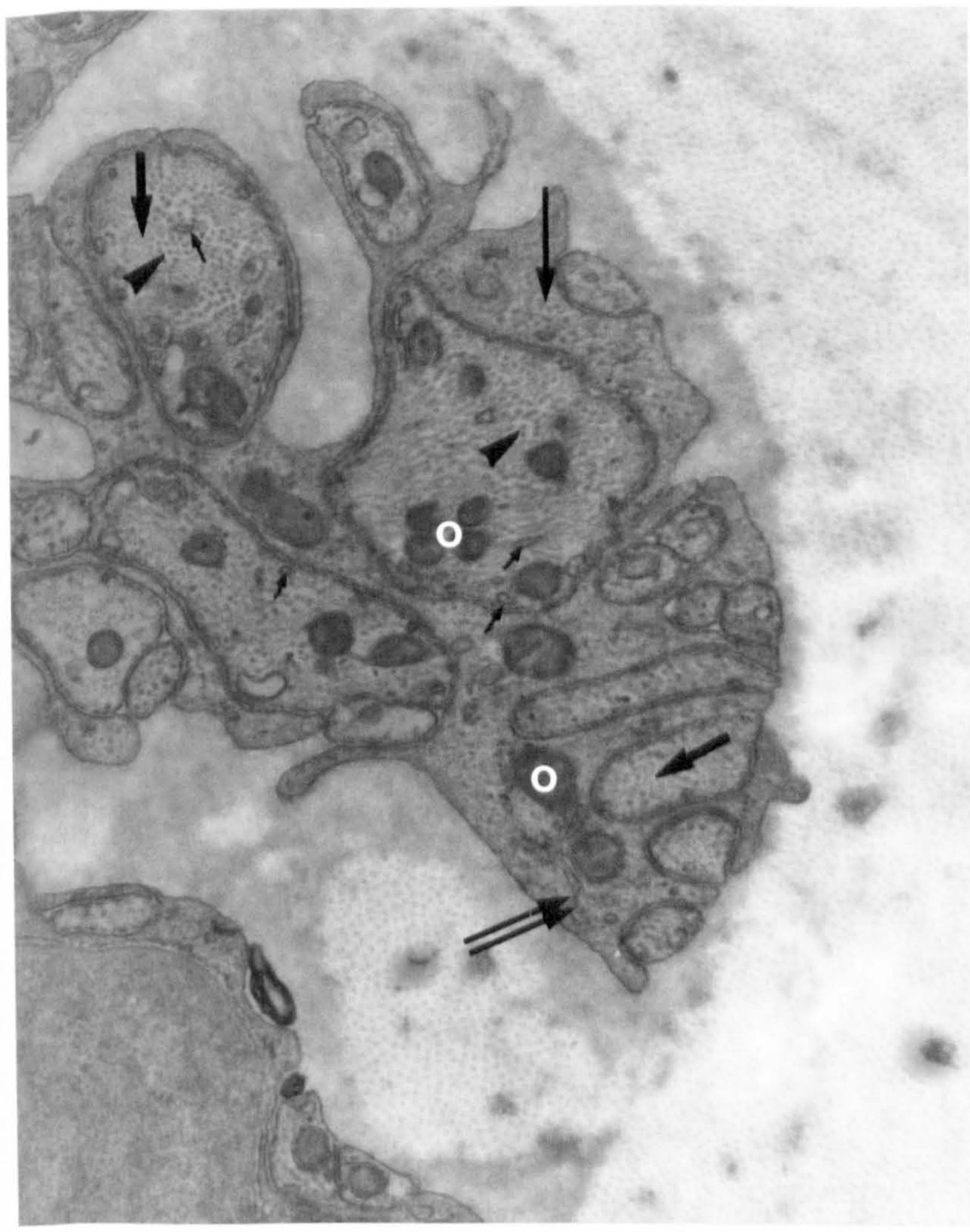


FIGURE 4.11

Transverse section of a mid-peripheral corneal nerve. The Schwann cell cytoplasm contains large mitochondria (circle), microtubules (long arrow) and traces of rough endoplasmic reticulum (double arrow). Axons are unmyelinated and contained in Schwann cell processes. The axoplasm contains microtubules (arrowheads), neurofilaments (thick arrows), vesicles (thin arrows) and mitochondria (circle) which are smaller than those seen in the Schwann cell. The basement membrane appears electron lucid. (Control : 50,000 X)

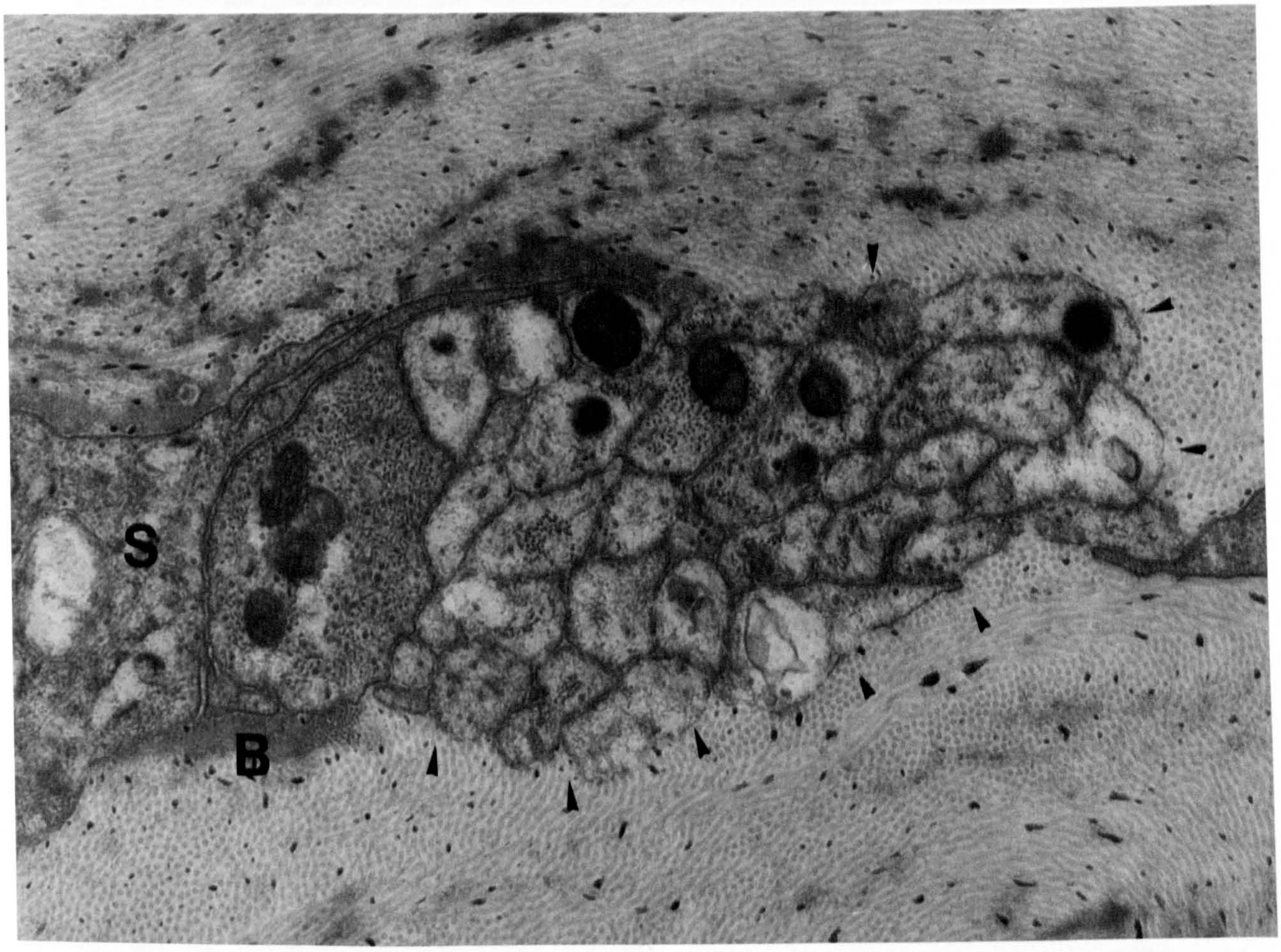


FIGURE 4.12

Transverse section of a central corneal nerve containing many axons. A basal lamina (B) surrounds the Schwann cell (S) but is absent from much of the bundle perimeter (arrowheads).

(Keratoconus : 18,000 X)

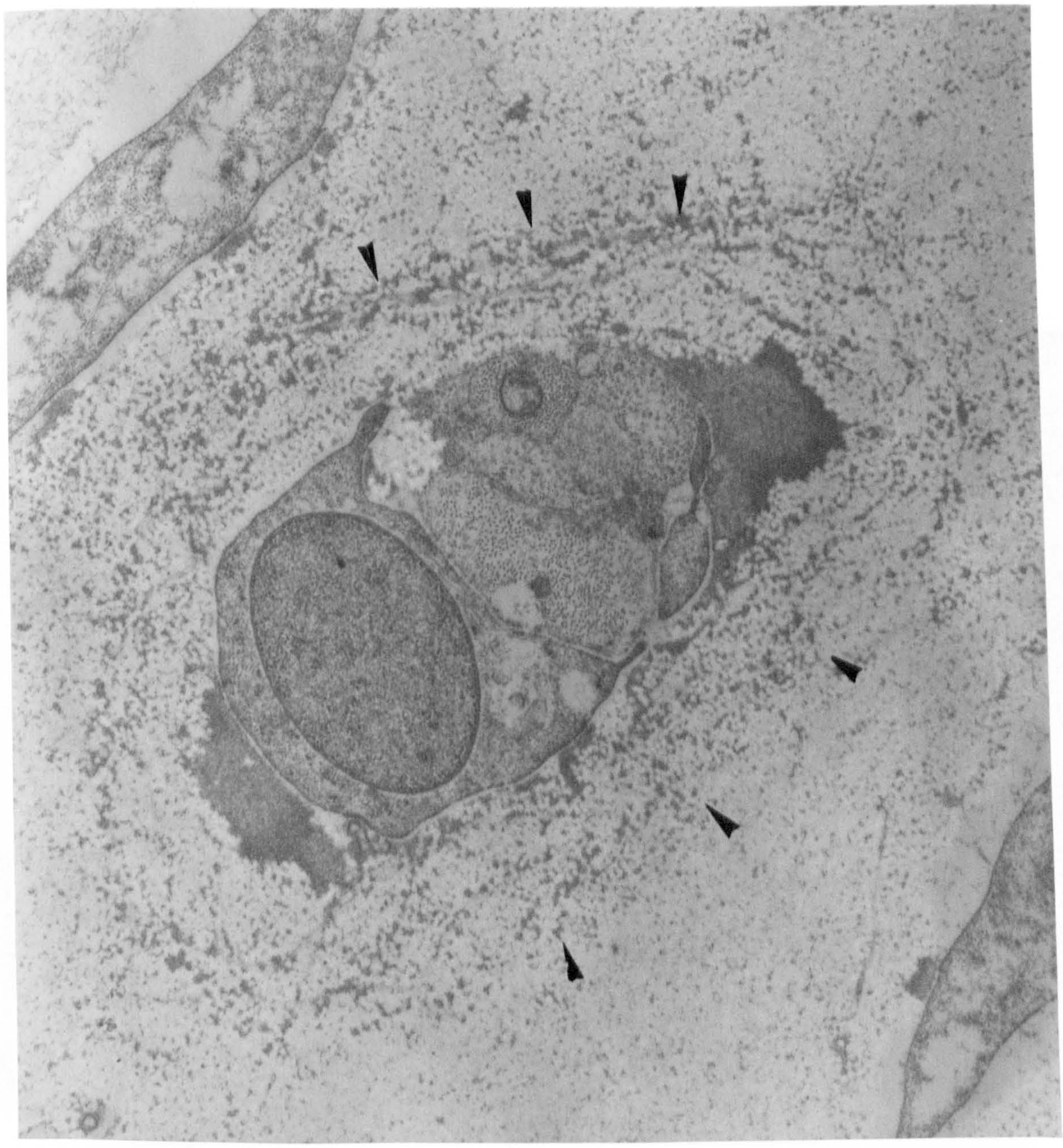


FIGURE 4.13

Transverse section of a central corneal nerve. A halo of electron dense material (arrows) is seen surrounding the nerve bundle. The material appeared similar to the basal lamina in electon density and structure. (Control : 17,600 X)

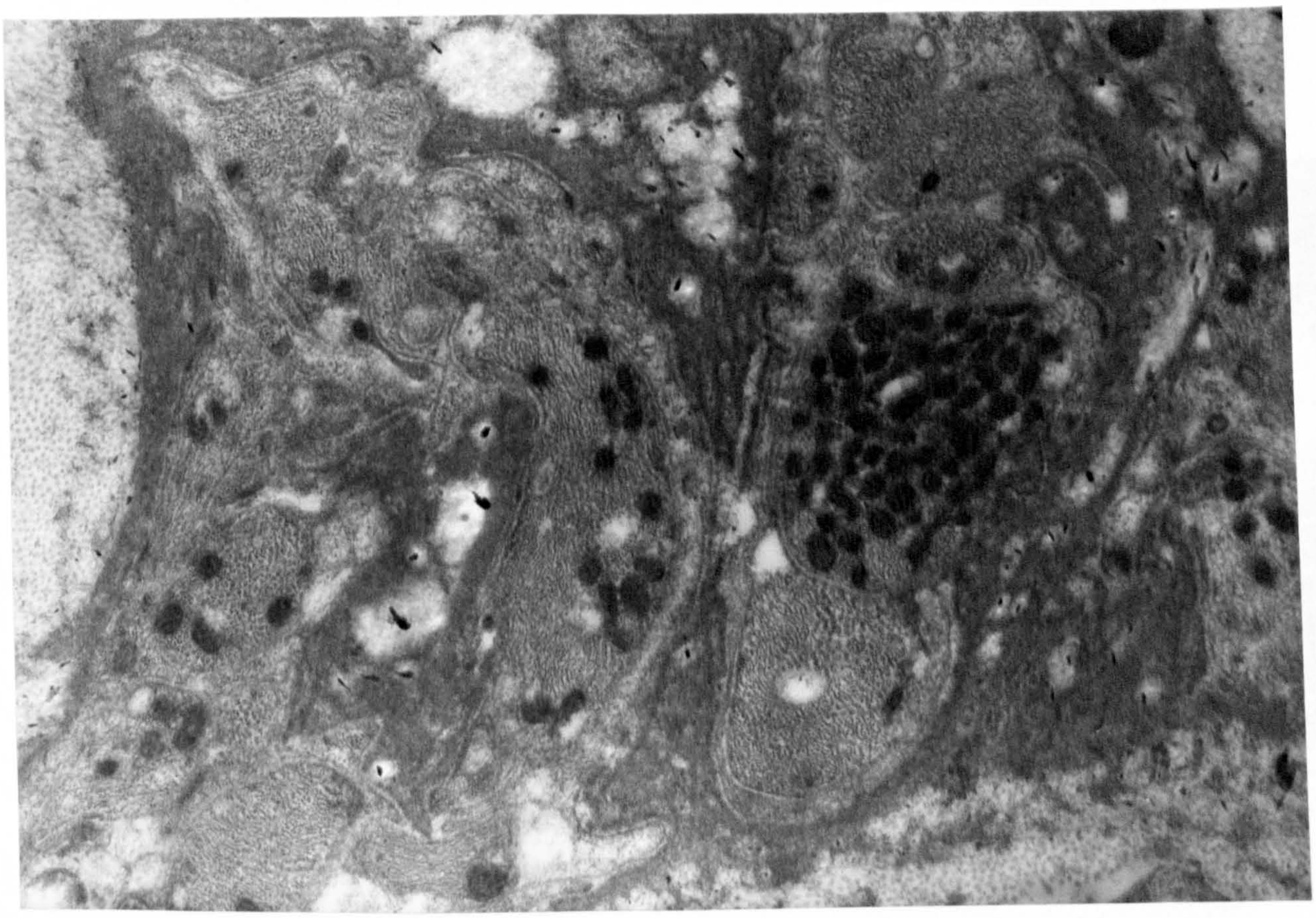


FIGURE 4.14

Central stromal nerve showing an axon varicosity packed with mitochondria but without vesicles. (Keratoconus : 24,000 X)



FIGURE 4.15

Mid-peripheral stromal nerve where two axon varicosities (VA) are seen to be packed with vesicles and mitchondria.

(Keratoconus : 31,800 X)

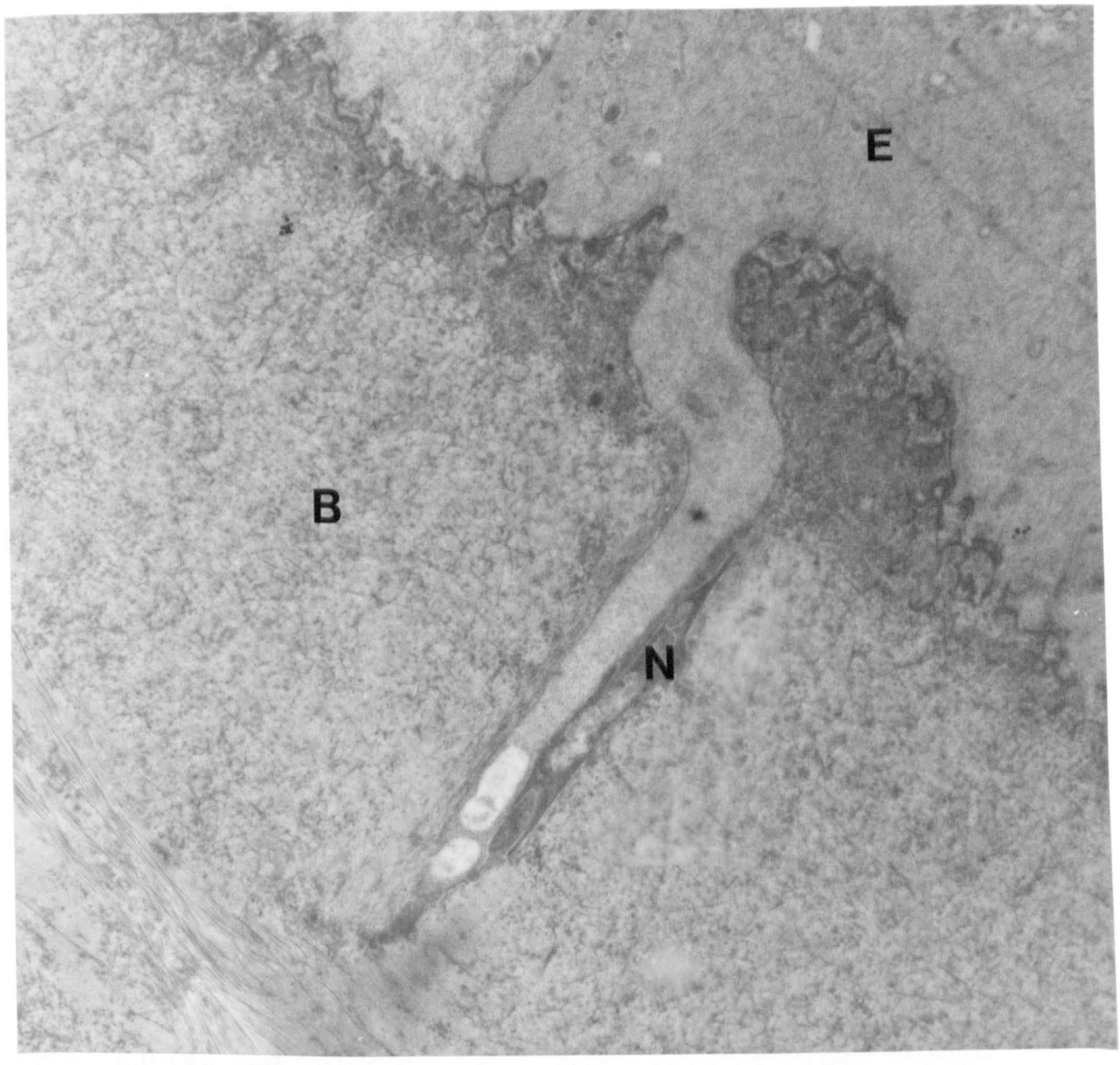


FIGURE 4.16

Penetration of Bowman's layer (BM) by epithelial cell (E) and nerve process (N) (see main text). The basement membrane is considerably thicker in the localised area of penetration. (Control : 12,000 X)

4.3 EPITHELIAL NERVE MORPHOLOGY

4.3.1 Axon Position

Epithelial axons or groups of axons were most frequently identified in the basal epithelial cell layer and very close to the basement membrane. Axon processes could not be identified above the basal epithelial cell layer. On occasions where a group of axons was sectioned longitudinally the axons were usually running parallel to the basement membrane. On one occasion the axons were observed to run at 90° to the plane of the basement membrane.

Epithelial axons were found in either of three positions relative to basal epithelial cells: Basal-buried (BB), inter-cellular (IC) or lateral-buried (LB). Basal-buried axons were situated in an epithelial cell infolding originating from the base of the cell (Figure 4.17 & 4.18). Inter-cellular axons or group of axons were located between neighbouring epithelial cells (Figure 4.19 & 4.25). Basal-buried and inter-cellular axons were the most frequent types found in the keratoconic and control epithelium. Lateral-buried axons were rarely encountered and were situated in infoldings originating from the epithelial cell membrane facing another epithelial cell (Figure 4.20). The cumulative frequency distribution of basal-buried, inter-cellular and lateral-buried axons for the

mid-peripheral and central corneal positions in keratoconus and control are shown in Figure 4.21. Keratoconic corneas have a larger proportion of axon groups located inter-cellularly.

Basal-buried axons occasionally showed desmosomes located at the opposing membranes of the same epithelial cell (Figure 4.22), and occasionally a gap junction could be identified (Figure 4.23).

4.3.2 Axon Quantity

Groups containing several axons were frequently encountered in keratoconic epithelium (Figures 4.19, 4.26 & 4.27) in contrast to control epithelium where axons usually appeared single (Figures 4.17, 4.18, 4.22 & 4.23). The groups which contained many axons were found inter-cellularly whereas single axons usually appeared in the basal-buried position. Quantitative data on density of epithelial axons and variation in axon diameter are presented separately in Chapter 5.

4.3.3 Content of Epithelial Axons

Axoplasm of epithelial axons contained microtubules, neurofilaments, mitochondria and occasionally vesicles (Figure 4.19, 4.24, 4.26 & 4.27). In the control material, mitochondria appeared pale in a number of axons. This may be secondary to inadequate fixation by the formalin used or be

due to a delay in fixing the tissue. Although these factors were beyond control, they did not interfere with the overall quality of the material.

Local enlargements or varicosities of epithelial axons were encountered in keratoconic and control material. They were identified in groups of axons when sectioned transversely or longitudinally and showed accumulations of mitochondria with vesicles frequently being present (Figures 4.24, 4.26 & 4.27).

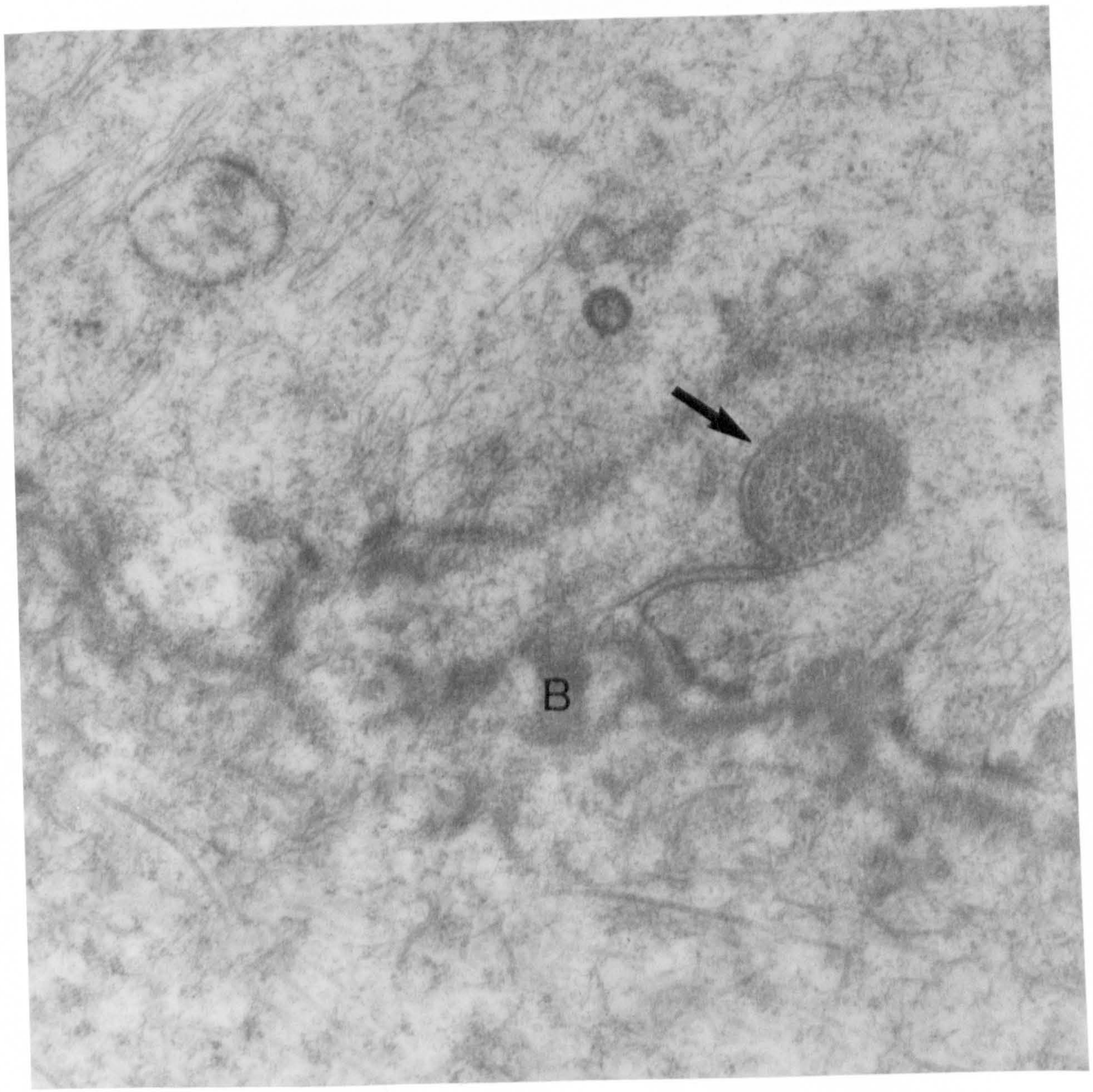


FIGURE 4.17

Single epithelial axon (arrow) invaginating the base of an epithelial cell (basal-buried type). The axon is located close to the basement membrane (BM). (Control : 60,000 X)

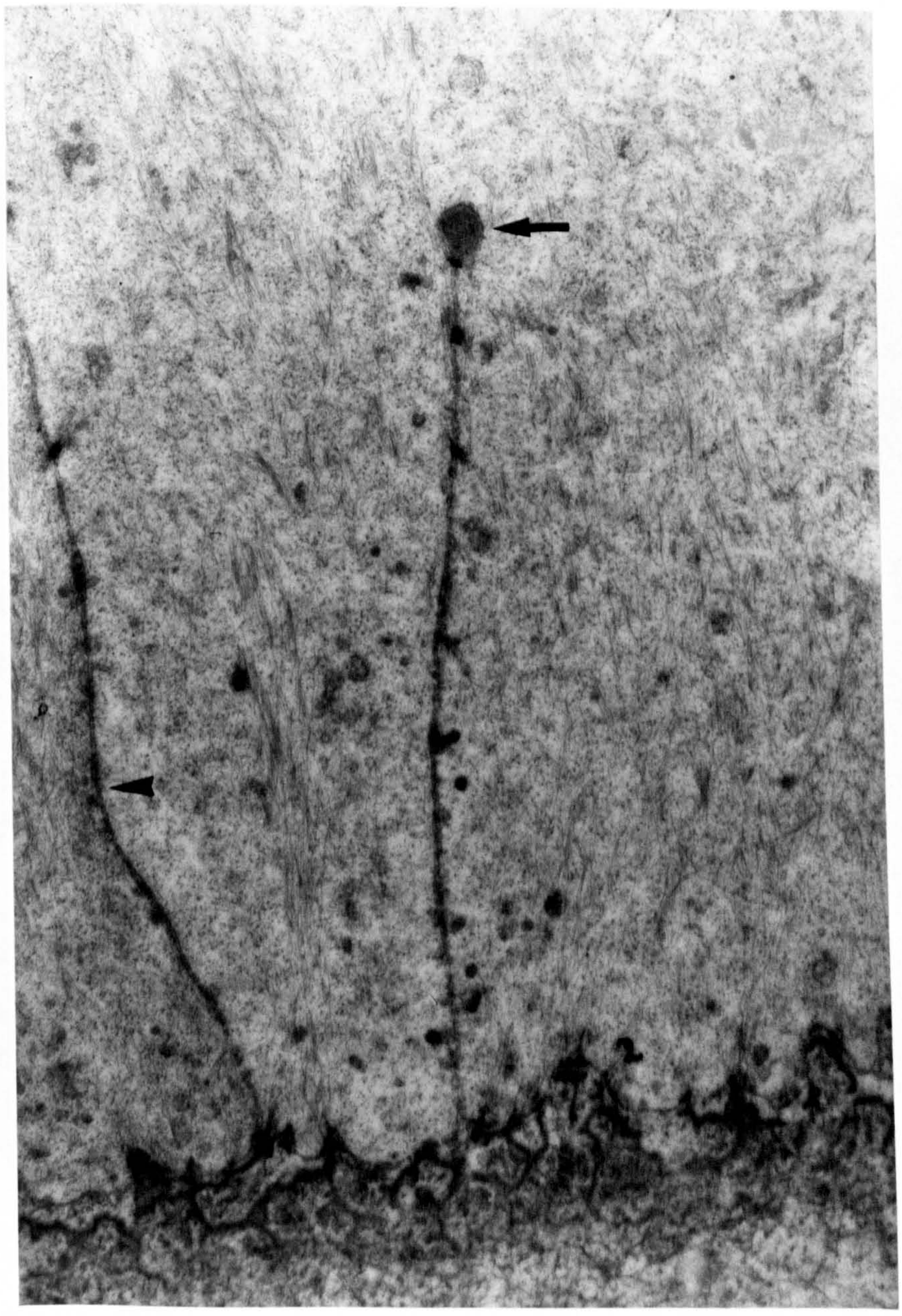


FIGURE 4.18

Single epithelial axon - basal buried type (arrow) but located unusually far from the basement membrane (BM) in the basal epithelial cell. The arrowhead indicates the border between two epithelial cells. (Control : 13,300 X)



FIGURE 4.19

Group of epithelial axons located between epithelial cells (inter-cellular axon types). Mitochondria (m) and microtubules (arrowheads) are abundant. The group of naked axons are located very close to the basement membrane (BM).
(Keratoconus : 29,200 X)

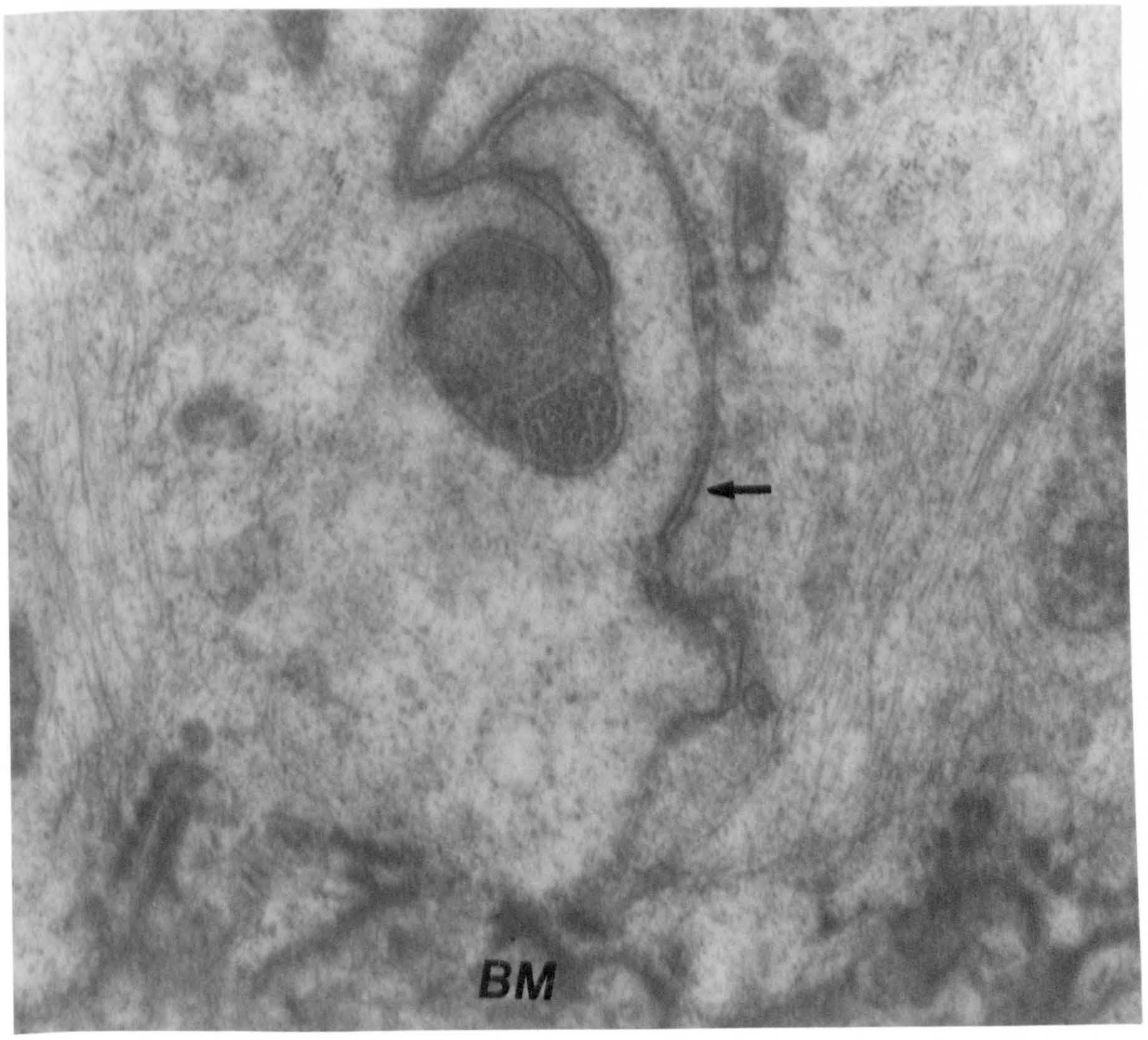


FIGURE 4.20

Epithelial axons located in an infolding from the lateral border of an epithelial cell (lateral-buried type). The axons seen are only a short distance from the basal membrane (BM). The arrow indicates the border between two epithelial cells. (Control : 50,000 X)

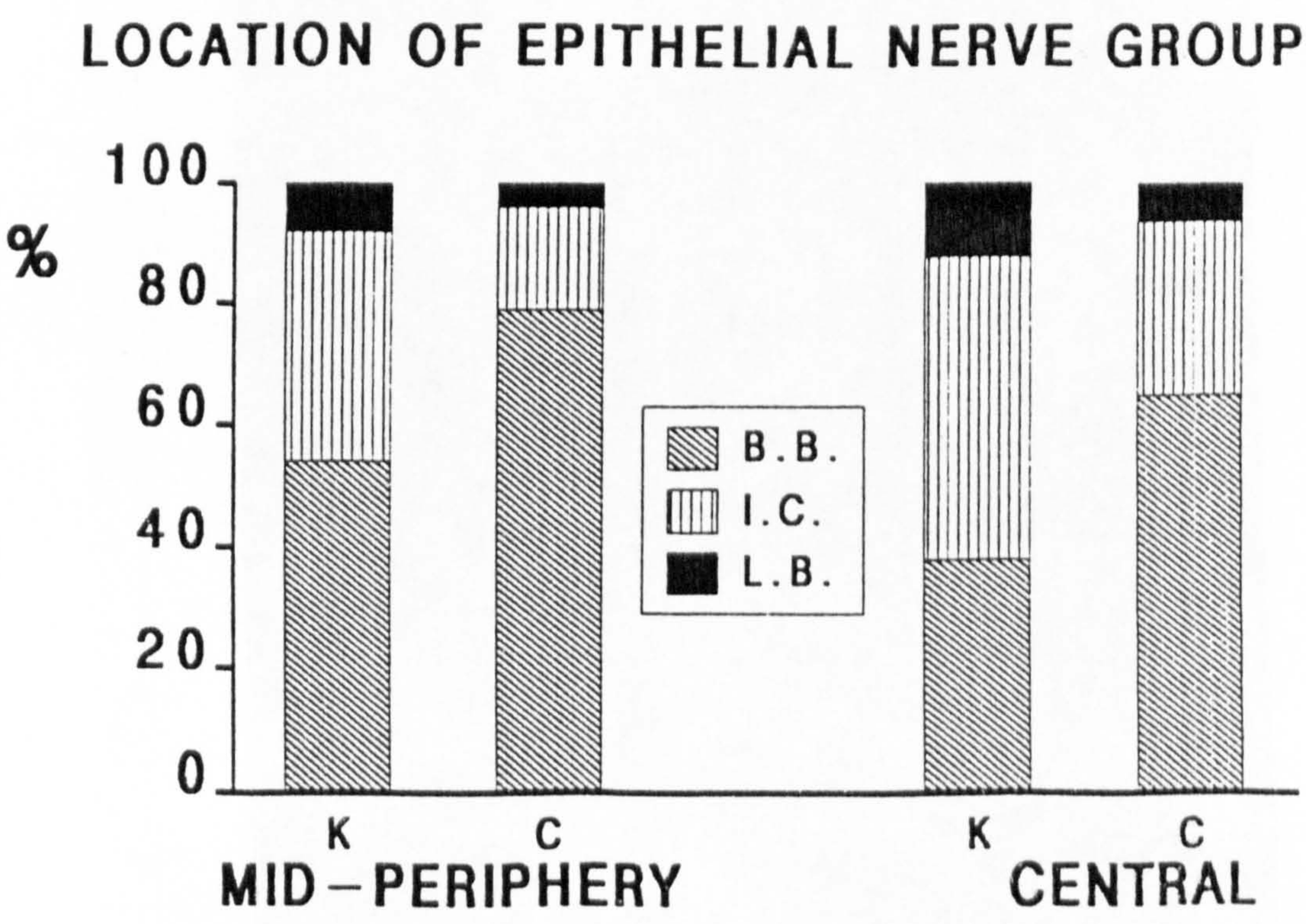


FIGURE 4.21

Cumulative frequency distribution of basal-buried (BB), inter-cellular (IC) and lateral-buried (LB) axons for the keratoconic (K) mid-peripheral (n=295) and central (n=177) positions and control corneas (C) for the mid-peripheral (n=507) and central (n=221) positions.



FIGURE 4.22

Single epithelial axon (Basal Buried type) showing desmosome attachment (arrowhead) of adjacent cellular membranes belonging to the same epithelial cell. (Control : 46,700 X)

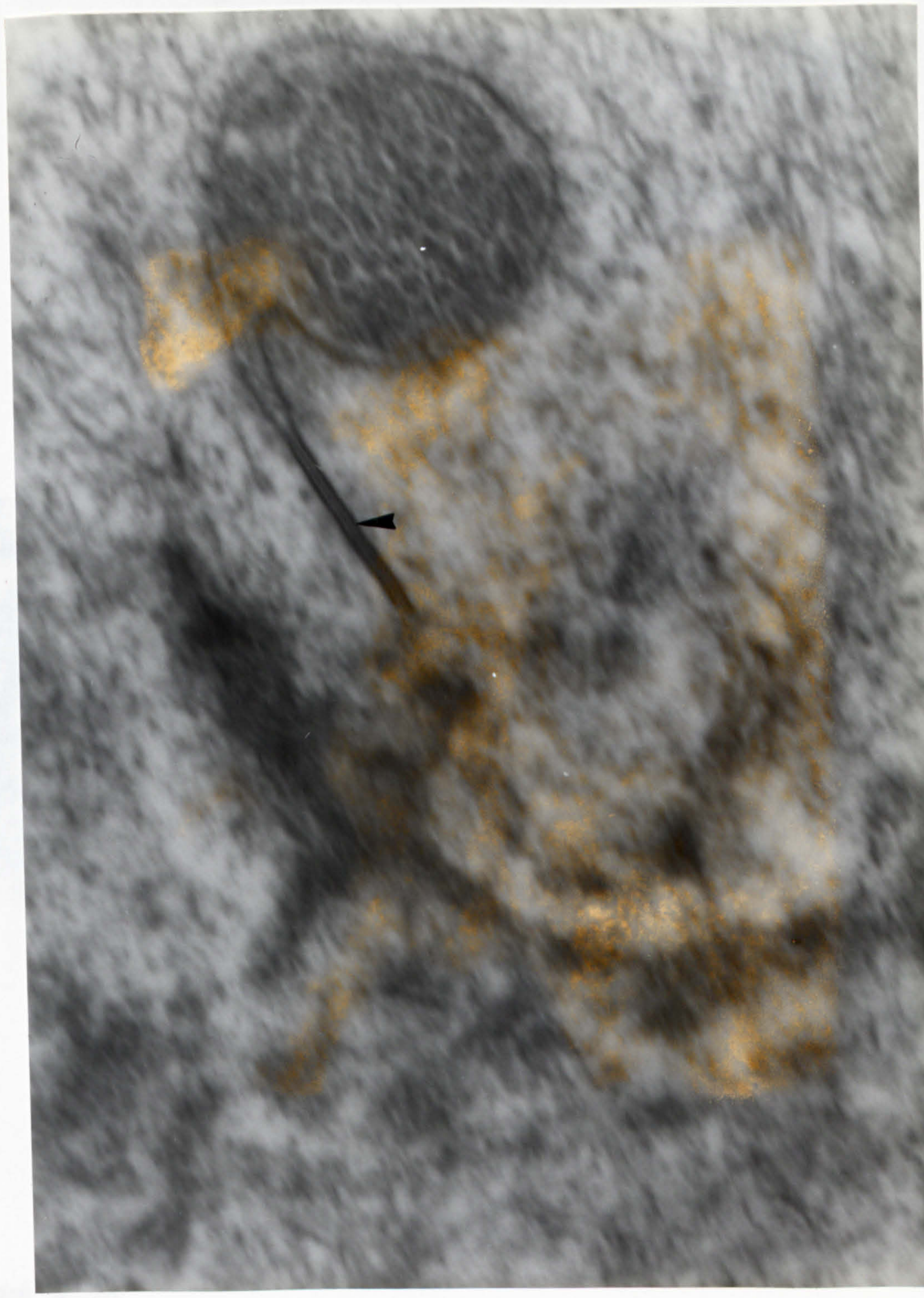


FIGURE 4.23

A single basal epithelial cell show a gap junction (arrowhead) in association with a single epithelial axon (Basal Buried type). (Control : 125,000 X)

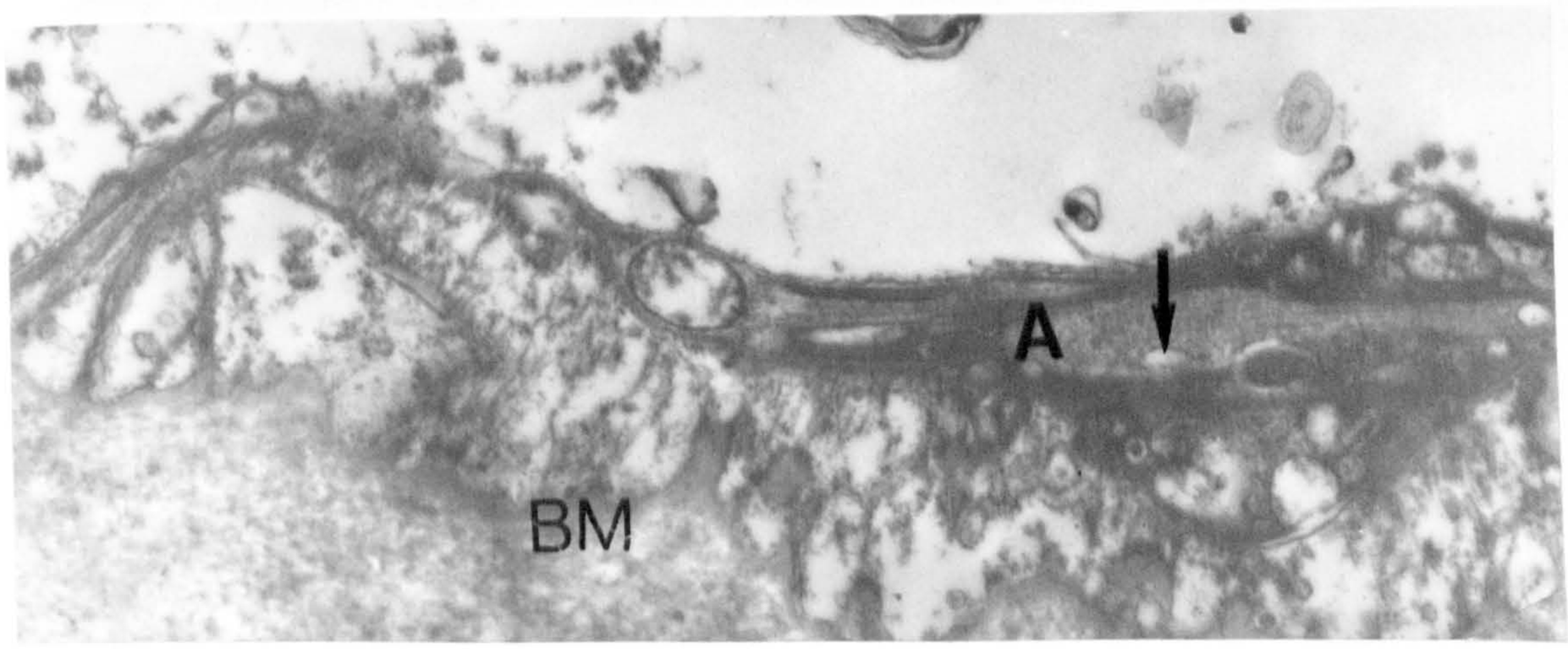


FIGURE 4.24

Group of epithelial axons in longitudinal section. The axons (A) run parallel to the basal membrane (BM). A varicosity is seen containing mitochondria and vesicles (arrow).

(Keratoconus : 16,700 X)

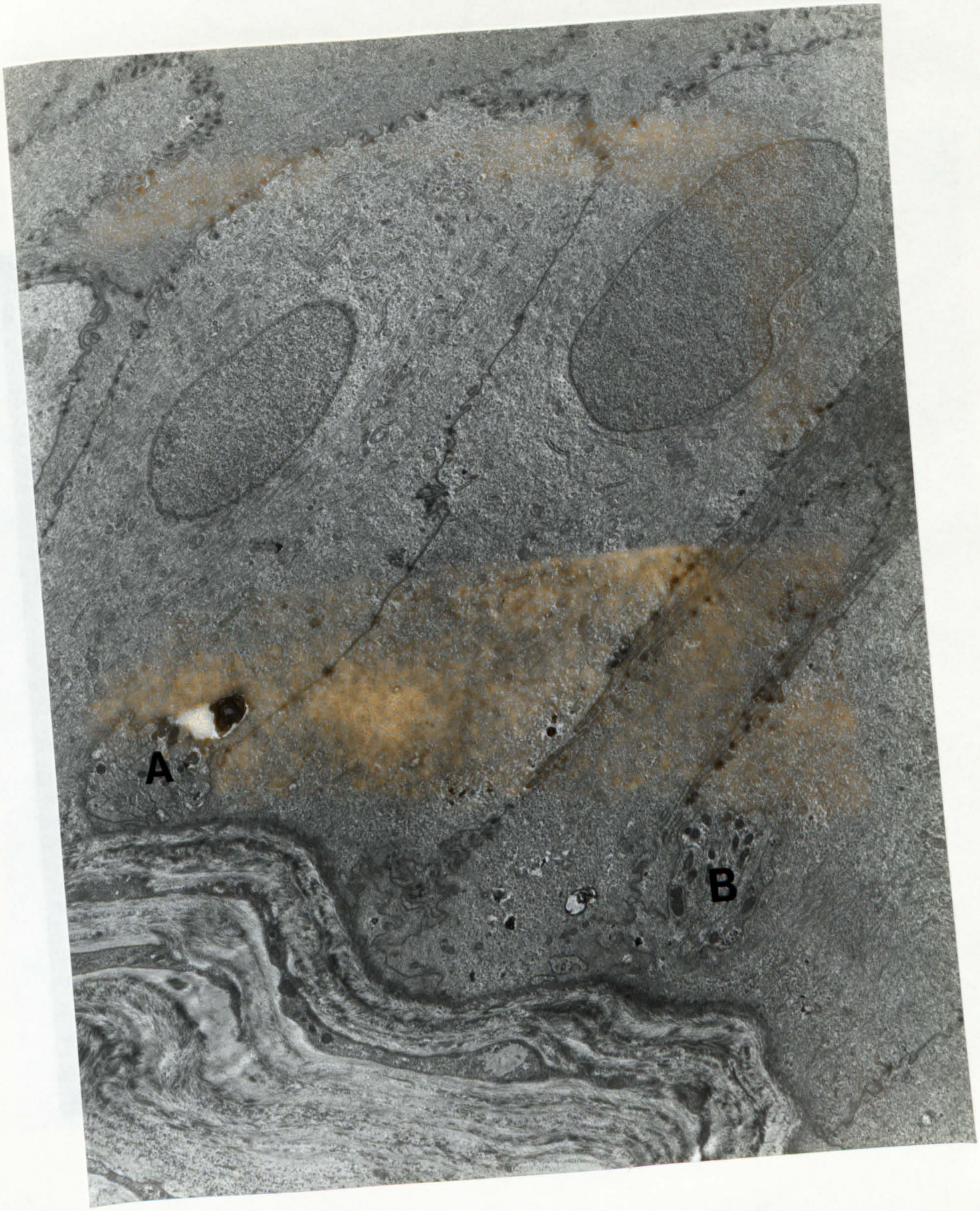


FIGURE 4.25

Two large groups of axons (A & B) are seen in the basal epithelial cell layer close to the basement membrane. Both axon groups are located between epithelial cells (Inter Cellular type). (Keratoconus : 7,500 X)

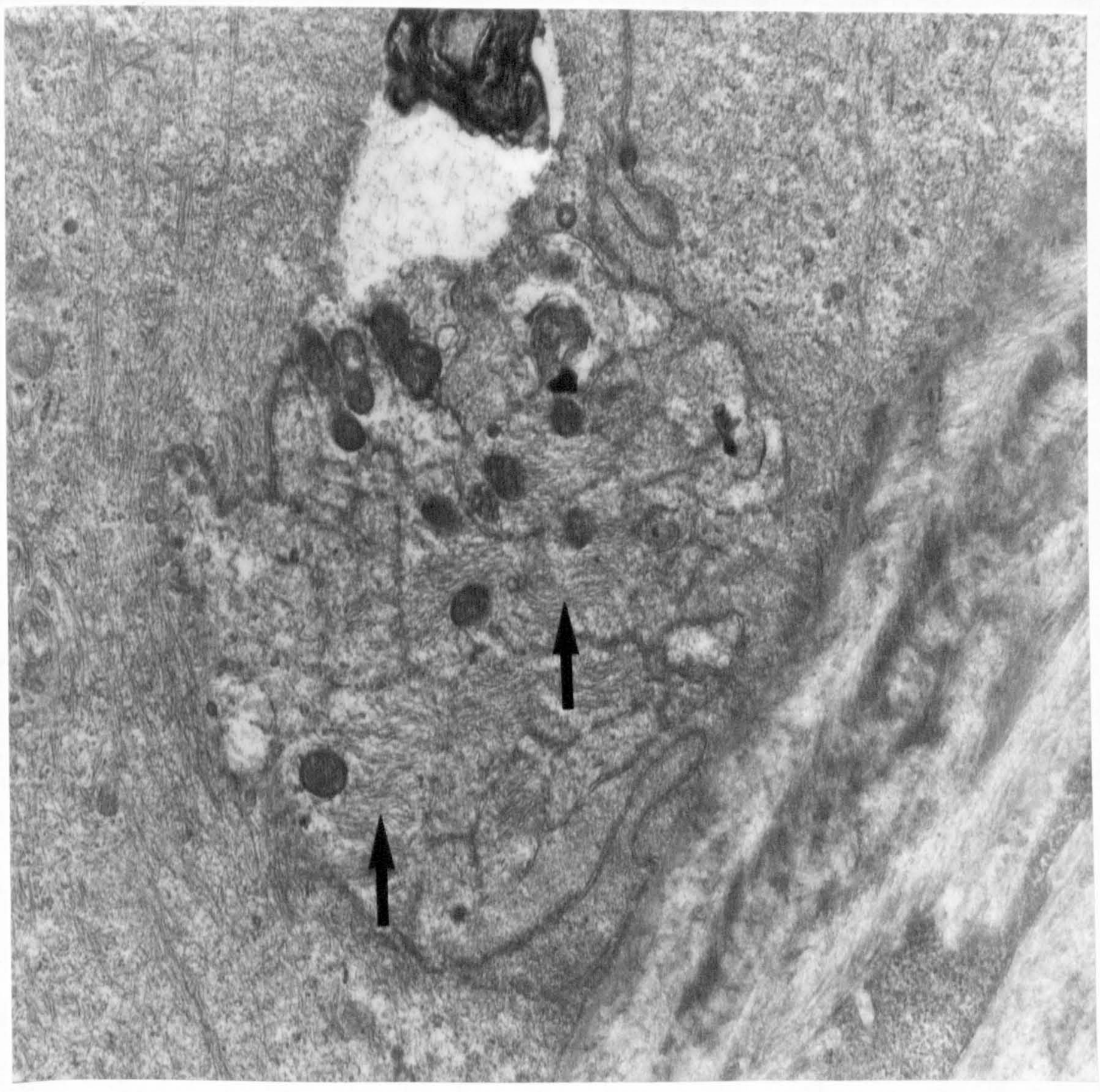


FIGURE 4.26

High magnification of axon group A illustrated in Figure 4.25. Neurofilaments (arrows) and mitochondria are seen in the large number of axons. (Keratoconus : 35,000 X)

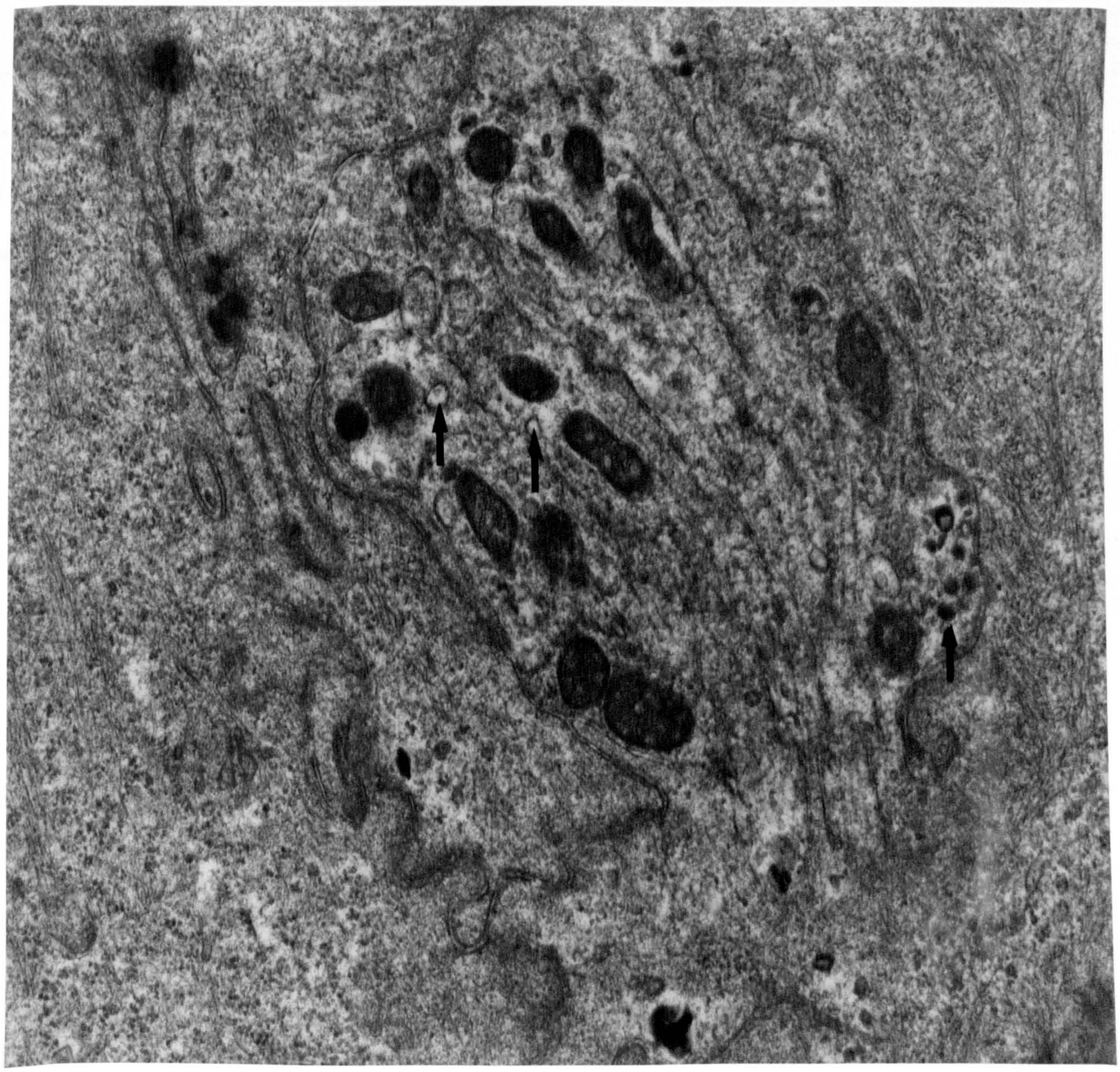


FIGURE 4.27

High magnification of axon group B illustrated in Figure 4.25. Vesicles are present in some of the axons (arrows).

(Keratoconus : 35,000 X)

4.4 DESENSITISED KERATOCONIC MATERIAL.

The desensitised keratoconic cornea showed clear signs of reinnervation having taken place. No axons were observed without a Schwann cell support, and no empty Schwann cell sheaths were encountered. The Schwann cells rarely demonstrated a complete content of axons, and many "almost empty spaces" were seen (Figure 4.28). A sparse amount of electron lucid material could be seen in these "empty spaces", and is thought to be cellular remnants. The Schwann cells appeared to be in very good condition with the normal organelles being present. The axons were small in diameter, but otherwise contained the normal organelles of mitochondria, neurofilaments, microtubules and vesicles. This specimen was treated differently during fixation (see Chapter 2) and a normal keratoconic cornea was treated in a similar manner as a control. This control keratoconic material showed the same neural content as glutaraldehyde fixed keratoconus material with the exception that some mitchondria appeared a little pale (Figure 4.29). It can be concluded that the differences observed in the desensitised cornea were not induced by a different procedure of fixation. The desensitised keratoconic cornea was analysed for neural content with regard to number of axons per mm as well as axon diameter. These results are dealt with separately in Chapter 5.

No results could be obtained for the epithelium due to the poor state of the epithelial cells.

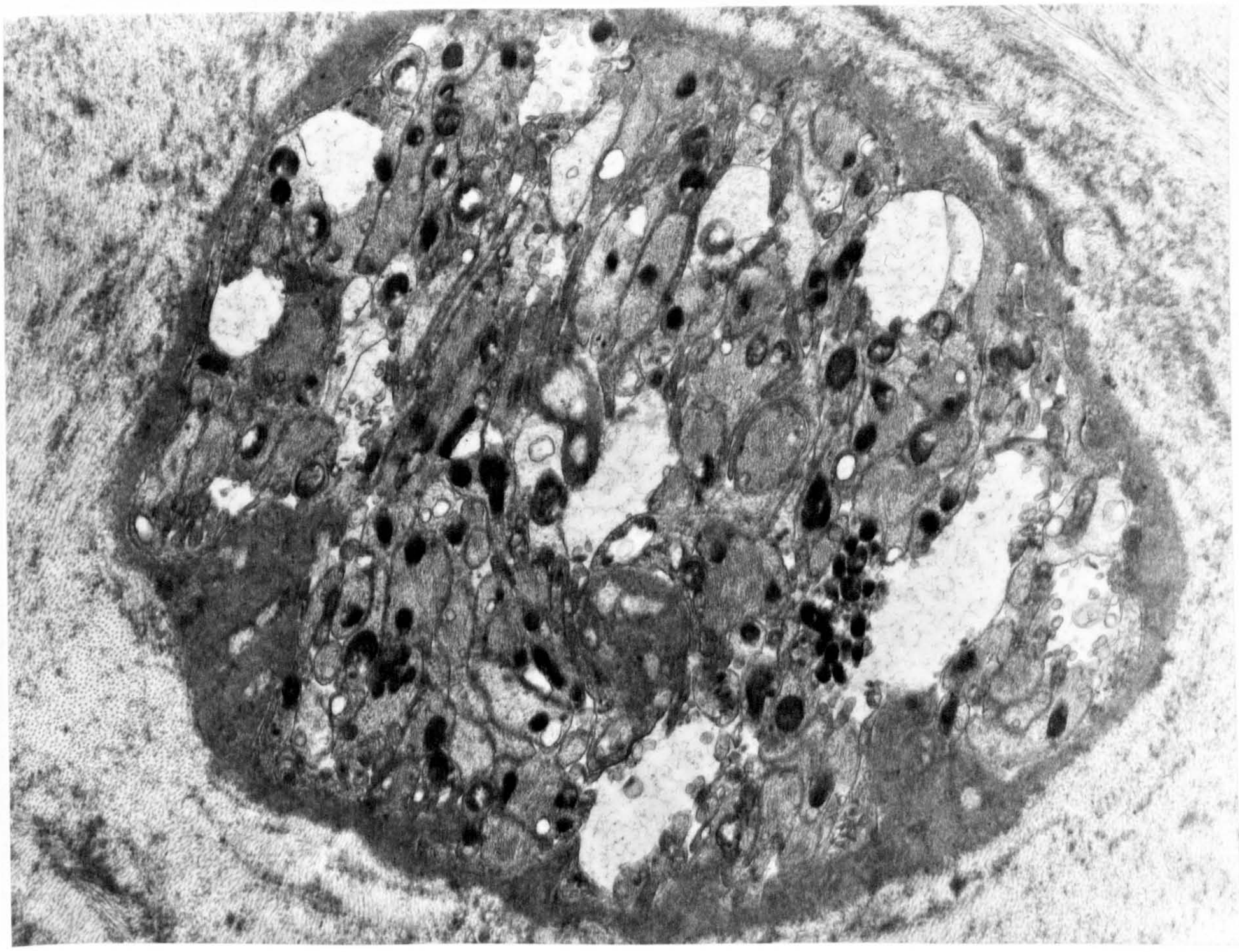


FIGURE 4.28

Stromal nerve bundle containing a large number of axons. Axons are small in diameter, and mitochondria and vesicles can be identified. The Schwann cell cytoplasm appears normal. Several areas are electron lucent with traces of flocculent material but without identifiable cellular content.

(Desensitised keratoconus - formal saline fixative : 11,500 X)

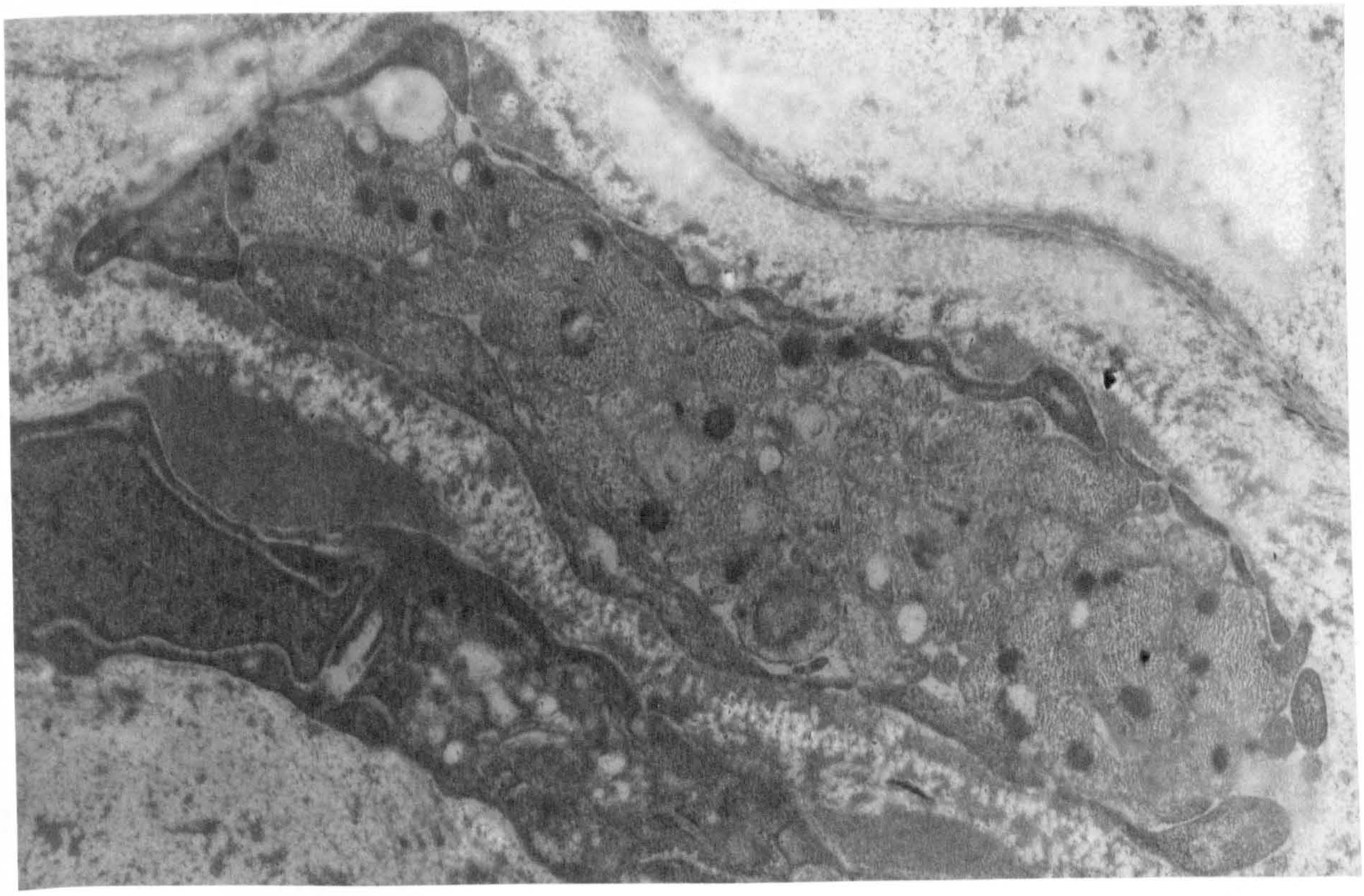


FIGURE 4.29

Stromal nerve bundle containing Schwann cell cytoplasm and axons in good condition. Microtubules, neurofilaments are visible and the mitchondria appear normal. The nerve does not contain such large areas with translucent material as seen in Figure 4.28.

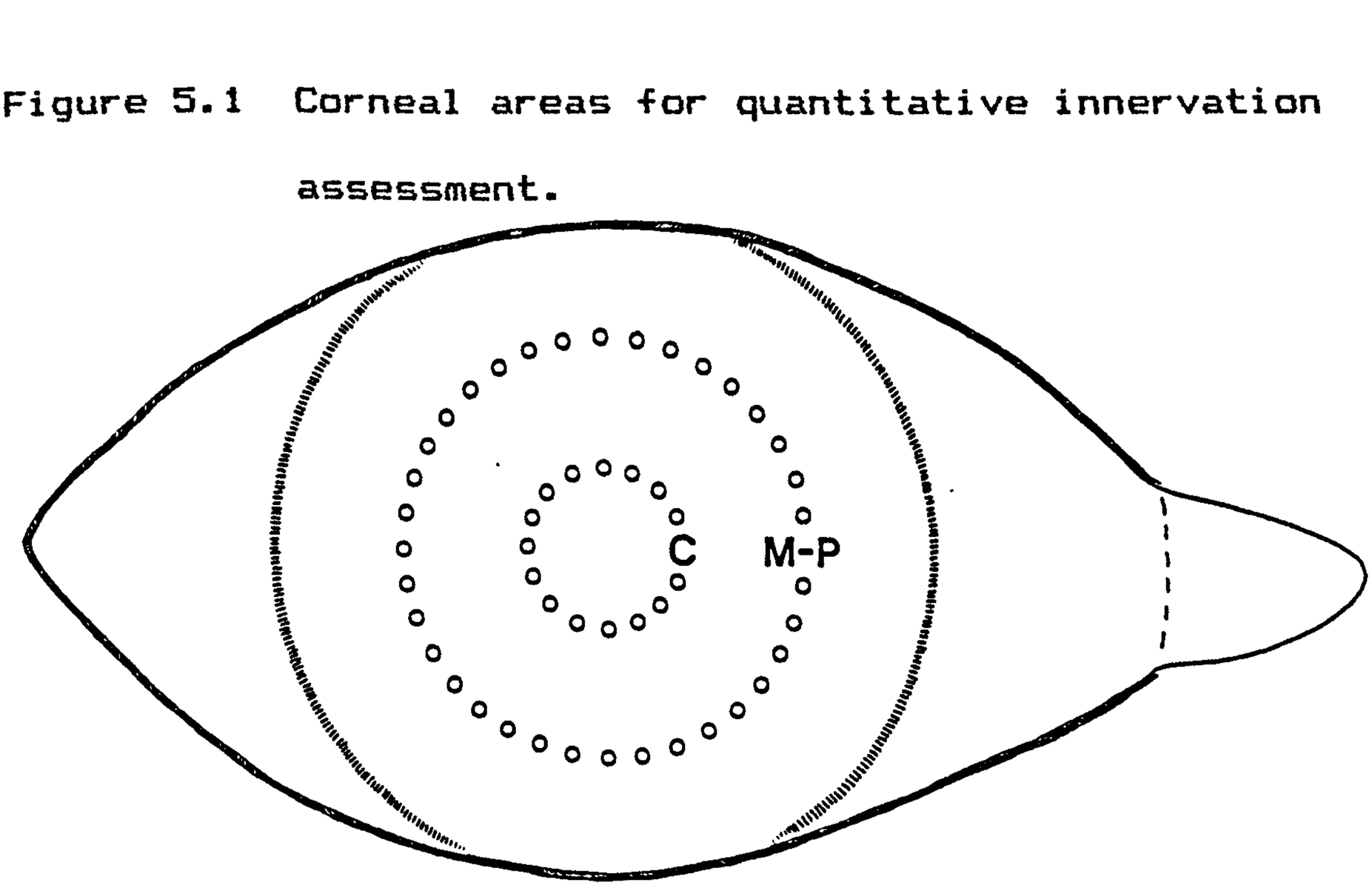
(Normal keratoconus - formal saline fixative : 20,000 X)

CHAPTER 5

QUANTITATIVE ASSESSMENT OF CORNEAL INNERVATION

Six keratoconic, five control and one desensitised cornea were used for this part of the study. The method of sampling has been described in detail in Chapter 2 and involves the mid-peripheral and central corneal areas as illustrated in Figure 5.1. Randomly selected electronmicrographs were used to test the repeatability of quantitative assessment of corneal innervation. The two independant assessments by Professor G.L.Ruskell and the author were in total agreement.

Figure 5.1 Corneal areas for quantitative innervation assessment.



M-P & C represent the mid-peripheral and central areas.

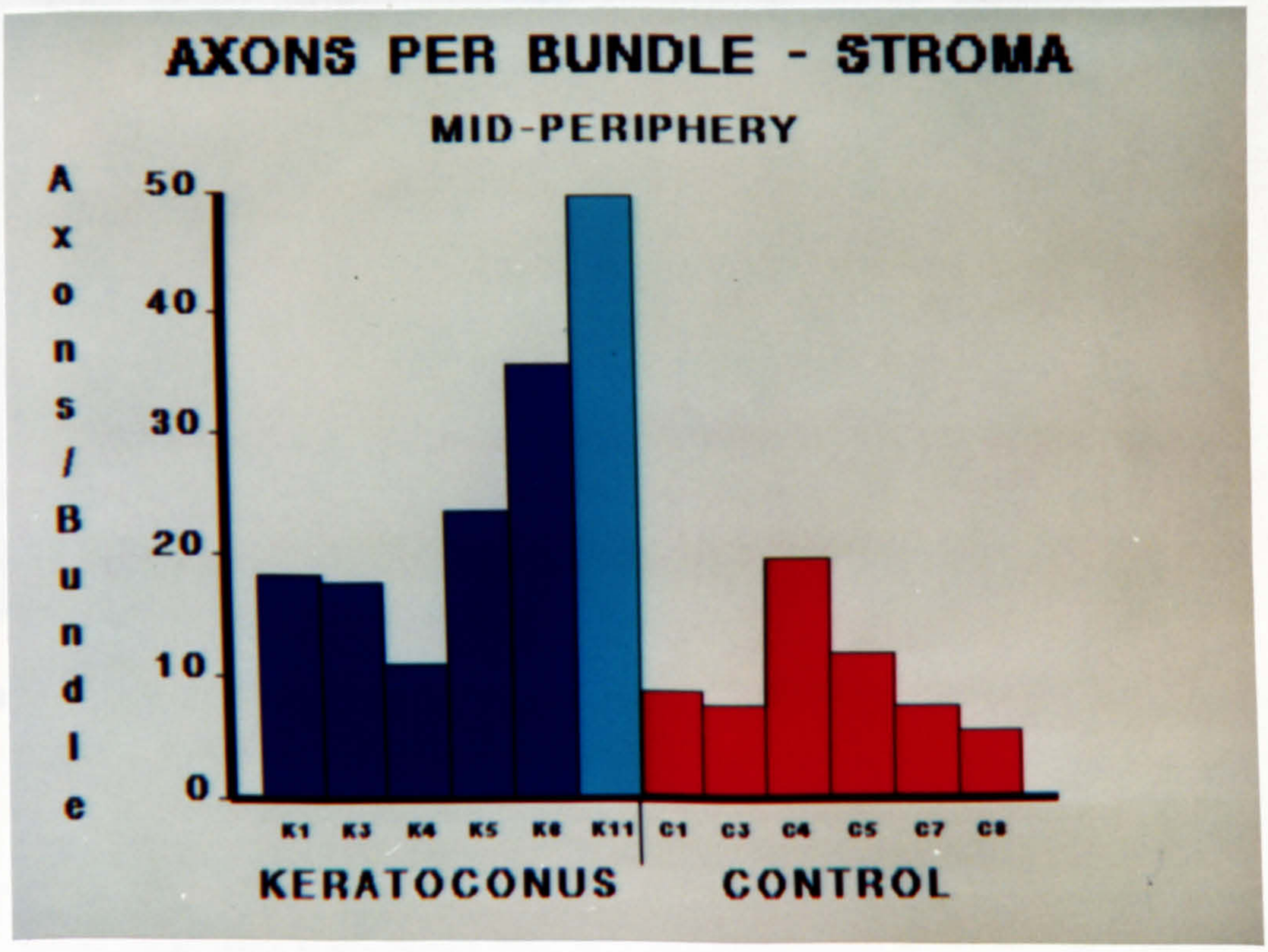
5.1 INNERVATION OF CORNEAL STROMA

5.1.1 Axons per nerve fibre bundle.

The axon content per bundle varied with the size of the bundle, corneal sampling position and the specimen studied. For the mid-periphery the range of axons per bundle was 1-73 for keratoconic, 1-56 for control corneas and 9-117 for the desensitised keratoconic cornea (K11). The central range was 1-204 for keratoconic, 1-25 for control corneas and 8-73 for K11. The mean axon content per nerve fibre in each specimen is illustrated Fig 5.2 & 5.3 for mid-peripheral and central cornea respectively. Appendix 10 provides detail of variability of axon content in nerve fibres within each specimen. The means are summarised in Fig 5.18, with the keratoconic group showing a higher mean than the control group of approximately twofold in the mid-peripheral and threefold in the central cornea. The desensitised keratoconic cornea showed the highest mean and variation of axon content per nerve fibre for mid-peripheral and central corneal areas (Table 5.1). For the mid-periphery, the keratoconic, control and desensitised corneas were all significantly different ($P<0.05$). This was not the case for the central corneal area where the only significant difference was between the desensitised and the control corneas ($P<0.001$). On average the axon content per nerve fibre was highest for the mid-peripheral compared to the central corneal area, although this was not statistically

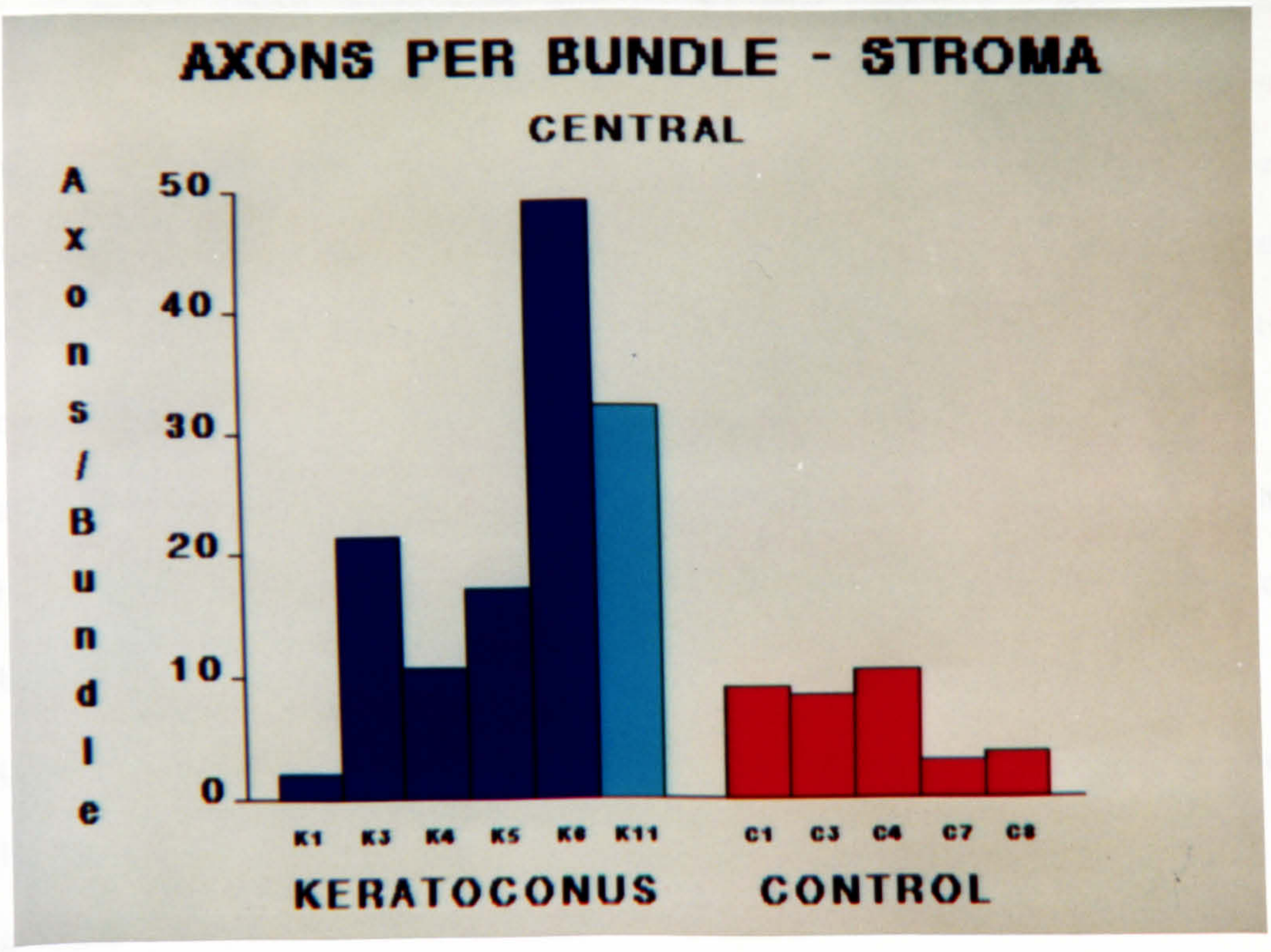
significant.

Figure 5.2



Stromal axon density per nerve fibre for mid-peripheral cornea.

Figure 5.3



Stromal axon density per nerve bundle for central cornea.

Table 5.1 Stromal axon content per nerve fibre bundle (axons/bundle).

	Mid-periphery	Central
Keratoconus	21.1±9.2	20.1±17.8
Control	10.1±5.1	6.9±3.3
K 11	49.4±30.9	32.4±25.0

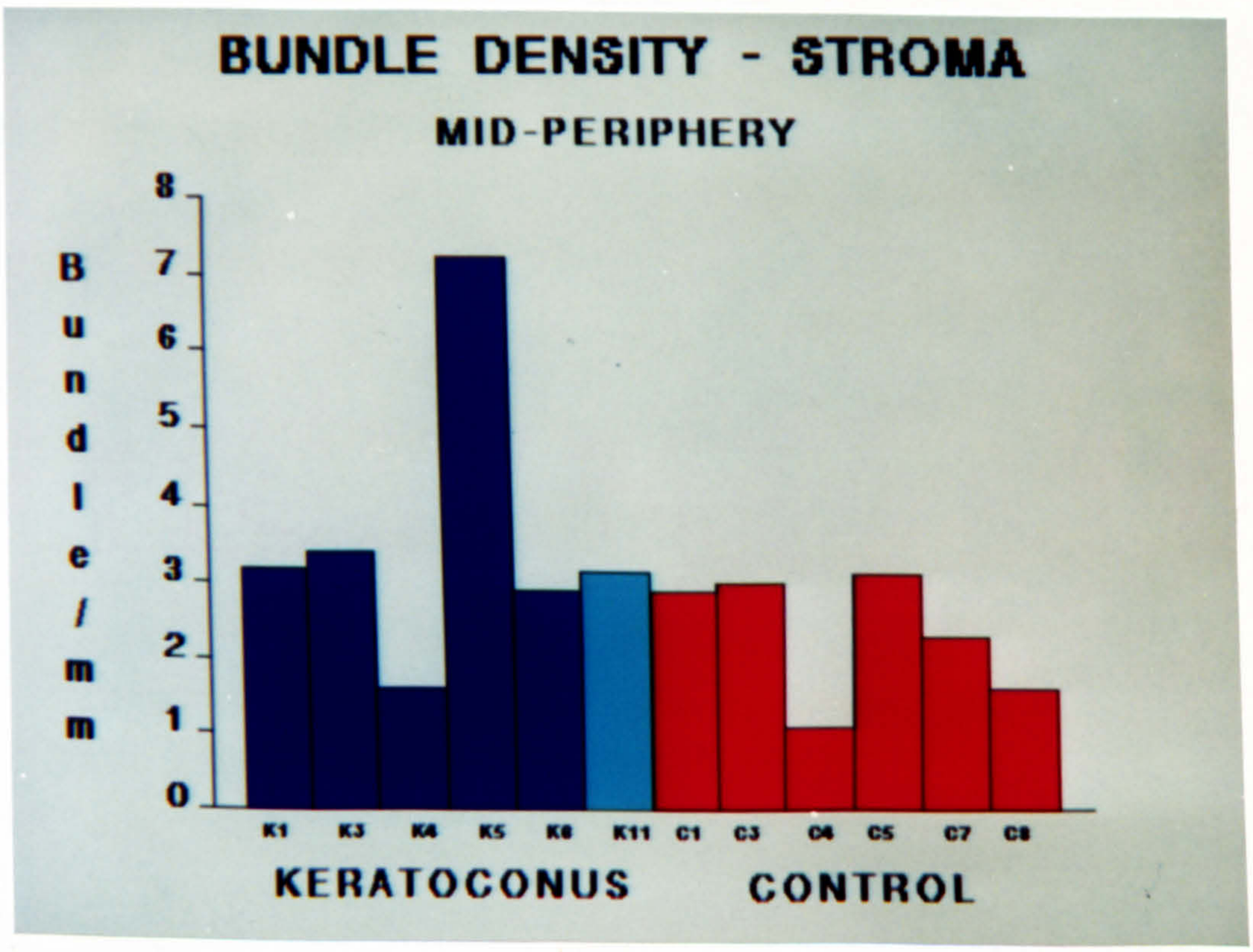
K 11 : Desensitised keratoconic cornea.

5.1.2 Frequency of Nerve Fibre Bundles (bundles/mm).

The frequencies of nerve fibre bundles in the corneal stroma

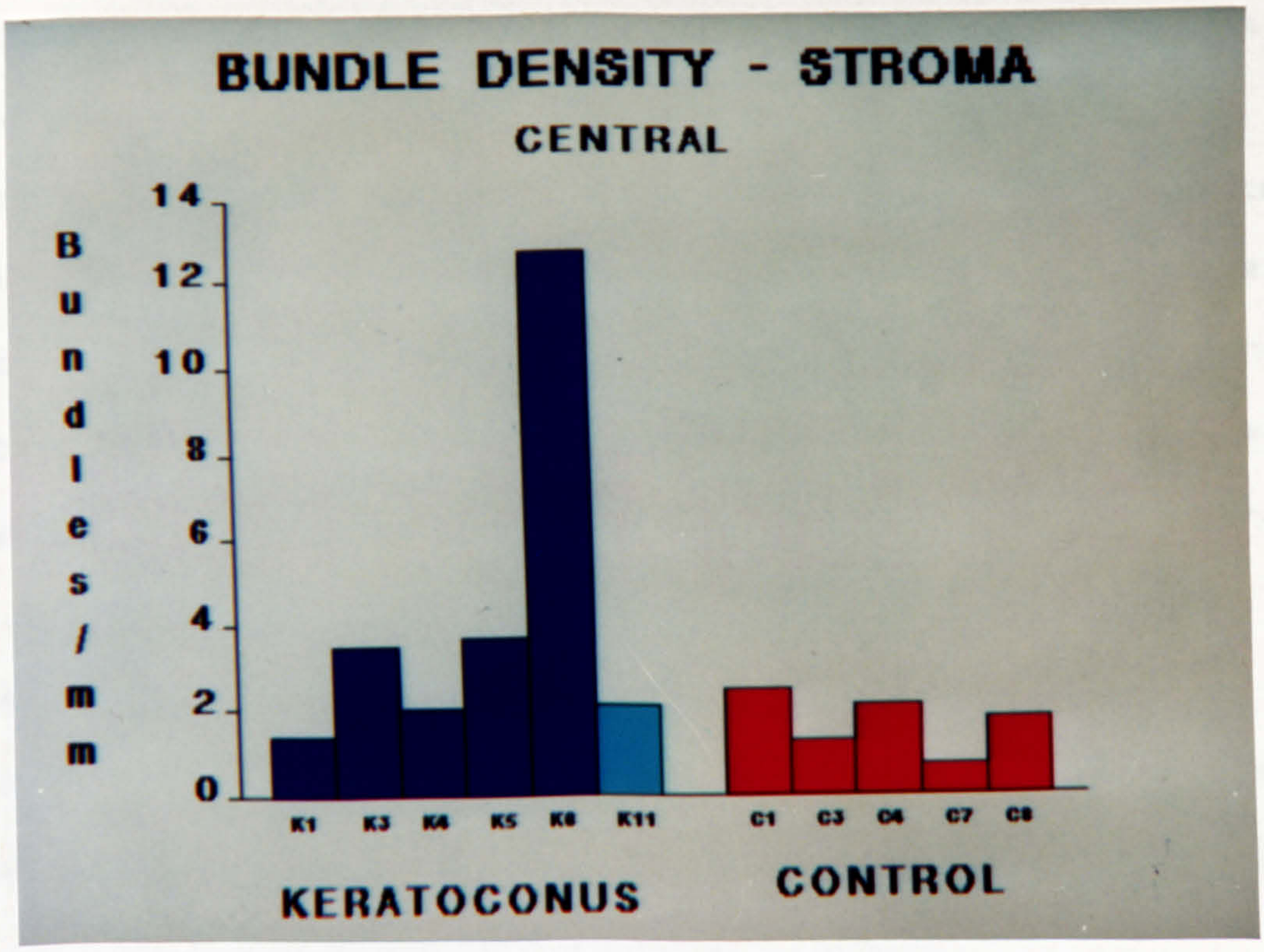
were similar in keratoconic, control and desensitised corneas (Table 5.2 & Fig. 5.19). In one keratoconic specimen (K5) a higher than average nerve fibre density was noted in the mid-peripheral area. On macroscopic examination this specimen did not appear different from the other keratoconic corneas and there was no apparent aetiology for an increase in nerve fibre density. One keratoconic specimen (K6) showed an exceptional high mean density of nerve fibres in the central cornea (12.7/mm) compared to an average of 2.7/mm for the other keratoconic corneas (Fig 5.4 & 5.5). The dense full thickness central corneal scarring noted in K6 may be of significance. See Appendix 10 for details of nerve bundle densities.

Figure 5.4



Stromal nerve fibre bundle density per unit length of tissue in mid-peripheral cornea.

Figure 5.5



Stromal nerve fibre bundle density per unit length of tissue in central cornea.

Table 5.2 Frequency of stromal nerve fibre bundles (bundles/mm).

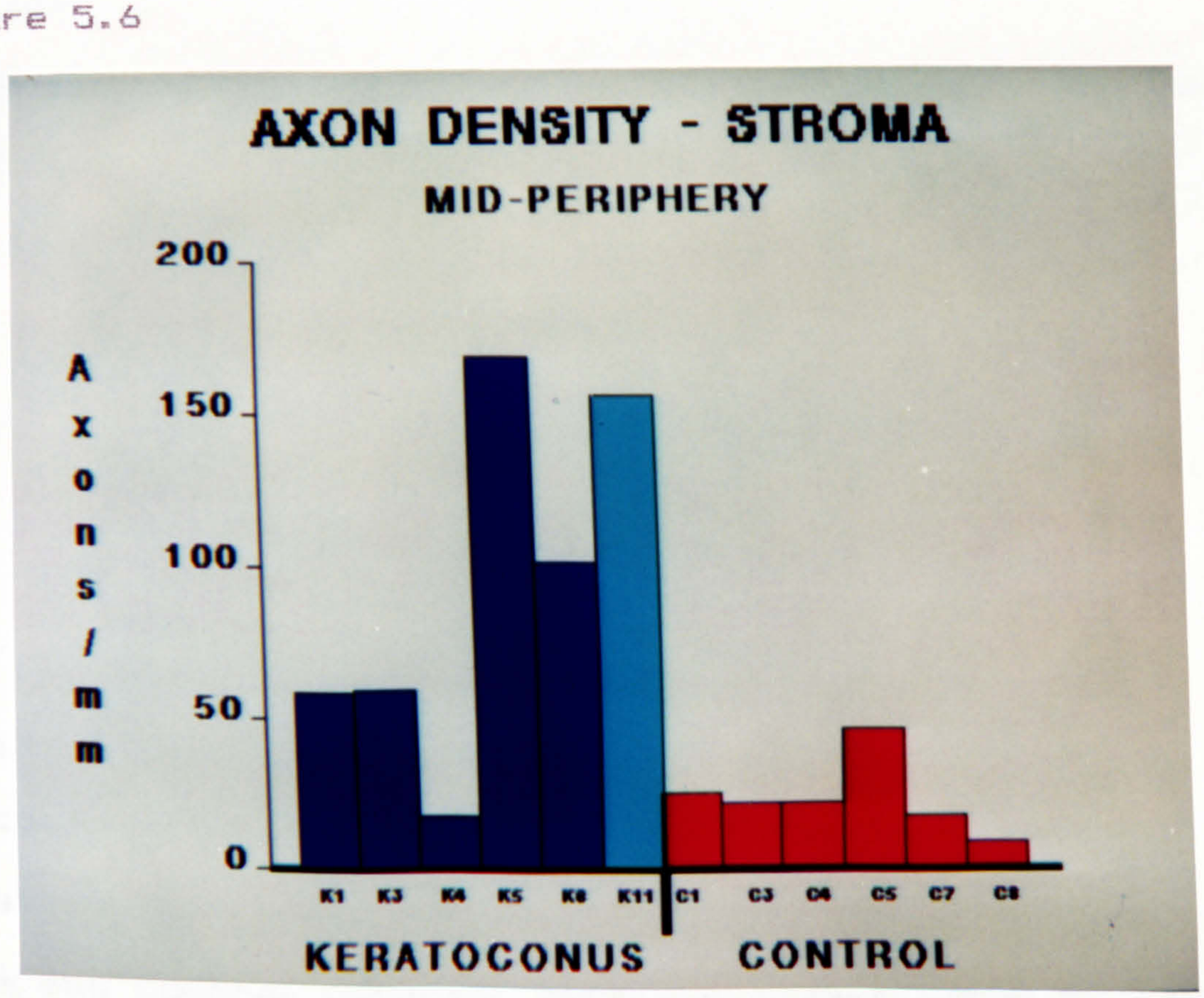
	Mid-periphery	Central
Keratoconus	3.7±2.1	4.7±4.6
Control	2.3±0.8	1.7±0.7
K11	3.2	2.1

5.1.3 Axons per unit length of tissue (axons/mm).

A large variation in axons/mm was noted among the keratoconic specimens. The axons/mm ranged from 16.9 to 168.3 in the mid-peripheral cornea (Fig 5.6) and 2.9 to

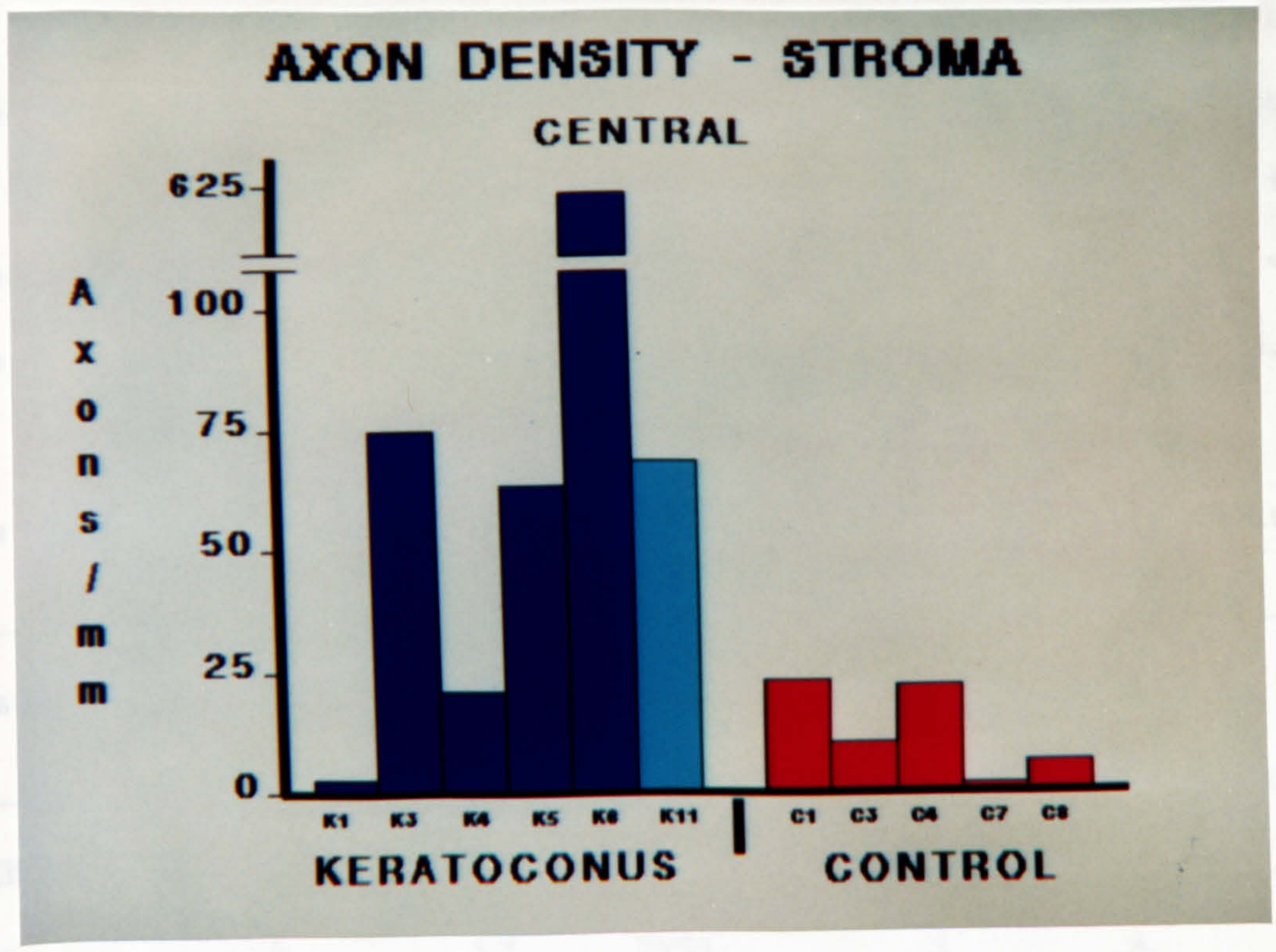
622.9 in the central cornea (fig 5.7). The very large variation for the central cornea was due to one specimen (K6) which was fifteenfold the average of the other keratoconic corneas and may be related to the dense central scarring observed. The control specimens showed relatively little variation in axons/mm. The mean values are summarised in Table 5.3 and Fig 5.20 and show approximately a threefold increase for keratoconus in the mid-periphery over control and a thirteenfold increase for the central cornea. The desensitised cornea showed a higher count than the keratoconic corneas at the mid-periphery but a lower count at the central area. The control corneas remained the lowest mean for mid-peripheral and central cornea.

Figure 5.6



Stromal axon density per unit length of tissue in mid-peripheral cornea.

Figure 5.7



Stromal axon density per unit length of tissue in central cornea.

Table 5.3 Frequency of corneal stromal axons (axons/mm).

	Mid-periphery	Central
Keratoconus	80.9±57.4	157.2±262.0
Control	23.4±12.3	12.7±9.3
K11	155.7	68.5

5.1.4 Axon diameter distribution.

Electron micrographs gave rise to early impressions that the keratoconic corneas contained a larger number of small axons than the control corneas. This was investigated by random selection of a number of specimens and nerve fibre bundles.

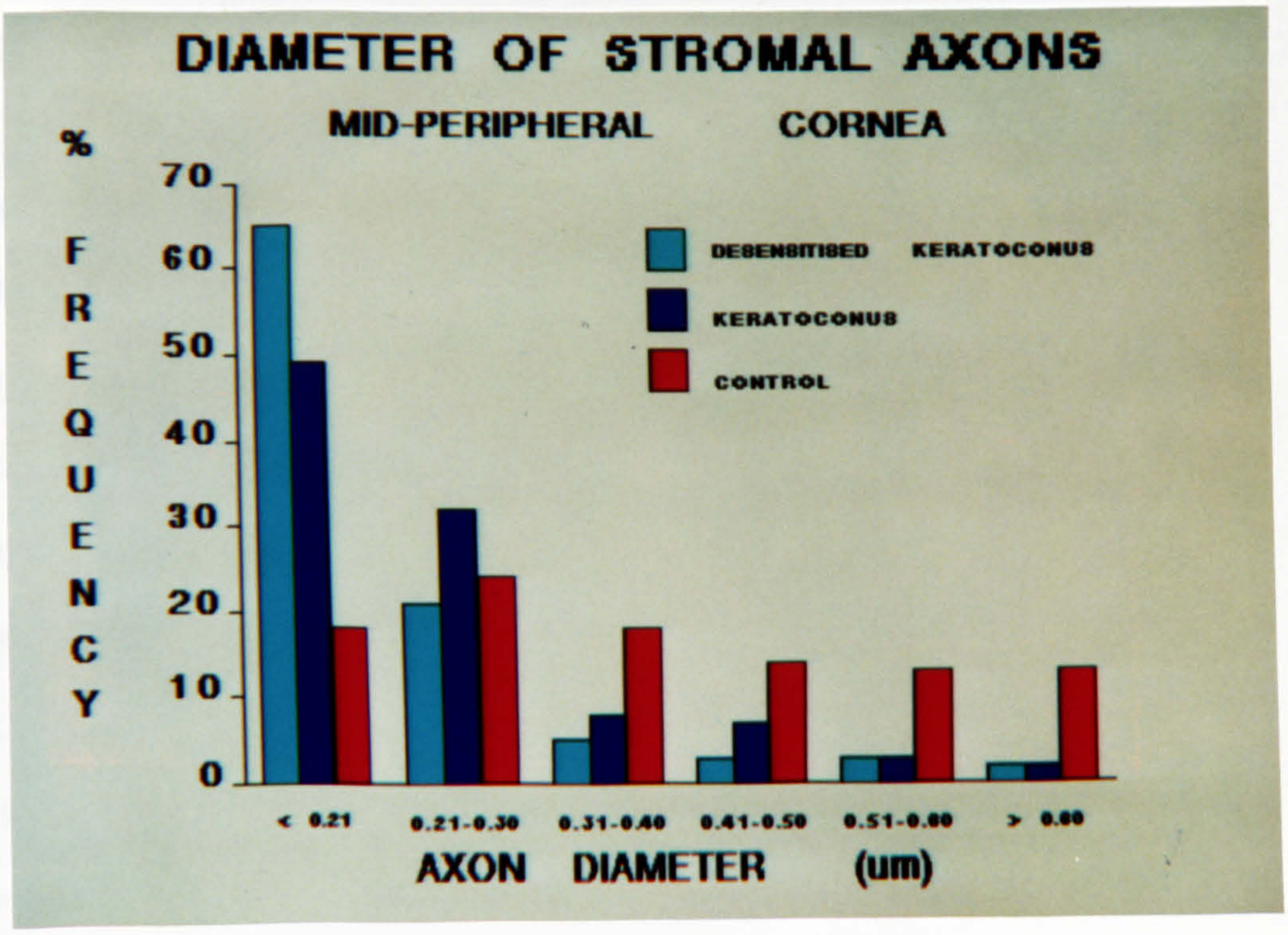
Good quality electron micrographs were used to measure the least diameter of axons. All axons were measured in each of the selected nerve fibre bundles. The mid-peripheral and central corneal areas were investigated separately for the stroma and the epithelium. The number of specimens, nerve fibre bundles and axons measured can be seen in Table 5.4.

Table 5.4 Specimens used for axon diameter measurements.

Tissue	Mid-peripheral			Central		
	specimens	bundles	axons	specimens	bundles	axons
STROMA						
K	4	17	551	3	6	308
C	4	21	215	2	6	33
K11	1	5	337	1	10	312
EPITHELIUM						
K	2	61	201	3	23	150
C	3	43	183	3	41	134
K11		N/R			N/R	

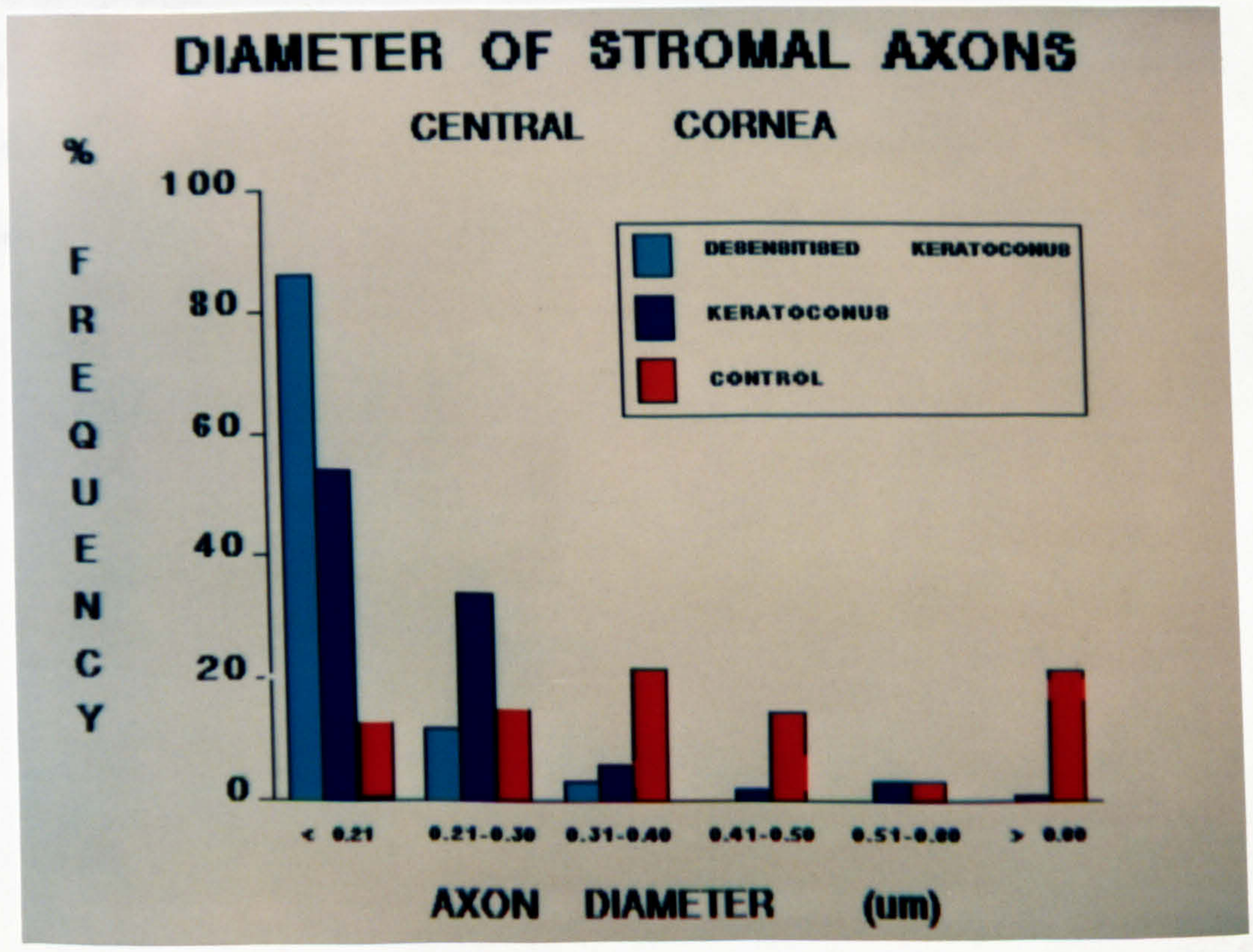
The axon diameter distribution is different for the three tissue types investigated. The control corneas show a larger proportion of large diameter axons in contrast to the skewed distributions of very small axons in the keratoconic and desensitised keratoconic corneas (Fig 5.8 & 5.9). There is little variation between the mid-peripheral and central distributions for each tissue type.

Figure 5.8



Axon diameter in mid-periphery of corneal stroma.

Figure 5.9



Axon diameter in central corneal stroma.

5.2 INNERVATION OF CORNEAL EPITHELIUM

5.2.1 Epithelial axon content (axons/group).

When axons are found together in the epithelium it is appropriate to refer to groups of axons as they lack the Schwann cells of conventional bundles. The mean axon content per nerve group in each specimen is illustrated Fig 5.10 & 5.11 for mid-peripheral and central cornea respectively. The axon contents per nerve group varied with the specimens studied but on average they were similar for the mid-peripheral and the central corneal areas within the keratoconic and control groups respectively (Fig 5.18). For the mid-periphery the range of axons per group was 1-96 for keratoconic and 1-28 for control corneas. The central range was 1-26 for keratoconic and 1-14 for control corneas.

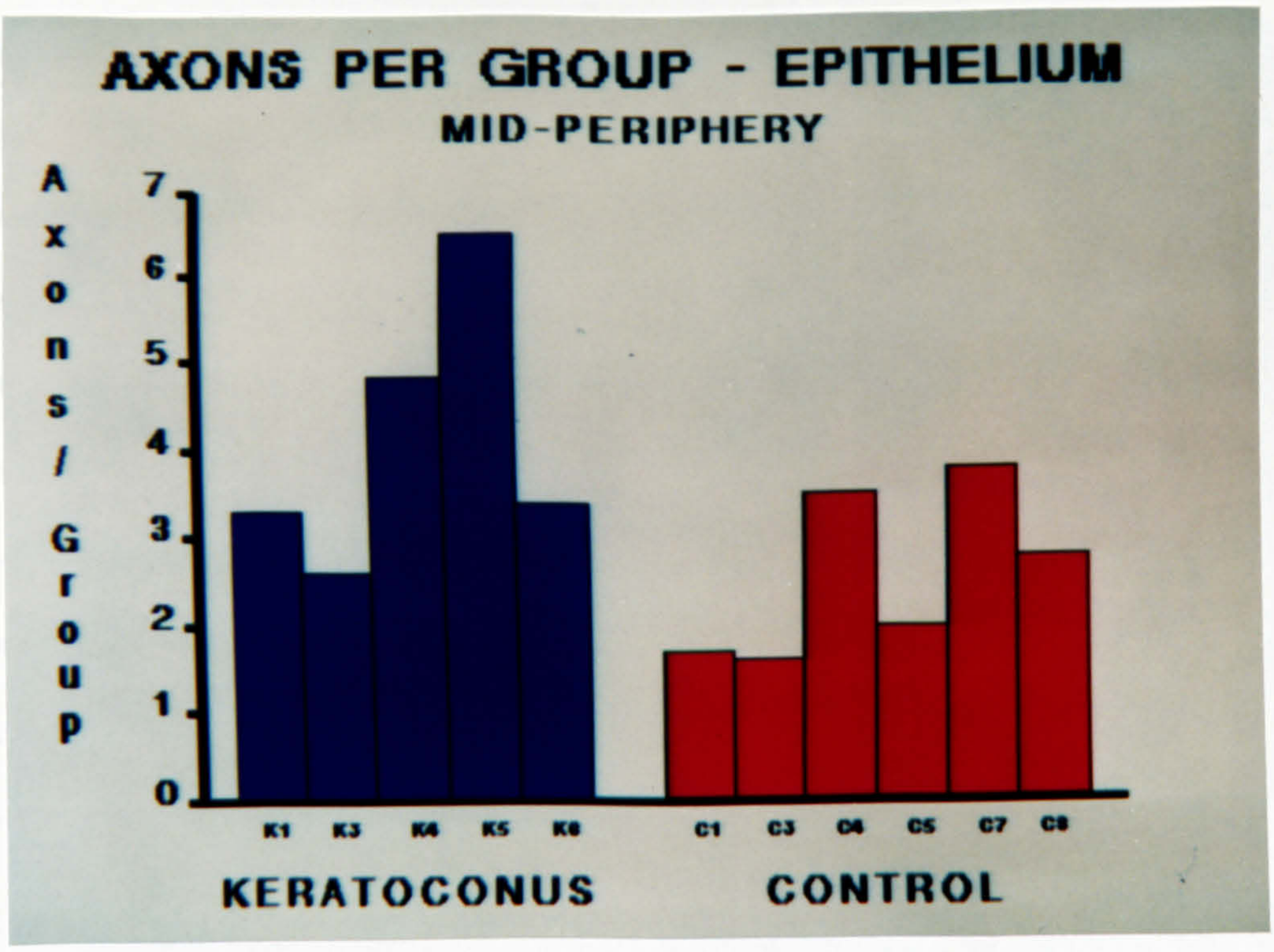
Appendix 10 provides detail of variability of axon content of nerve groups. The keratoconic group show a small but significantly higher mean over the control group for the mid-peripheral and central corneal areas (Table 5.5).

Table 5.5 Epithelial axon content per nerve fibre group (axons/group).

	Mid-periphery	Central
Keratoconus	4.1±1.6	4.4±1.2
Control	2.6±0.9	2.8±1.2
Significance *	P<0.05	P<0.05

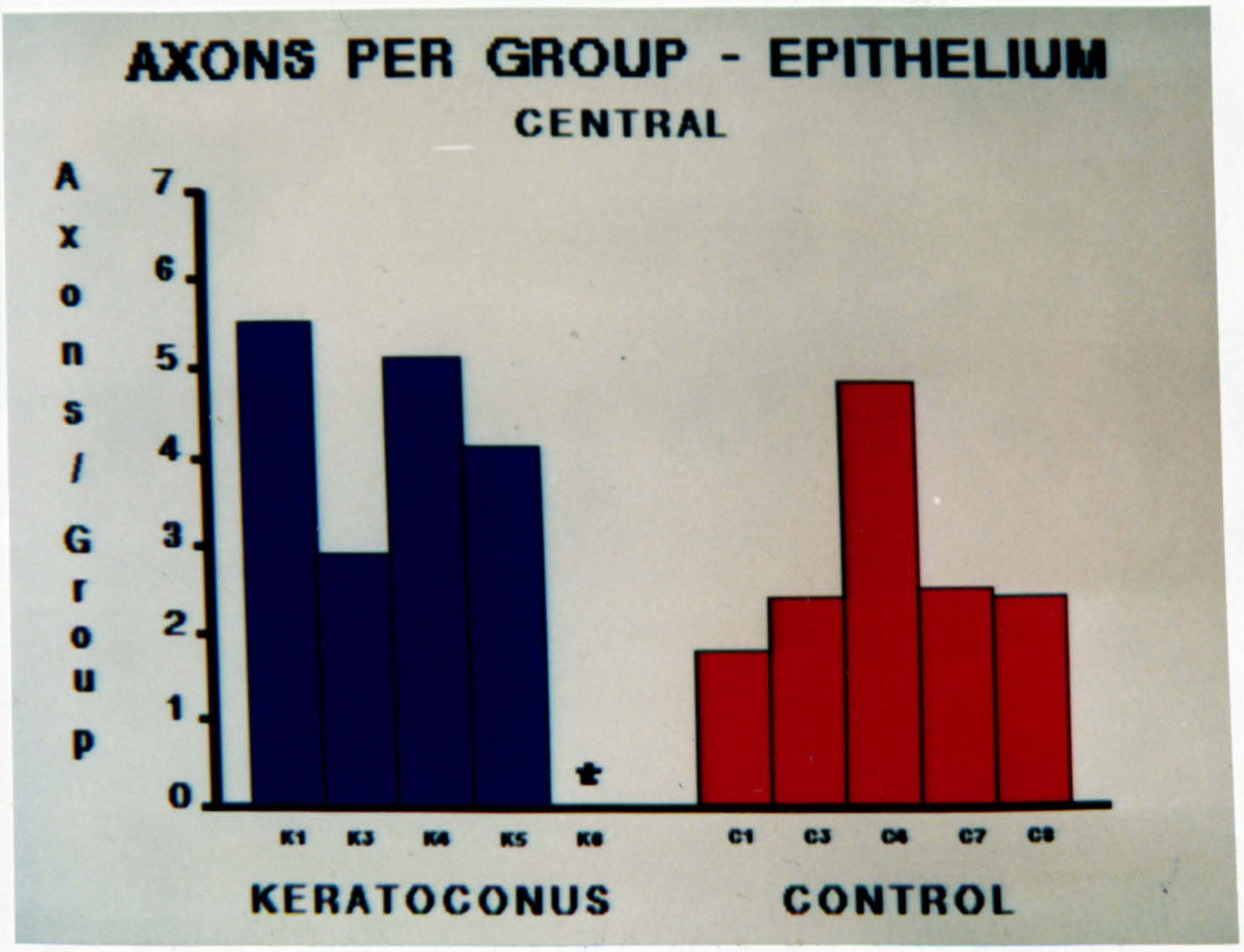
* Student T-test (one-tail).

Figure 5.10



Epithelial axon density per nerve group for mid-peripheral cornea.

Figure 5.11



Epithelial axon density per nerve group for central cornea.
(*) No count was possible on K6 due to poor epithelium.

5.2.2 Frequency of Nerve Fibre Groups (groups/mm).

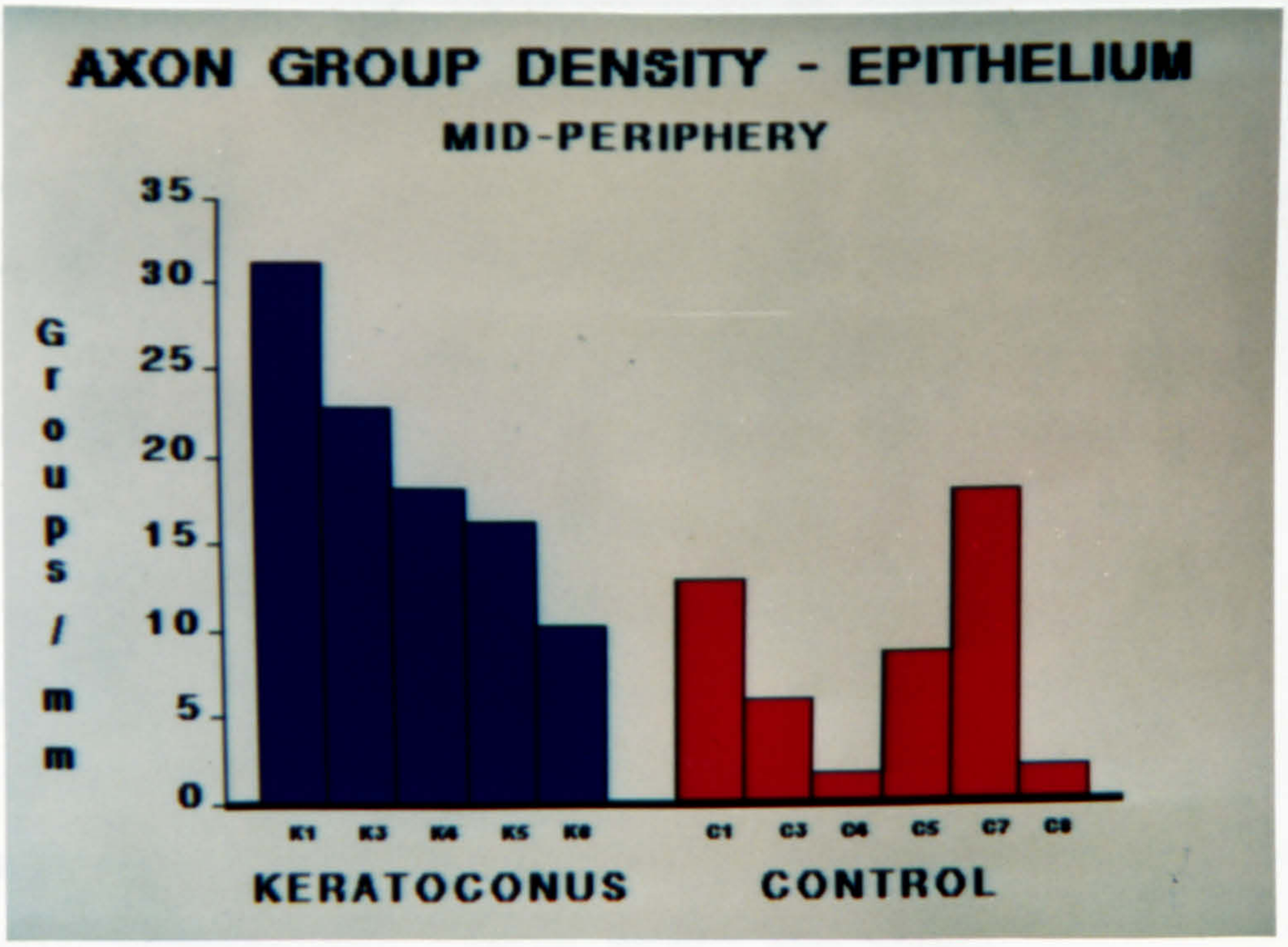
The frequency of nerve fibre groups in the corneal epithelium varied between specimens in keratoconic and control corneas (Fig 5.12 & 5.13). On average the frequency of nerve fibre groups was similar for the mid-peripheral and the central corneal area within the keratoconic and control groups respectively (Fig 5.19). The keratoconic group, however, showed a significant higher mean of approximately twofold that of the control group (Table 5.6). See Appendix 10 for details of nerve group densities.

Table 5.6 Frequency of epithelial nerve fibre groups (bundles/mm).

	Mid-periphery	Central
Keratoconus	19.8±7.8	19.1±4.3
Control	8.2±6.4	8.6±6.1
Significance *	P<0.01	P<0.01

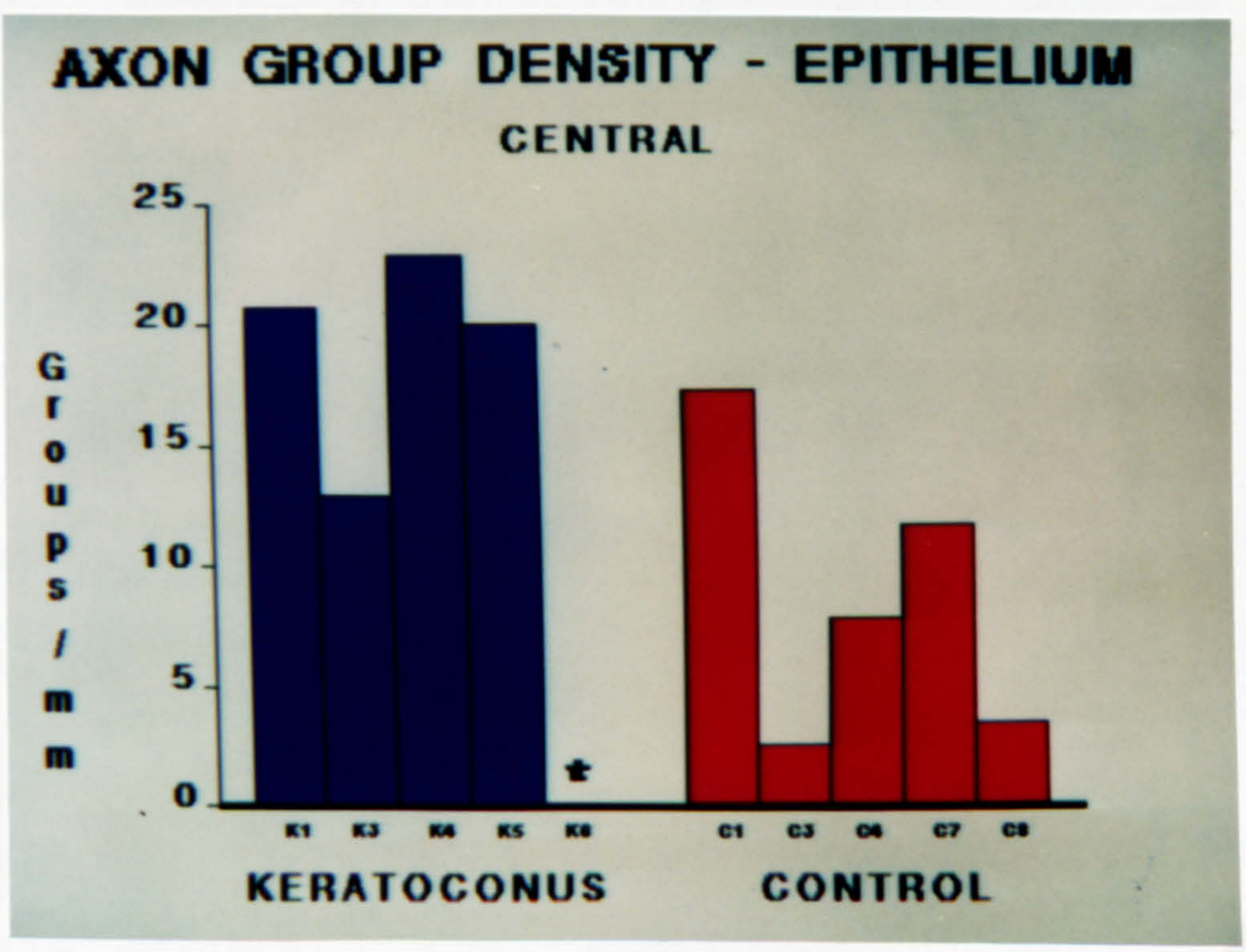
* Student T-test (one-tail).

Figure 5.12



Epithelial nerve group density per unit length of tissue in mid-peripheral cornea.

Figure 5.13



Epithelial nerve group density per unit length of tissue in central cornea. (*) No count was possible on K6.

5.2.3 Axons per Unit Length of Tissue (axons/mm).

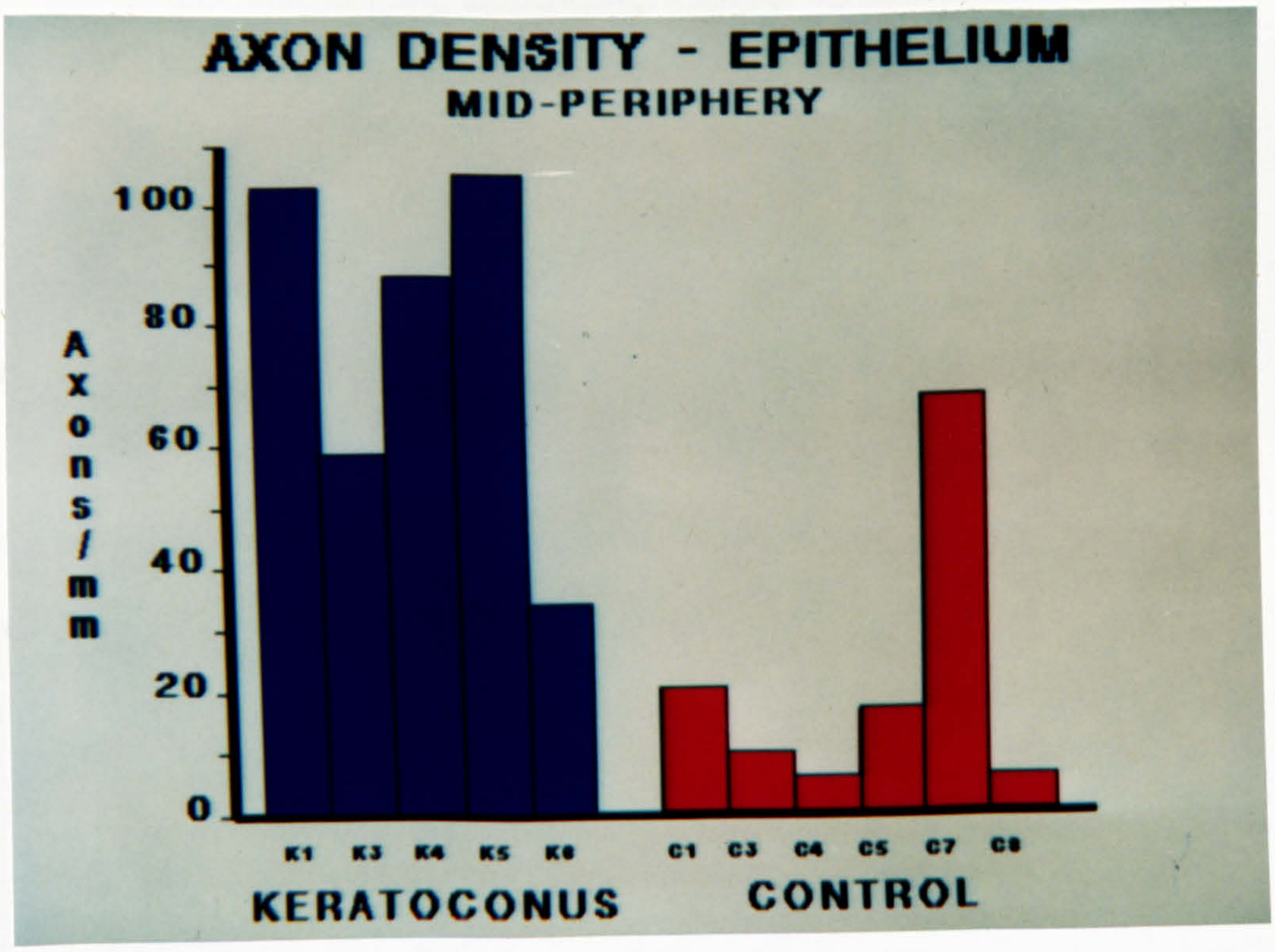
The variation in axons/mm was higher among the keratoconic specimens than the control. For the keratoconic corneas, the axons/mm ranged from 34.4 to 104.9 in the mid-peripheral cornea (Fig 5.14) and 37.4 to 115.9 in the central cornea (fig 5.15). Unfortunately, the poor state of the epithelial tissue made it impossible to obtain any count in specimen K6. The control specimens showed relatively little variation except for one specimen (C7) which had an exceptional high number of axons/mm in the mid-peripheral corneal region (Fig 5.14). The difference between the keratoconic and control group is highly significant (Table 5.7), and shows approximately a threefold increase for keratoconus over the control in the mid-periphery and the central cornea. The number of axons per mm is similar for the mid-peripheral and central corneal areas within the keratoconic and control groups respectively (Fig 5.20).

Table 5.7 Frequency of corneal epithelial axons (axons/mm).

	Mid-periphery	Central
Keratoconus	77.9±30.4	86.9±36.7
Control	21.4±23.5	22.1±14.2
Significance *	P<0.001	P<0.001

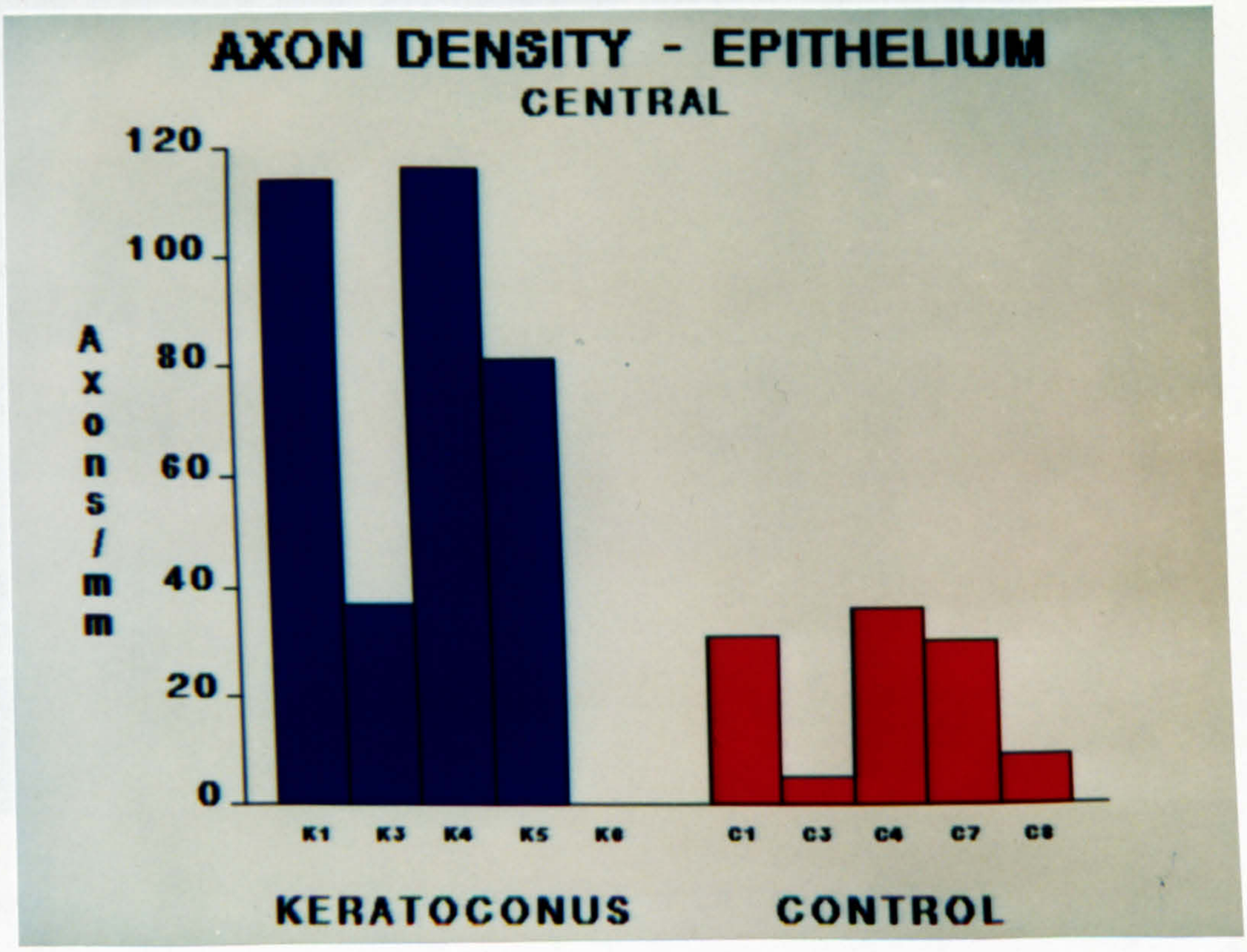
* Student T-test (one-tail).

Figure 5.14



Epithelial axon density per unit length of tissue in mid-peripheral cornea.

Figure 5.15

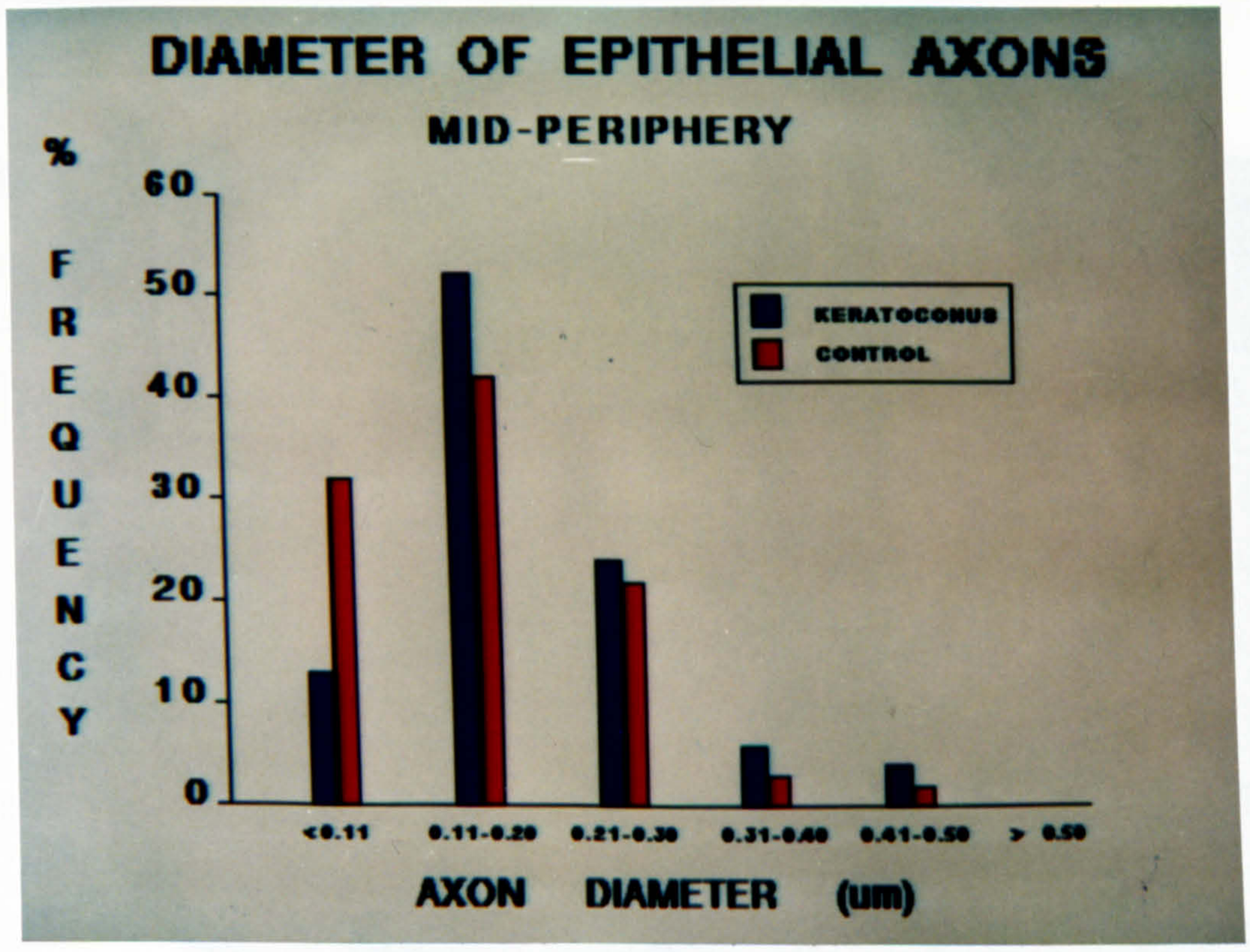


Epithelial axon density per unit length of tissue in central cornea.

5.2.4 Axon Diameter Distribution.

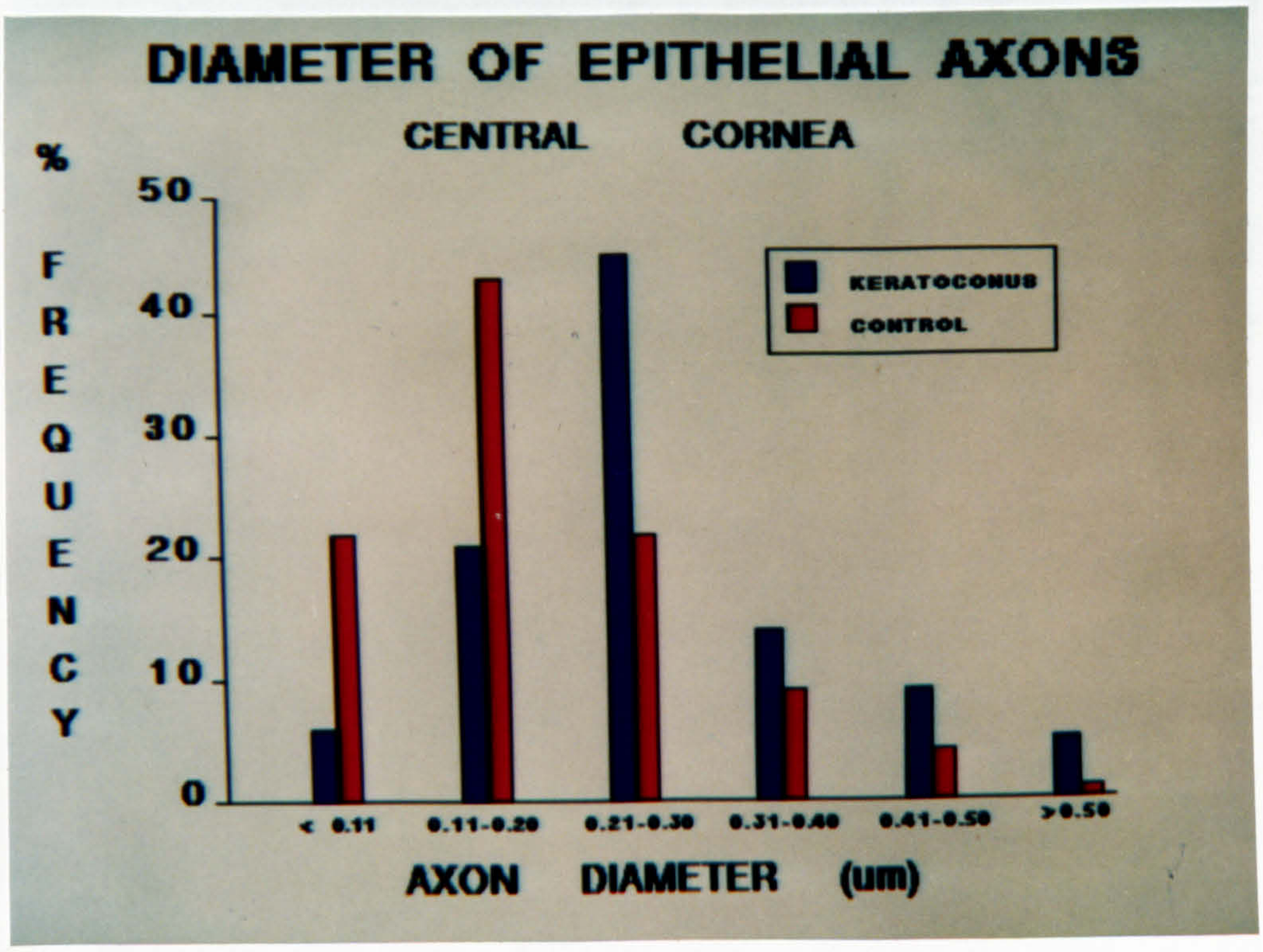
Although the keratoconic and control groups show similar axon diameter distributions in the epithelium, the control corneas show a slightly larger proportion of small diameter axons. The distributions are similar for the mid-periphery and central corneas and are skewed towards the small diameters in a similar manner to the stroma (Fig 5.16 & 5.17).

Figure 5.16



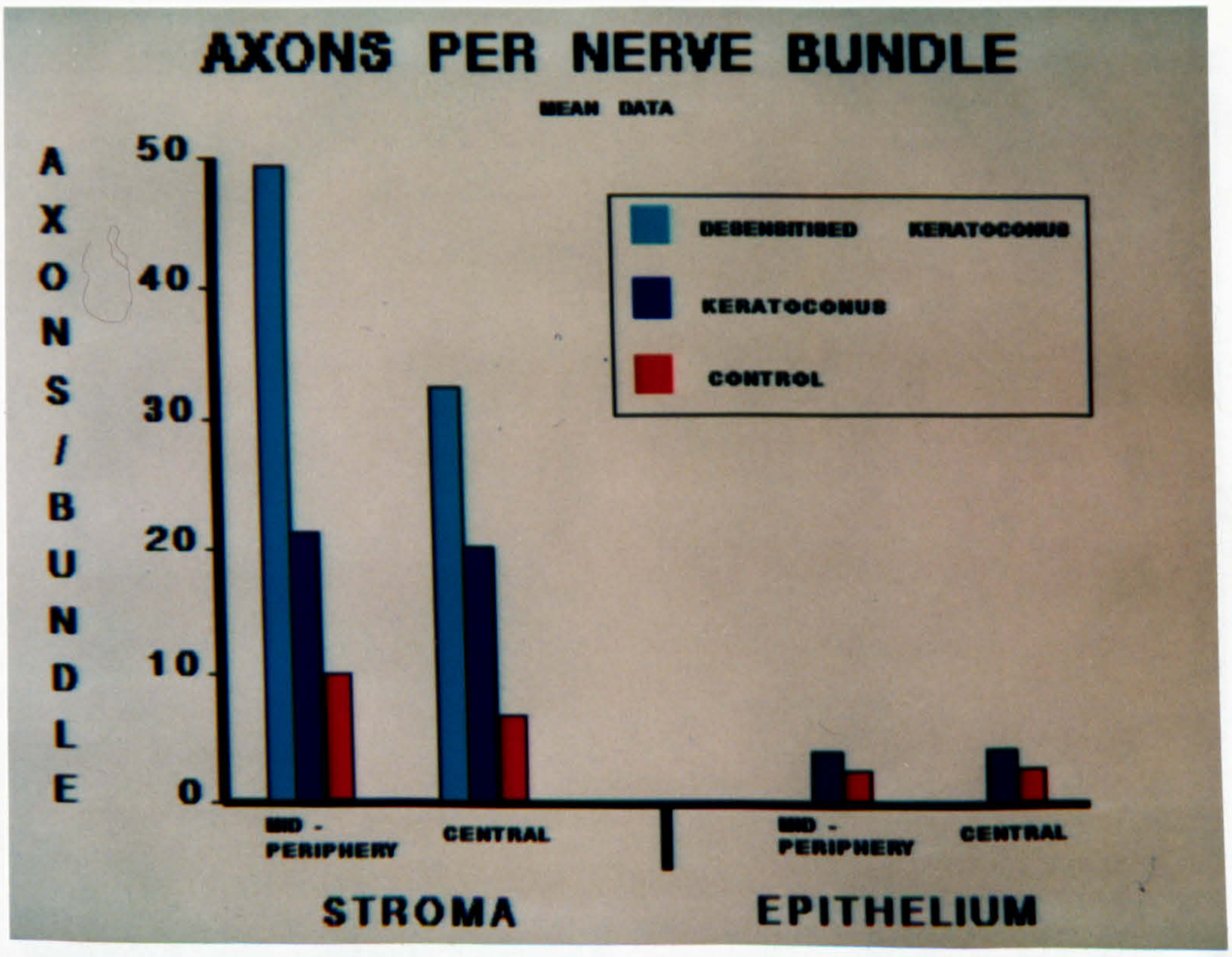
Axon diameter in mid-peripheral corneal epithelium.

Figure 5.17



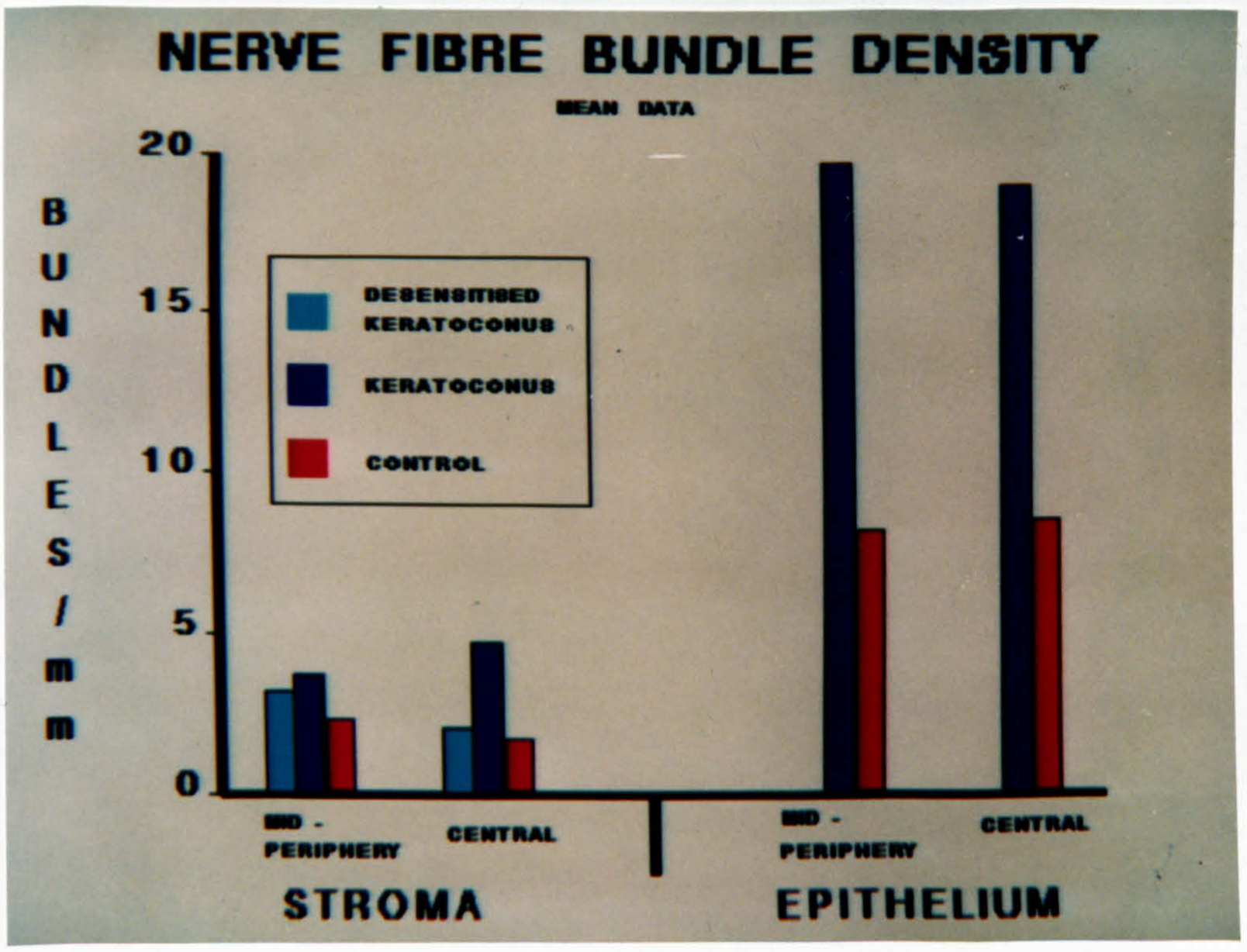
Axon diameter in central corneal epithelium.

Figure 5.18



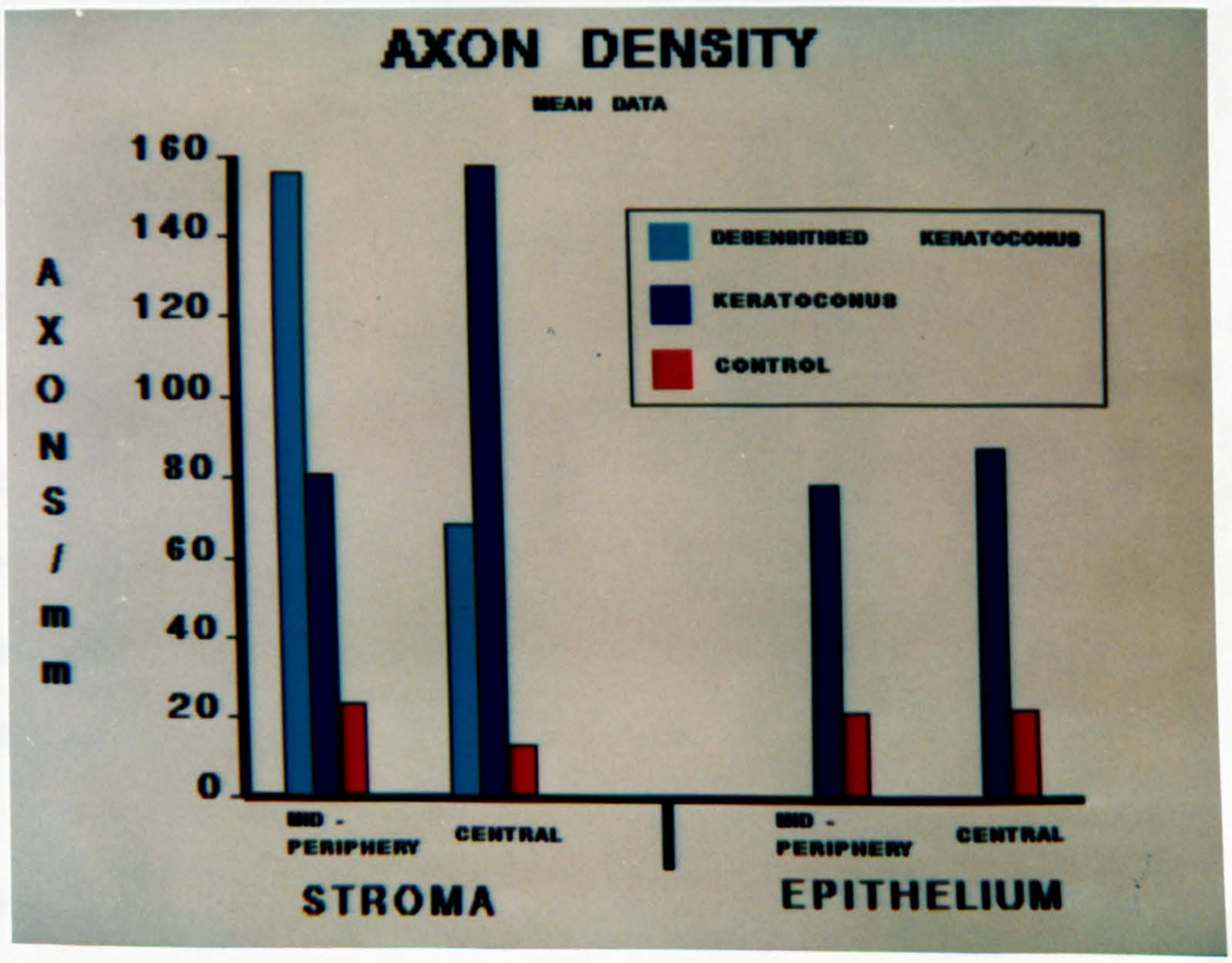
Group means for axon/bundle for stroma and axon/group for epithelium.

Figure 5.19



Group means for bundles/mm for stroma and groups/mm for epithelium.

Figure 5.20



Group means for axon/mm for stroma and epithelium.

CHAPTER 6

DISCUSSION AND CONCLUSIONS

6.1 PATIENT POPULATION.

The patient population used for the corneal sensitivity measurements was matched for age in the experimental and control groups. Furthermore only young caucasian males participated who had not worn contact lenses for more than 5 years. Controlling the experimental design in this manner assisted in reducing the number of variable factors which have been shown to affect corneal touch threshold measurements (Boberg-Ans 1955,1956, Draeger 1984, Millodot 1969,1971,1972,1974,1977). The only other study which has dealt with corneal sensitivity in a keratoconic population did not show the same strict control over these variables (Millodot & Owens 1983).

The maternal age of 27.3 years (±5.5) in the whole keratoconic group was found to be lower than that of the age matched control group 30.7 (±6.5) (Fig. 3.2). This difference was significant (One-way ANOVA $P<0.05$) and in agreement with the mean keratoconic maternal age of 27.0 years (±4.8) found by Millodot & Owens. Despite Millodot & Owens not mentioning data on a control group, the two studies are in disagreement with Woodward (1981). He found

that 60% of his keratoconic group had a maternal age of above 30 years. Also lacking a control group he used data from the Registrar-General's statistical review to claim a lower maternal age for the general population. The sample was larger in Woodward's study (n=150) compared to that of Millodot & Owens (n=38) and the present study (n=50). Woodward also claimed a correlation between high social class, maternal age and keratoconus. The control group in the present study was drawn on a population of undergraduates and graduate students and accordingly may represent a higher social class than the general population. Despite the control group being smaller in the present study, the results bring into question Woodward's deductions concerning maternal age and keratoconus.

6.2 SENSITIVITY

6.2.1 Central Corneal Touch Threshold (CTT).

Corneal sensitivity was reduced centrally in keratoconus and the results support the findings of Millodot & Owen (1983). Furthermore, a significant relationship was found between CTT and corneal curvature, corneal distortion, corneal thickness, corneal scarring and severity.

Corneal curvature and distortion were measured along the visual axis and may therefore not necessarily represent the steepest part of the cornea (Marchall-Courtois 1965; Tomlinson et al 1980; Wesley 1982). However, the steepening

of corneal curvature was the most significant single sign to correlate with an increase in central CTT. Corneal distortion, when graded with respect to the quality of mire image, related positively to the increase in CTT, however, the difference in corneal curvature between the two principal meridians (corneal astigmatism) and the angle between the two principal meridians did not individually correlate to the increase in central CTT. Millodot & Owens (1983) used the photokeratoscopic image as an index for corneal distortion and found a correlation with increase in central CTT. They did not take into account the other major disease characteristic of corneal thinning, and for a more complete grading of the keratoconic condition, corneal thinning was combined with the whole range of distorted surface characteristics into a severity index in the present study. The severity index showed a positive relationship with increase in CTT despite the fact that the individual components of corneal astigmatism and angle between principal meridians lacked any correlation with CTT. Furthermore, corneal thickness, considered alone demonstrated a negative correlation with CTT. The severity index combined with central CTT measurements provide good clinical tools for the diagnosis of keratoconus.

Corneal scarring also significantly increased the CTT and was very much related to the local area of scarring with CTT reaching normal keratoconic levels immediately outside the

scarred area. Similar reductions in sensitivity have been reported in scarred areas and scarring induced by surgical procedures (Boberg-Ans 1955, Cochet-Bonnet 1960, Draeger 1984, Ruben et al 1979) and is not unique to keratoconus.

Central CTT did not show any correlation with the duration of the condition. The keratoconic patients were referred from many different sources and it was difficult to establish the date of first diagnosis accurately. Accordingly, the lack of correlation with duration of the disease and CTT has to be interpreted with caution.

Corneal nerve fibres were significantly more visible in the keratoconic patients ($P<0.005$), however, the highly visible nerve fibres did not correlate with the increase in central CTT. Such lack of correlation has not been commented on by other researchers.

6.2.2 Peripheral Corneal Touch Threshold (CTT).

The four peripheral corneal positions demonstrated similar CTT in keratoconic and control patients. This indicates that the disease does not have the same traumatic effect on neural functions in the peripheral cornea as it does in the central cornea. This compares with other diagnostic signs being prominent in the central cornea and insignificant in the peripheal cornea. It does not necessarily follow that nerve integrity differs in the two positions and later it

will be argued that the reduction in central corneal sensitivity can more readily be attributed to epithelial and stromal tissue changes.

6.2.3 Lid Margin Touch Thresholds (LTT).

The possibility that the keratoconic disease may induce a change in touch threshold of the lid margins was investigated. Although statistically no difference could be demonstrated in LTT between upper and lower lid margins the contact lens wearing keratoconus group showed a higher LTT of the upper lid margin compared to the lower lid margin. This not being the case for the non-contact lens wearing keratoconus group may suggest an aetiology in incomplete recovery following contact lens wear, as an increase in LTT is normally induced by hard contact lens wear (Dixon 1964; Lowther & Hill 1968). For hard contact lens wear of a duration up to 5 years recovery in LTT has been estimated to be complete following a 24 hours period without lens wear (Millodot 1982). The present study incorporated a 48 hour period without contact lens wear preceding the measurement of LTT, and none of the patients exceeded 5 years of contact lens wear.

The non-contact lens wearing control group demonstrated a similar non significant difference between upper and lower lid margin, and as no statistical difference was found between the contact lens wearing keratoconic group and the

non-contact lens wearing control group, one can conclude that, within the experimental error of the technique, any possible contact lens induced changes in LTT are reversible in keratoconus, the lid movement over the keratoconic cone does not have any effect on LTT and the disease itself does not affect the LTT.

Strughold (1953) tested corneal, conjunctival and lid margin touch thresholds using hairs and found that for the lid margin "The thresholds ... correspond approximately to those of the pressure sense on the skin of the lower arm, being slightly higher than the thresholds on the outer skin of the eyelid." Comparable results were obtained later using the Cochet-Bonnet aesthesiometer (Dixon 1964). However, Lowther and Hill (1968), using a modified version of the Cochet-Bonnet aesthesiometer obtained central lower lid thresholds that were comparable to values found for the central cornea, indicating identical sensitivity of lid margin and central cornea. In the present study LTT was slightly higher for both upper and lower lid margin compared to central CTT and was of similar magnitude to the thresholds found at the inferior and nasal corneal positions. The normal LTT in conjunction with the non-optimal fitting characteristics of contact lenses in keratoconus may explain the frequent complaints of discomfort with contact lenses in this group of patients.

The descrepency between the studies is likely to be due to the difference in methodologies. The results are not as repeatable as for corneal measurements due to the constant mobility of the lid. The lid motion may push the filament against the cornea thus inducing a false positive response. In order to reduce this problem in the present study a thin high water content hydrogel lens was used to protect the cornea. The contact lens had a diameter which enabled the edge to remain behind the eyelids and was allowed to settle until the patient was comfortable. It can, however, be argued that the lens may have induced adaptation of the lid margin and raised the touch threshold.

Larger samples including a control contact lens wearing group (rigid lenses), control of the anatomical site of stimulation, control of the lid margin conditions and a standardised technique in measuring LTT is called for in order to investigate the touch threshold of the lid margins more precisely.

6.3 HISTOLOGY.

The general histological pattern of keratoconus was confirmed although this was not the primary aim of the present study. As many investigators have concerned themselves with the histopathology of keratoconus, including the detailed studies by Jakus (1962) and Teng (1963), no further comments are necessary here.

6.3.1 Stromal Nerves.

Stromal nerve bundles were observed exclusively in the anterior two-thirds of the cornea in agreement with previous work (Zander & Weddell 1951a). Due to the nature of the keratoconic material, sampling had to be limited to the central 7 to 8 mm i.e. the mid-peripheral and central cornea. Nerve bundles became progressively flattened and smaller as the centre of the cornea was approached. The nerve bundles demonstrated a similar appearance in the keratoconic and control tissue except where they appeared irregular in association with the irregular lamellae and grossly abnormal and scarred stromal collagen.

A quantitative neural investigation was carried out on 5 keratoconic, 6 control and 1 desensitised keratoconic specimen. The mid-peripheral corneal position showed a slight tendency for more nerve bundles per unit length of tissue in keratoconic compared to control material, 3.7±2.1 and 2.3±0.8 respectively. For most specimens (keratoconic and control) the frequency of nerve bundles was in the region of 3 per mm and one specimen (K5) had an abnormally high score of just over 7 bundles per mm. The desensitised keratoconic material gave a similar result to most of the other keratoconic specimens (3.2 bundles per mm). The central corneal positions showed slightly more bundles per mm in the keratoconic material. The high score for keratoconic cornea

(4.7±4.6) relative to a more moderate score for control specimens (1.7±0.7) is due to one keratoconic specimen (K6) containing an extreme high frequency of bundles (approximately 13 per mm). Excluding the extreme samples the nerve bundle frequency was only slightly increased in keratoconus.

6.3.2 Axon Content per Nerve Bundle in the Stroma.

The number of axons per nerve bundle in keratoconus was approximately twice that of control. This was apparent for the mid-peripheral and central corneal positions despite inter-specimen variation in axon content. As a result, an axon density of 75-80 axons per mm in keratoconus was found to be almost four times greater than that found in control material. Lim (1979) in a study on monkey material found the peripheral stromal axon density to be 33±2 which was slightly higher than the mid-peripheral density of 23±12 for the human control material in the present study.

6.3.3. Diameter of Stromal Axons.

The axon diameter distribution was found to be different for the keratoconic and control materials. The keratoconic axons were smaller than the control axons in a manner that approximately half of the keratoconic axons measured 0.20 um or less whereas only approximately one sixth of the control axons were below 0.20 um. The trend was similar for the mid-peripheral and central corneal positions.

The origin of the increase in axon density in keratoconus is unknown. Rerouting of existing nerve bundles from the limbal plexus would have resulted in an increase in nerve bundle density. The nerve bundle density was indeed increased in keratoconus, however, only by approximately 50 % and could therefore only partially account for the four-fold increase in axon density. The initial impression of a patchy distribution of bundles in keratoconus compared with a regular distribution in normals could not be confirmed. Furthermore, there was no evidence of axonal degeneration and accordingly there is little reason to suspect that nerve fibre death followed by replacement had occurred. Nerve cell division in the trigeminal ganglion is unlikely as in most mature nerve cells, cell division is no longer possible (Schwartz 1985) and there is no evidence in the literature to suggest that this could occur. The sprouting ability of nerves offers the most likely explanation. This could take place at any level along the route from the nasociliary nerve. Large diameter collateral sprouts have been reported arising from intact nerves following experimental keratectomy, however, these sprouts are known to survive for a short period only and have been shown to possess hyperexcitability sensory capabilities (Beuerman et al 1985). Formation of long term neuron regrowth is associated with small axon diameters and the large number of small axons present in keratoconic material of the present study

is indicative of immature axons of regenerating sprouts (Spencer & Ochoa 1981).

Animal studies on the formation of axon sprouts have all concerned sudden traumatic lesions in the form of surgical procedures (Kondo 1972,1973a,1973b, Rexed et al 1951a,1951b, Tanaka 1969, Zander & Weddell 1951a). Formation of axon sprouts in the absence of such surgical procedures must have a different aetiology. The answer may be in the unknown aetiology of the keratoconic disease and be related to some form for enzyme released by degenerating basal epithelial cells (Teng 1963). There are, however, no reports of axon sprouting in other collagen diseases and the trauma from longterm mechanical effects in the form of increased lid pressure due to corneal ectasia, scarring and contact lens wear may be the primary reason for inducing neural changes. A reduced corneal rigidity induces less tension and stress at the apex of the cone (Andreassen 1980; Brooks 1984; Poole 1973), which would then also exert less stress on the corneal nerves, however, increased sliding of collagen bundles which form the lamellae (Edmund 1989) may induce trauma to the nerve fibres.

The age of the control tissue ranged from 1 to 59 years (mean 42 years) compared to the keratoconic tissue which ranged from 17 to 38 years (mean 27 years). Increasing signs of decay of unmyelinated fibres in human sural nerve have

been reported in a small series starting in the fourth decade of life (Ochao et al 1969). The signs of irregular basal lamina and increase in the number of smaller axons described in the sural nerve are minor compared to those axons in the keratoconic material and the higher age of the control tissue is an improbable explanation for the ultrastructural appearance in the keratoconic nerve fibres.

6.3.4 Morphology of Stromal Nerves.

Axons contained the normal complement of organelles of microtubules, neurofilaments, mitochondria and vesicles in keratoconic and control tissue. The size of mitochondria varied in the axoplasm and Schwann cell cytoplasm and was not consistently larger in either of the cell types. Schwann cell cytoplasm became progressively less apparent as the centre of the cornea was approached and the number of mitochondria decreased accordingly. Cellular organelles and cytoplam occasionally appeared pale and somewhat structureless. However, as this was apparent in keratoconic and control tissue for axons, Schwann cells and keratocytes alike this was thought to be caused by inadequate fixation of the tissues rather than evidence of axon degeneration.

Varicosities containing vesicles were frequently encountered. These have been described previously (Hoyes & Barber 1976; Lim 1979; Matsuda 1968) and appeared in similar form and numbers in keratoconic and control tissue.

Functional classification has been attempted by several investigators with respect to the presence of mitochondria and clear and dense cored vesicles (Bergmanson 1978; Cauna 1959; Hoyes & Barber 1976; Matsuda 1968; Tervo et al 1981; Ueda et al 1989 and others). However, sensory or autonomic morphologic criteria still have to be regarded as unreliable.

The basement membrane frequently varied in thickness and was often incomplete around Schwann cells of keratoconic stromal nerve bundles. This phenomenon has been linked to axon degeneration of unmyelinated nerve fibres (Spencer & Ochoa 1981).

The extracellular matrix of the stroma comprises basal lamina, fibrillar collagen, microfibrils and various amorphous materials (Carlson et al 1988; Lahav et al 1982; Pouliquen et al 1968). In the present study scattered patches of electron dense material were often seen to surround stromal nerve bundles like a halo. As no biochemical analysis were performed on the structure it is difficult to determine the exact nature of this material, however, it did appear to extend from and have the same structure as the basement membrane of the nerve bundles. Furthermore, the halo tended to be more marked around nerve bundles which had a thin basal membrane or where it was missing in places. It appeared as if the basal membrane had

dispersed among the collagen fibrils in the surrounding ground substance. It was frequently seen in keratoconic material but was also present in the control and was useful as a clue to locate small nerve bundles in the disorganised collagen using relative low magnification under the electron microscope. The presence of haloes surrounding nerve fibre bundles have not been reported earlier.

6.3.5 Epithelial Nerves.

The axon groups were observed in three types of locations in the basal epithelial layer. In an infolding originating from the base of an epithelial cell (basal-buried), in the intercellular space of two neighbouring epithelial cells (inter-cellular) and in an infolding originating from the lateral wall of an epithelial cell (lateral-buried). The larger axon groups tended to be of the intercellular type and were more common in the keratoconic epithelium. The basal-buried type axon group was most frequently seen in control material whereas the lateral-buried type was rare in keratoconic and control material.

All epithelial axons were identified in the basal epithelial cell layer and is consistant with the presence of axon leashes previously described (Rozsa & Beuerman 1982; Schimmelpfennig 1982). Epithelial axons have been reported above the level of basal epithelial cells (Rozsa & Beuerman 1982), however, the material used in the present study did

not demonstrate such axon profiles. The quality of surface cells and cells of the wing layer was frequently not of a standard which would allow single axon terminals to be identified. The disease is the probable cause of this epithelial disturbance in keratoconus, whereas tissue drying on removal from the host or poor and inadequate fixation may be the cause in control tissue.

6.3.6 Nerve Group Frequency.

The frequency of axon groups was similar for the mid-peripheral and central corneal positions. In the keratoconic tissue, however, axon groups were encountered with more than twice the frequency compared to the controls. Furthermore in keratoconus the groups frequently contained 4-5 axons and occasionally many more whereas the number of axons per group averaged slightly less (2-3) in the control tissue. As a result the keratoconic basal epithelium was innervated by approximately four times the population of axons seen in control tissue. In one control specimen (C7) the axon frequency in the mid-peripheral basal epithelium approached the high level seen in keratoconus. This result could not be explained by the age of the tissue nor by the sampling or other experimental technique. In the monkey Lim (1979) found the population of epithelial axons (3 axons/mm) to be much less than our study in man (control: 22 axons/mm; keratoconus 78-87 axons/mm).

6.3.7 Diameter of Epithelial Axons.

When axons were cut obliquely the smallest diameter was assumed to be the axon diameter. The keratoconic axon population demonstrated a slightly greater proportion of large diameter axons than was seen in control material. This was the reverse of the pattern seen in the stroma. The majority of axons had a diameter in the region of 0.1-0.3 um. It was often difficult to measure axon diameter in keratoconus as pathology of the basal epithelial cells made the axon membranes indistinct.

6.3.8 Morphology of Epithelial Axons.

Epithelial axons also contained the normal complement of organelles of microtubules, neurofilaments, mitochondria and vesicles. Varicosities containing mitochondria and vesicles were identified in transverse and longitudinal sections. These were similar in keratoconic and control material and have been described in detail in previous studies (Lim 1979; Matsuda 1968; Zander & Weddell 1951).

6.4 DESENSITISED KERATOCONIC CORNEA.

The desensitised keratoconic specimen was comparatively similar to keratoconic and control material with 2.1 bundles per mm. The axon content of nerve bundles in the desensitised keratoconic specimen, however, was increased more than four fold over the control material. This resulted in a very high number of axons per mm for the mid-peripheral

(155 axons/mm) and central (68.5 axons/mm) corneal positions. An excess of nerve fibres has been reported in re-innervation of corneal grafts (Rexed 1951), however, the lower frequency of axons per mm in the central compared to the mid-peripheral cornea may be due to the desensitising operative procedure 10 years previously. The cornea was incised by a 5 and a 10 mm trephine and sampling for quantitative analysis was taken in the mid-peripheral cornea (between the two incisions) and centrally. In order to reach the central cornea nerves would have to penetrate two scarred areas which both act as barriers for reinnervation (Escapini 1948, Rexed et al 1950, Zander & Weddell 1951b).

The majority of axons in the mid-periphery had a diameter of less than 0.21 um. The high number of axons per nerve bundle and their small diameter indicates that these axons are of a sprouting nature (Beuerman & Rozsa 1984, Dyck et al 1972, Rexed et al 1950). The scar from the second incision gives rise to a difficult barrier for these new axons to cross and the number of axons is consequently reduced. Similar results have been obtained experimentally in animals previously (Rozsa et al 1983). At no stage were naked axons encountered and accordingly all axons were located within Schwann cell sheaths. Furthermore no empty Schwann cells were encountered.

6.5 NERVE FIBRE VISIBILITY.

Stromal nerves are often clearly visible in keratoconus and there are no reports as to the aetiology of this phenomenon. The histological investigation of the present study revealed stromal nerve bundles adopting the irregular shape of the surrounding collagen. It is, however, unlikely for the shape solely to account for the increased visibility as the nerves are most visible in the peripheral cornea where the collagen is normal and regular. Amyloid deposition in association with the nerves has been suggested (Tervo 1987), however, such deposits were not identified in the present study. The axon content of organelles was observed to be normal, however, in keratoconus, histological changes were seen in the increase of the number of axons per nerve bundle and the irregular appearance of the basement membrane surrounding the nerve bundles. It is possible that the increase in nerve bundle axon content gives rise to the bundles increasing in size and accordingly becomes more visible. The basement membrane in a large number of keratoconic nerve bundles showed inconsistent electron density and thickness. In many keratoconic nerve bundles the basement membrane was missing and appeared to be scattered in the surrounding ground substance. These features may result in nerve bundles becoming more visible when viewed clinically by biomicroscopy. Fibrillary lines have been observed to make contact with stromal nerves in keratoconic and normal epithelium (Bron et al 1975) and the fibrils may represent

subepithelial nerve aborizations previously described (Zander & Weddell 1951).

6.6 SENSITIVITY IN RELATION TO INNERVATION.

Previous reports (Lim 1979) have suggested that corneal touch and pain thresholds may not be dependant upon axon density. Lim found the innervation to be sparse in monkeys whereas previous researchers have reported abundant innervation in the rabbit cornea. For keratoconic corneas the present study revealed a significant correlation between corneal touch threshold and epithelial innervation with regard to the number of axons per mm tissue ($P<0.05$). No such correlation could be demonstrated for the stromal innervation.

The use of electrophysiological techniques testing the function of sensory nerve fibres distal to the sensory ganglia indicate a progressive deficit of nerve impulse conduction with age. The sensory nerve action potentials decrease in amplitude and propagate at slower velocities from the third decade (Spencer & Ochoa 1981). High conduction velocity has been linked to large diameter axons (McLeod et al 1967, Spencer et al 1981). In the cornea the influence of ageing has been shown to increase the corneal touch threshold (Boberg-Ans 1955, Millodot 1977). The present study showed that ultrastructural changes in

keratoconic nerve fibres result in a large number of small stromal axons which may not be functional or resemble the changes seen in ageing neurons and therefore explain the increase in corneal touch threshold found in keratoconic corneas. Accordingly, corneal touch threshold may depend more on the size and functional quality of axons than on the volume of axons despite the significant correlation between corneal touch threshold and epithelial innervation revealed by the present study.

6.7 SUMMARY.

Corneal touch threshold (CTT) was found to be increased in the central cornea of keratoconus. The peripheral keratoconic cornea did not demonstrate any abnormality of touch threshold.

Central CTT did not show any correlation with the duration of the condition nor the nerve fibre visibility. It did correlate with corneal curvature, corneal distortion, corneal thickness, corneal scarring and the severity of the disease.

The site for the impairment of tactile corneal sensitivity in keratoconus is most likely to be localised as the function of axons is compromised and does not necessarily involve higher centres.

Neural changes are suggested to be secondary to the progression of the keratoconic condition although no definite correlation could be demonstrated.

The increase in visibility of corneal nerve fibres is thought to be related to the increase in axonal content and changes observed in the basement membrane surrounding the nerve bundles in keratoconus. A new technique using a confocal microscope (Cavanagh et al 1990) allows corneal nerves to be observed in vivo and may assist in providing an answer to the visibility of nerve fibres.

The origin of the increase in axon density in keratoconic epithelium and stroma is unknown. It can be speculated that the pathology of basal epithelial cells of the central cornea has a direct traumatic effect on the epithelial axons, and that a long-term mechanical trauma may be the trigger for the changes seen in stromal axons.

The epithelial terminals are generally regarded as serving the sensory function of the cornea. It is also generally agreed that the central epithelium is served by axons penetrating Bowman's membrane from the underlying stroma (Schimmelpfennig 1982, Rozsa et al 1982). It is therefore reasonable to suggest that pathology of the stromal axons would have an effect on the action potentials originating in

the epithelial axon terminals and being transmitted along the stromal axons. The large population of small diameter stromal axons can be compared to the axon sprouts observed in regenerating nerves following injury, and is indicative of a large group of immature axons. It is therefore likely that, despite the increase in axon population, corneal sensitivity is reduced in keratoconus because of these axons being incapable of transmitting action potentials. The use of monoclonal antibodies as markers for neurite growth can be useful in detecting antigens which are specifically associated with regions of neural sprouting. Their application might provide an answer to this question.

APPENDIX

List of abbreviations used in Appendix

K	:	Keratoconus
C	:	Control
HG	:	Control
COL	:	Control
M	:	Male
F	:	Female
Glut	:	3 to 5 % Glutaraldehyde fixative
Form	:	10 % Formaldehyde fixative
R/Form	:	Isotonic Ringer / 10 % Formal saline
EM	:	Electron microscopy
Count	:	Quantitative assessment
AChE	:	Acetylcholinesterase
CC	:	Control with contact lens wear
CS	:	Control without contact lens wear
KC	:	Keratoconus with contact lens wear
KS	:	Keratoconus without contact lens wear

APPENDIX 1

DETAILS OF MATERIAL

	REFERENCE	AGE	SEX	NATURE OF MATERIAL	FIX	PROCEDURE
KERATOCONUS	K-1	30	M	Central 8 mm	Glut	EM / Count
	K-2	27	M	Central 7 mm	Glut	AChE
	K-3	25	M	Central 8 mm	Glut	EM / Count
	K-4	17	M	Central 8 mm	Glut	EM / Count
	K-5	26	M	Central 8 mm	Glut	EM / Count
	K-6	27	M	Central 8 mm	Glut	EM / Count
	K-7	38	F	Central 8 mm	Glut	AChE
	K-P1	?	?	Central 8 mm	Glut	AChE
	K-P2	?	?	Central 8 mm	Glut	AChE
	K-11	39	M	1/2 Central 7 mm	R/Form	EM / Count
	K-12	37	M	Central 8 mm	R/Form	EM / Count
CONTROL	C-1	59	?	1/2 Cornea Melanoma	Form	EM / Count
	C-2	?	?	1/2 Cornea Melanoma	Form	AChE
	C-3	58	?	1/2 Cornea Melanoma	Form	EM / Count
	C-4	56	?	1/2 Cornea Melanoma	Form	EM / Count
	C-5	33	?	4mm P-cor. Melanoma	Form	EM / Count
	C-7	44	?	1/3 Cornea Melanoma	Form	EM / Count
	C-8	1	?	1/3 Cornea Melanoma	Form	EM / Count
	HG-1	1	?	1/3 Cornea Glaucoma	Glut	EM
	HG-2	20	?	2 mm, Glaucoma	Glut	EM
	HG-3	43	?	1.5 mm, Melanoma	Glut	EM
	COL-1	N/A	?	Peripheral Cornea	Glut	EM
	COL-2	N/A	?	Peripheral Cornea	Glut	EM
	COL/C1	N/A	?	Peripheral Cornea	Glut	EM

APPENDIX 2

KERATOCONUS GROUP WITH CONTACT LENS WEAR

CODE		BIOMETRIC DATA					CONTACT LENS DATA			
		AGE	AGE @ DIAG	M-AGE	I-COL 1-3,A-D	ALLERGY A-D	CL A-E	WT Hr	M	S-CL Hr
KC01	R	23	16	35	1-B	C	B	16	60	18
KC02	R	34	30	22	1-BC	A,C	E	12	6	48
	L						E	12	6	48
KC03	R	21	20	21	1-B	A,B	B	4	4	48
	L						E	4	4	48
KC04	R	25	24	42	2-B	NIL	B	8	2	48
	L						B	8	2	48
KC05	R	29	21	22	2-B	B	B	12	6	56
	L						B	12	6	56
KC06	R	34	32	30	2-B	NIL	E	10	12	48
KC07	R	18	17	20	1-B	A,B,C	E	15	3	48
	L						E	15	3	48
KC08	R	33	31	35	1-B	B,D	B	14	12	58
	L						E	14	12	58
KC09	R	21	19	27	2-AB	C	B	12	18	42
	L						B	12	18	42
KC10	R	28	22	29	2-D	NIL	B	16	60	38
	L						B	16	60	38
KC11	R	29	24	26	2-CD	C	E	7	24	48
	L						E	7	24	48
KC12	R	15	12	23	2-D	C	B	14	9	46
	L						B	14	9	46
KC13	R	26	20	27	2-BC	NIL	E	4	16	144
	L						E	4	16	144

KEY: M-AGE ..... Maternal age

I-COL ..... 1. Light 2. Medium 3. Dark
A. Grey B. Blue C. Green D. Brown

S-CL ..... Hours without CL wear prior to experiment

Allergy ..... A. Asthma B. Eczema C. Hayfever D. Other (ie penicillin)

CL ..... A. Nil B. PMMA C. Hydrogel (38% HEMA) D. Scleral E. Gas-permeable

/Cont'd..

APPENDIX 2

KERATOCONUS GROUP WITH CONTACT LENS WEAR continued

CODE		BIOMETRIC DATA					CONTACT LENS DATA			
		AGE	AGE @ DIAG	M-AGE	I-COL 1-3,A-D	ALLERGY A-D	CL A-E	WT Hr	M	S-CL Hr
KC14	L	16	13	25	1-BC	NIL	B	8	30	60
KC16	R	20	17	32	1-B	D	B	15	24	48
	L						B	15	24	50
KC17	R	17	16	25	1-A	NIL	B	4	0	48
	L						B	4	0	48
KC18	R	32	29	24	1-B	NIL	E	9	30	48
	L						D	9	30	48
KC19	R	39	38	22	1-B	C	E	3	0	168
	L						E	3	0	168
KC20	R	17	16	34	2-CD	A,B,C	E	15	8	50
	L						E	15	8	50
KC22	R	26	19	31	2-C	NIL	E	8	5	50
	L						E	8	5	50
KC23	L	25	9	30	2-AB	A,C	B	8	21	96
KC24	R	39	38	30	2-AB	NIL	D	1	12	168
KC26	R	26	18	22	2-D	B,C	B	16	9	72
	L						B	16	9	72
KC27	R	29	28	26	1-C	NIL	E	10	1	48
	L						E	10	1	48
KC28	L	25	19	25	1-BC	C	B	13	12	48
KC29	R	19	15	24	1-A	C	E	12	30	72
	L						B	12	30	72
KC30	R	21	14	27	1-B	C	B	16	18	56
	L						B	16	18	56

KEY: M-AGE ..... Maternal age

I-COL ..... 1. Light A. Grey
2. Medium B. Blue
3. Dark C. Green
D. Brown

S-CL ..... Hours without CL wear prior to experiment

Allergy ..... A. Asthma C. Hayfever
B. Eczema D. Other (ie penicillin)

CL ..... A. Nil D. Scleral
B. PMMA E. Gas-permeable
C. Hydrogel (38% HEMA)

/Cont'd.

APPENDIX 2

KERATOCONUS GROUP WITHOUT CONTACT LENS WEAR

CODE		BIOMETRIC DATA AGE	AGE @ DIAG	M-AGE	I-COL 1-3,A-D	ALLERGY A-D	CONTACT LENS DATA CL A-E	WT Hr	WT M	S-CL Hr
KS01	R	18	12	30	1-BC	NIL	A	-	-	-
	L						A	-	-	-
KS02	R	21	20	24	1-AB	NIL	A	-	-	-
	L						A	-	-	-
KS03	R	21	20	34	1-AB	A,C	A	-	-	-
	L						A	-	-	-
KS04	R	23	23	26	1-B	C	A	-	-	-
	L						A	-	-	-
KS05	R	19	17	35	2-CD	A,B	A	-	-	-
	L						A	-	-	-
KS06	R	18	18	38	1-BC	C	A	-	-	-
	L						A	-	-	-
KS07	R	23	20	30	1-AB	C	A	-	-	-
	L						A	-	-	-
KS08	R	25	17	40	1-BC	B	A	-	-	-
	L						A	-	-	-
KS09	R	28	20	28	1-AB	A,B,D	A	-	-	-
	L						A	-	-	-
KS10	R	18	15	30	1-A	C	A	-	-	-
	L						A	-	-	-
KS11	R	22	15	26	2-CD	C	A	-	-	-
	L						A	-	-	-
KS12	R	23	22	26	1-AB	NIL	A	-	-	-
	L						A	-	-	-
KS13	R	19	18	33	1-AC	A,C	A	-	-	-
	L						A	-	-	-

KEY: M-AGE ..... Maternal age

Allergy ..... A. Asthma C. Hayfever B. Eczema D. Other (ie penicillin)

I-COL ..... 1. Light A. Grey; 2. Medium B. Blue; 3. Dark C. Green; D. Brown

CL ..... A. Nil D. Scleral; B. PMMA E. Gas-permeable; C. Hydrogel (38% HEMA)

S-CL ..... Hours without CL wear prior to experiment

/Cont'd...

APPENDIX 2 Continued

CODE		BIOMETRIC DATA					CONTACT LENS DATA			
		AGE	AGE @ DIAG	M-AGE	I-COL 1-3,A-D	ALLERGY A-D	CL A-E	WT Hr	M	S-CL Hr
KS14	R	18	14	22	1-B	C	A	–	–	–
	L						A	–	–	–
KS15	R	27	26	25	1-AB	A,C	A	–	–	–
	L						A	–	–	–
KS16	R	18	14	20	2-CD	NIL	A	–	–	–
	L						A	–	–	–
KS17	R	34	33	21	1-AC	C	A	–	–	–
	L						A	–	–	–
KS18	R	21	16	26	1-C	NIL	A	–	–	–
	L						A	–	–	–
KS19	R	26	21	32	1-B	B,C	A	–	–	–
	L						A	–	–	–
KS20	R	29	28	21	1-D	A,C	A	–	–	–
	L						A	–	–	–
KS21	R	23	23	20	1-B	NIL	A	–	–	–
	L						A	–	–	–
KC01	L	23	16	35	1-B	C	A	–	–	–
KC06	L	34	32	30	2-B	NIL	A	–	–	–
KC14	R	16	13	25	1-BC	NIL	A	–	–	–
KC15	R	33	29	23	1-AB	NIL	A	–	–	–
	L						A	–	–	–
KC21	R	24	17	21	1-AB	A,C	A	–	–	–
	L						A	–	–	–
KC23	R	25	9	30	2-AB	A,C	A	–	–	–
KC24	L	39	38	30	2-AB	NIL	A	–	–	–
KC28	R	25	19	25	1-BC	C	A	–	–	–

KEY: M-AGE ..... Maternal age

I-COL ..... 1. Light A. Grey
2. Medium B. Blue
3. Dark C. Green

S-CL ..... Hours without CL wear prior to experiment

Allergy ..... A. Asthma C. Hayfever
B. Eczema D. Other (ie penicillin)

CL ..... A. Nil D. Scleral
B. PMMA E. Gas-permeable
C. Hydrogel (38% HEMA)

/Cont'd...

APPENDIX 2

CONTROL GROUP WITH AND WITHOUT CL WEAR

CODE		BIOMETRIC DATA					CONTACT LENS DATA			
		AGE	AGE @ DIAG	M-AGE	I-COL 1-3,A-D	ALLERGY A-D	CL A-E	WT Hr	M	S-CL Hr
CS01	R	20	-	35	2-A	NIL	A	-	-	-
	L						A	-	-	-
CS02	R	22	-	20	1-D	NIL	A	-	-	-
	L						A	-	-	-
CS03	R	21	-	40	1-D	C	A	-	-	-
	L						A	-	-	-
CS04	R	23	-	N/A	1-BC	NIL	A	-	-	-
	L						A	-	-	-
CS06	R	18	-	25	2-D	D	A	-	-	-
	L						A	-	-	-
CS07	R	19	-	20	2-D	NIL	A	-	-	-
	L						A	-	-	-
CC08	R	19	-	26	1-B	A,C,D	C	14	36	48
	L						C	14	36	48
CS09	R	23	-	35	1-AB	A,B,D	A	-	-	-
	L						A	-	-	-
CC10	R	22	-	35	1-B	C	C	20	2	96
	L						C	20	2	96
CS11	R	33	-	22	1-CD	NIL	A	-	-	-
	L						A	-	-	-
CS12	R	19	-	32	2-A	NIL	A	-	-	-
	L						A	-	-	-

KEY: M-AGE ..... Maternal age

Allergy ..... A. Asthma C. Hayfever
B. Eczema D. Other

I-COL ..... 1. Light A. Grey
2. Medium B. Blue
3. Dark C. Green
D. Brown

CL ..... A. Nil D. Scleral
B. PMMA E. Gas-permeable
C. Hydrogel (38% HEMA)

S-CL ..... Hours without CL wear prior to experiment

/Cont'd...

APPENDIX 2 Continued ...

CODE		BIOMETRIC DATA AGE	AGE @ DIAG	M-AGE	I-COL 1-3,A-D	ALLERGY A-D	CONTACT LENS DATA CL A-E	WT Hr	M	S-CL Hr
CS13	R	31	-	36	2-CD	NIL	A	-	-	-
	L						A	-	-	-
CS14	R	26	-	24	1-AB	B	A	-	-	-
	L						A	-	-	-
CC15	L	34	-	34	1-AB	A,B,C	E	6	2	120
CS16	R	23	-	32	1-A	C	A	-	-	-
	L						A	-	-	-
CC17	R	25	-	41	1-AB	NIL	C	16	6	56
	L						C	16	6	56
CS18	R	23	-	36	1-AB	NIL	A	-	-	-
	L						A	-	-	-
CS19	R	31	-	36	1-BC	B	A	-	-	-
	L						A	-	-	-
CS20	R	31	-	27	2-AD	C	A	-	-	-
	L						A	-	-	-
CC21	R	24	-	28	1-B	NIL	A	-	-	-
	L						A	-	-	-

KEY: M-AGE ..... Maternal age

Allergy ..... A. Asthma C. Hayfever
B. Eczema D. Other (ie penicillin)

I-COL ..... 1. Light A. Grey
2. Medium B. Blue
3. Dark C. Green
D. Brown

CL ..... A. Nil D. Scleral
B. PMMA E. Gas-permeable
C. Hydrogel (38% HEMA)

S-CL ..... Hours without CL wear prior to experiment

APPENDIX 3

KERATOCONIC GROUP WITH CONTACT LENS WEAR.

		SLITLAMP EXAMINATION				KERATOMETRY		
CODE		F-R x/360°	STRIAE	SCAR	N-VIS	FLAT	STEEP	CODE
KC01	R	0	0	0	0	7.74 @ 12	7.32 @ 94	3
KC02	R	0	1	0	0	7.84 @ 47	7.41 @ 145	4
	L	0	1	0	0	8.10 @ 180	8.04 @ 90	1
KC03	R	?	?	?	?	7.35 @ 38	6.73 @ 142	3
	L	?	?	?	?	6.03 @ 158	5.68 @ 92	3
KC04	R	?	?	?	?	8.40 @ 12	7.24 @ 102	2
	L	?	?	?	?	7.30 @ 122	7.17 @ 32	2
KC05	R	?	3	1	?	7.60 @ 152	7.49 @ 38	4
	L	?	0	0	?	7.84 @ 10	7.72 @ 75	3
KC06	R	80	0	0	?	7.00 @ 32	6.61 @ 142	3
KC07	R	0	3	1	AB-2	7.76 @ 24	5.84 @ 114	3
	L	0	3	1	AB-2	7.00 @ 150	6.02 @ 70	3
KC08	R	0	1	1	AB-2	7.58 @ 150	7.16 @ 50	4
	L	0	0	0	A-1	8.20 @ 118	7.82 @ 36	3
KC09	R	0	1	1	AB-2	8.01 @ 8	6.47 @ 102	4
	L	0	2	1	0	8.03 @ 168	6.44 @ 80	3
KC10	R	0	0	0	?	7.96 @ 134	7.92 @ 44	2
	L	100	3	2	?	6.30 @ 115	5.90 @ 180	5
KC11	R	120	0	0	AB-2	7.09 @ 54	6.44 @ 150	3
	L	0	0	0	AB-2	7.19 @ 33	7.18 @ 123	2
KC12	R	100	0	0	AB-1	7.38 @ 18	6.70 @ 116	4
	L	170	0	0	AB-2	6.60 @ 165	5.50 @ 85	5
KC13	R	30	0	0	ABC-3	7.52 @ 16	6.88 @ 106	2
	L	260	0	0	AB-3	6.98 @ 150	5.30 @ 60	4

N-VIS ..... A. peripheral 1. just visible
(nervefibre) B. mid-periphery 2. visible
(visibility) C. central 3. very visible

F-R ..... Fleischer's ring in degrees of arc

Striae .... 0. none 2. 4-10
1. 1-3 faint 3. >10 marked

Keratometry .. 1. clear image, no distortion
(code) 2. slightly irregular image, good alignment
3. irregular image, not at right angles
4. very irregular image, reading possible
5. reading not reliable

Scarring .. 0. none 3. 26-50%
1. 1-10% 4. 51-75%
2. 11-25% 5. >75%

/Cont'd...

APPENDIX 3 Continued

CODE		SLITLAMP EXAMINATION F-R x/360°	STRIAE	SCAR	N-VIS	KERATOMETRY FLAT	KERATOMETRY STEEP	CODE
KC14	L	0	0	0	ABC-3	7.73 @ 142	6.86 @ 61	3
KC16	R	0	0	0	0	7.20 @ 30	6.38 @ 120	3
	L	135	0	0	AB-3	6.29 @ 174	5.60 @ 90	3
KC17	R	280	0	0	A-1	7.74 @ 50	7.08 @ 150	5
	L	290	0	0	A-1	7.66 @ 120	7.04 @ 40	4
KC18	R	0	0	0	?	7.90 @ 30	7.44 @ 134	4
	L	20	2	0	?	7.66 @ 168	6.74 @ 86	3
KC19	R	10	0	0	?	7.72 @ 50	7.16 @ 140	2
	L	0	0	0	?	7.68 @ 180	7.55 @ 90	1
KC20	R	230	3	2	0	5.80 @ 180	?4.70 @ (90)	5
	L	0	0	0	0	8.04 @ 168	7.10 @ 80	5
KC22	R	80	0	0	0	7.88 @ 164	7.84 @ 48	3
	L	250	0	0	0	7.54 @ 50	7.52 @ 122	4
KC23	L	260	3	1	?	7.15 @ 140	6.16 @ 54	5
KC24	R	300	3	5	0	?4.00 @ 160	?3.90 @ 70	5
KC26	R	35	0	0	AB-2	7.70 @ 15	7.35 @ 118	4
	L	280	3	3	ABC-3	5.60 @ 170	4.50 @ 60	5
KC27	R	135	0	0	ABC-2	7.03 @ 55	6.88 @ 80	3
	L	0	1	0	AB-2	7.01 @ 10	6.50 @ 108	3
KC28	L	80	0	0	ABC-3	7.84 @ 98	7.74 @ 8	2
KC29	R	330	3	5	ABC-3	?5.40 @ 180	?5.40 @ 100	5
	L	60	0	0	AB-1	8.18 @ 128	7.76 @ 50	3
KC30	R	300	3	1	AB-2	6.40 @ 36	5.52 @ 120	4
	L	180	2	1	0	6.80 @ 160	5.72 @ 74	3

N-VIS ..... (nervefibre) (visibility)
A. peripheral
B. mid-periphery
C. central
1. just visible
2. visible
3. very visible

F-R ..... Fleischer's ring in degrees of arc

Keratometry .. (code)
1. clear image, no distortion
2. slightly irregular image, good alignment
3. irregular image, not at right angles
4. very irregular image, reading possible
5. reading not reliable

Striae .... 0. none 1. 1-3 faint 2. 4-10 3. >10 marked

Scarring .. 0. none 1. 1-10% 2. 11-25% 3. 26-50% 4. 51-75% 5. >75%

/Cont'd...

APPENDIX 3

KERATOCONIC GROUP WITHOUT CONTACT LENS WEAR.

		SLITLAMP EXAMINATION				KERATOMETRY		
CODE		F-R x/360º	STRIAE	SCAR	N-VIS	FLAT	STEEP	CODE
KS01	R	?	0	0	A-1	7.07 @ 12	6.10 @ 102	2
	L	?	0	0	A-1	6.74 @ 180	5.80 @ 98	3
KS02	R	?	1	0	A-1	6.98 @ 53	6.13 @ 145	3
	L	?	0	0	A-1	7.46 @ 95	7.14 @ 8	2
KS03	R	90	0	0	A-1	7.70 @ 40	6.87 @ 130	2
	L	90	1	0	AB-2	7.75 @ 154	6.71 @ 64	2
KS04	R	0	0	0	AB-3	6.88 @ 25	5.88 @ 125	3
	L	0	0	0	0	7.63 @ 106	7.36 @ 24	3
KS05	R	210	0	0	?	7.38 @ 156	6.64 @ 74	3
	L	360	3	3	?	5.20 @ 138	4.80 @ 44	5
KS06	R	55	0	0	ABC-3	7.87 @ 20	7.30 @ 110	3
	L	0	0	0	A-2	7.77 @ 146	7.51 @ 56	1
KS07	R	50	0	0	0	7.76 @ 50	7.68 @ 140	1
	L	100	0	0	0	7.42 @ 152	7.00 @ 68	4
KS08	R	150	1	0	AB-3	8.20 @ 45	7.26 @ 130	3
	L	240	3	0	ABC-3	8.04 @ 45	6.54 @ 128	5
KS09	R	0	3	0	0	8.01 @ 78	6.45 @ 168	2
	L	0	1	0	0	7.63 @ 110	6.72 @ 20	2
KS10	R	0	1	0	A-1	8.02 @ 12	6.53 @ 100	4
	L	0	0	0	A-1	7.84 @ 157	6.96 @ 67	3

/Cont'd...

N-VIS ..... A. peripheral 1. just visible
(nervefibre) B. mid-periphery 2. visible
(visibility) C. central 3. very visible

F-R ..... Fleischer's ring in degrees of arc

Striae .... 0. none 2. 4-10
1. 1-3 faint 3. >10 marked

Keratometry .. 1. clear image, no distortion
(code) 2. slightly irregular image, good alignment
3. irregular image, not at right angles
4. very irregular image, reading possible
5. reading not reliable

Scarring .. 0. none 3. 26-50%
1. 1-10% 4. 51-75%
2. 11-25% 5. >75%

APPENDIX 3 Continued

		SLITLAMP EXAMINATION				KERATOMETRY		
CODE		F-R x/360°	STRIAE	SCAR	N-VIS	FLAT	STEEP	CODE
KS11	R	360	3	0	AB-3	5.98 @ 10	5.20 @ 104	3
	L	180	2	0	A-2	7.40 @ 166	6.42 @ 80	4
KS12	R	280	3	0	AB-3	6.26 @ 8	5.20 @ 105	4
	L	330	3	3	0	<4.50 @ -	<4.50 @ -	5
KS13	R	0	0	0	AB-3	8.00 @ 46	7.62 @ 156	4
	L	235	2	1	ABC-3	8.45 @ 175	5.10 @ 90	5
KS14	R	70	0	0	A-1	7.62 @ 36	7.36 @ 138	3
	L	360	2	1	AB-2	7.01 @ 144	5.00 @ 54	5
KS15	R	0	1	0	AB-2	8.04 @ 42	7.70 @ 152	3
	L	0	0	0	AB-2	8.32 @ 112	7.93 @ 22	2
KS16	R	90	0	0	AB-1	6.95 @ 2	5.75 @ 92	2
	L	360	0	0	ABC-3	6.36 @ 168	5.50 @ 78	2
KS17	R	0	0	0	AB-1	8.04 @ 16	7.80 @ 106	2
	L	250	0	0	AB-1	7.73 @ 108	7.26 @ 26	4
KS18	R	130	0	0	A-1	7.64 @ 20	7.04 @ 116	3
	L	100	0	0	AB-2	7.49 @ 166	7.10 @ 76	2
KS19	R	0	0	1	AB-2	7.70 @ 26	7.64 @ 116	1
	L	330	0	1	AB-2	6.90 @ 152	5.86 @ 62	2
KS20	R	130	0	0	?	7.55 @ 38	6.82 @ 136	3
	L	230	1	0	?	7.71 @ 130	6.76 @ 40	3

N-VIS ..... A. peripheral 1. just visible
(nervefibre) B. mid-periphery 2. visible
(visibility) C. central 3. very visible

Keratometry .. 1. clear image, no distortion
(code) 2. slightly irregular image, good alignment
3. irregular image, not at right angles
4. very irregular image, reading possible
5. reading not reliable

F-R ..... Fleischer's ring in degrees of arc

Striae .... 0. none 2. 4-10
1. 1-3 faint 3. >10 marked

Scarring .. 0. none 3. 26-50%
1. 1-10% 4. 51-75%
2. 11-25% 5. >75%

APPENDIX 3 Continued

CODE		SLITLAMP EXAMINATION F-R x/360°	STRIAE	SCAR	N-VIS	KERATOMETRY FLAT	STEEP	CODE
KS21	R	110	1	0	A-3	7.38 @ 12	6.68 @ 110	3
	L	0	0	0	A-1	7.76 @ 166	7.42 @ 76	1
KC01	L	0	0	0	0	7.71 @ 60	7.19 @ 100	3
KC06	L	0	0	0	?	7.60 @ 144	7.36 @ 54	1
KC14	R	0	0	0	A-1	7.82 @ 180	7.80 @ 90	2
KC15	R	260	3	0	ABC-3	6.53 @ 26	5.86 @ 132	5
	L	360	3	0	ABC-3	7.12 @ 110	6.10 @ 36	5
KC21	R	0	0	0	AB-3	7.88 @ 18	7.36 @ 108	2
	L	0	0	0	AB-1	7.54 @ 150	6.56 @ 58	3
KC23	R	90	0	0	?	7.76 @ 30	7.02 @ 126	3
KC24	L	240	0	0	ABC-3	7.48 @ 168	7.00 @ 75	5
KC28	R	290	0	2	AB-2	7.98 @ 90	7.95 @ 180	1

N-VIS ..... (nervefibre) (visibility)
A. peripheral B. mid-periphery C. central
1. just visible 2. visible 3. very visible

F-R ..... Fleischer's ring in degrees of arc

Keratometry .. (code)
1. clear image, no distortion
2. slightly irregular image, good alignment
3. irregular image, not at right angles
4. very irregular image, reading possible
5. reading not reliable

Striae .... 0. none 1. 1-3 faint 2. 4-10 3. >10 marked

Scarring .. 0. none 1. 1-10% 2. 11-25% 3. 26-50% 4. 51-75% 5. >75%

APPENDIX 3

CONTROL GROUP WITH & WITHOUT CONTACT LENS WEAR.

		SLITLAMP EXAMINATION				KERATOMETRY		
CODE		F-R x/360°	STRIAE	SCAR	N-VIS	FLAT	STEEP	CODE
CS01	R	0	0	0	0	8.03 @ 180	7.94 @ 90	1
	L	0	0	0	AB-1	8.05 @ 180	7.94 @ 90	1
CS02	R	0	0	0	AB-2	8.28 @ 180	8.22 @ 90	1
	L	0	0	0	AB-2	8.28 @ 180	8.28 @ 90	1
CS03	R	0	0	0	0	7.90 @ 180	7.62 @ 90	1
	L	0	0	0	0	7.85 @ 162	7.54 @ 72	1
CS04	R	0	0	0	0	7.85 @ 180	7.85 @ 90	1
	L	0	0	0	0	7.85 @ 180	7.81 @ 90	1
CS06	R	0	0	0	0	8.20 @ 180	8.10 @ 90	1
	L	0	0	0	0	8.21 @ 160	8.14 @ 70	1
CS07	R	0	0	0	0	7.51 @ 170	7.41 @ 80	1
	L	0	0	0	A-1	7.46 @ 8	7.28 @ 98	1
CC08	R	0	0	0	AB-2	7.78 @ 165	7.55 @ 75	2
	L	0	0	0	AB-2	7.72 @ 180	7.46 @ 90	1
CS09	R	0	0	0	0	8.38 @ 180	8.23 @ 90	1
	L	0	0	0	0	8.34 @ 180	8.13 @ 90	1
CC10	R	0	0	0	A-1	8.21 @ 180	8.15 @ 90	1
	L	0	0	0	A-1	8.24 @ 90	8.19 @ 180	1
CS11	R	0	0	0	A-1	8.30 @ 180	8.28 @ 90	1
	L	0	0	0	A-1	8.30 @ 180	8.28 @ 90	1

/Cont'd...

N-VIS ..... A. peripheral 1. just visible
(nervefibre) B. mid-periphery 2. visible
(visibility) C. central 3. very visible

Keratometry .. 1. clear image, no distortion
(code) 2. slightly irregular image, good alignment
3. irregular image, not at right angles
4. very irregular image, reading possible
5. reading not reliable

F-R ..... Fleischer's ring in degrees of arc

Striae .... 0. none 2. 4-10
1. 1-3 faint 3. >10 marked

Scarring .. 0. none 3. 26-50%
1. 1-10% 4. 51-75%
2. 11-25% 6. >75%

APPENDIX 3 Continued

CODE		SLITLAMP EXAMINATION F-R x/360°	STRIAE	SCAR	N-VIS	KERATOMETRY FLAT	STEEP	CODE
CS12	R	0	0	0	A-1	8.20 @ 180	7.88 @ 90	1
	L	0	0	0	A-1	8.23 @ 180	8.00 @ 90	1
CS13	R	0	0	0	0	7.70 @ 170	7.53 @ 80	1
	L	0	0	0	0	7.68 @ 10	7.46 @ 100	1
CS14	R	0	0	0	0	7.80 @ 170	7.70 @ 80	1
	L	0	0	0	0	7.83 @ 23	7.76 @ 113	1
CC15	L	0	0	0	0	7.80 @ 180	7.54 @ 90	1
CS16	R	0	0	0	0	8.13 @ 18	7.95 @ 108	1
	L	0	0	0	0	8.14 @ 180	7.89 @ 90	1
CC17	R	0	0	0	AB-2	7.81 @ 42	7.77 @ 132	1
	L	0	0	0	AB-2	7.66 @ 90	7.61 @ 180	1
CS18	R	0	0	0	0	7.85 @ 180	7.82 @ 90	1
	L	0	0	0	0	7.92 @ 180	7.90 @ 90	1
CS19	R	0	0	0	A-1	8.26 @ 180	8.12 @ 90	1
	L	0	0	0	A-1	8.35 @ 180	8.16 @ 90	1
CS20	R	0	0	0	A-2	8.14 @ 12	7.90 @ 102	1
	L	0	0	0	A-2	8.05 @ 180	7.80 @ 90	1
CS21	R	0	0	0	A-1	7.80 @ 180	7.67 @ 90	1
	L	0	0	0	A-1	7.86 @ 180	7.74 @ 90	1

N-VIS ..... (nervefibre) (visibility)
A. peripheral
B. mid-periphery
C. central
1. just visible
2. visible
3. very visible

F-R ..... Fleischer's ring in degrees of arc

Keratometry .. (code)
1. clear image, no distortion
2. slightly irregular image, good alignment
3. irregular image, not at right angles
4. very irregular image, reading possible
5. reading not reliable

Striae .... 0. none 1. 1-3 faint 2. 4-10 3. >10 marked

Scarring .. 0. none 1. 1-10% 2. 11-25% 3. 26-50% 4. 51-75% 6. >75%

APPENDIX 4

KERATOCONIC GROUP WITH CONTACT LENSES

CODE		TOUCH THRESHOLD (mg/mm²) CORNEA C	N	T	I	S	LID I	S	CORNEAL THICKNESS Time	Central (um)
KC01	R	5	5	4.4	4.4	10	–	–	1450	416
KC02	R	2	5	5	2	14	–	–	1640	456
	L	3	5	5	10	6	–	–		478
KC03	R	40	17	14	17	17	–	–	1730	382
	L	40	17	17	22	30	4.4	14		351
KC04	R	10	8	3	6	10	–	–	1710	416
	L	4.4	6	10	14	30	–	–		420
KC05	R	5	4.4	4.4	8	8	–	–	1630	376
	L	8	5	4.4	14	40	–	–		433
KC06	R	22	12	22	17	12	–	–	1705	362
KC07	R	6	9	5	8	30	–	–	1435	436
	L	10	14	4.4	10	14	–	–		449
KC08	R	2	22	6	40	22	–	–	1435	444
	L	5	10	22	22	30	–	–		482
KC09	R	30	55	40	150	40	–	–	1430	456
	L	22	10	40	150	22	–	–		447
KC10	R	22	12	8	22	22	–	6	1425	462
	L	22	10	14	22	55	–	5		362
KC11	R	17	55	30	22	40	40	17	1635	431
	L	30	40	22	14	90	90	90		476
KC12	R	14	22	30	40	55	10	17	1550	480
	L	90	22	12	40	90	14	9		433
KC13	R	12	10	12	30	30	14	10	1620	464
	L	55	12	8	30	40	10	22		460

KEY: Corneal Touch Threshold (CTT)
C .... Central
N .... Nasal
T .... Temporal
I .... Inferior
S .... Superior

Lid Touch Threshold (LTT)
I .... Inferior
S .... Superior

/Cont'd...

APPENDIX 4 continued

CODE		AESTHESIOMETRY (mg/mm²) CORNEA C	N	T	I	S	LID I	S	PACHOMETRY Time	Central (um)
KC14	L	6	6	6	6	10	5	5	1445	484
KC16	R	150	17	22	55	40	22	40	1600	524
	L	90	150	150	90	90	150	150		380
KC17	R	40	40	30	150	90	17	5	1645	411
	L	30	55	30	90	90	10	14		378
KC18	R	9	17	17	55	22	90	90	1415	449
	L	30	150	17	150	90	90	90		422
KC19	R	10	22	17	55	55	14	17	1640	489
	L	7.5	55	17	40	40	40	12		496
KC20	R	150	22	17	22	40	40	40	1540	447
	L	40	55	30	40	40	150	90		456
KC22	R	14	17	14	17	12	8	17	1605	478
	L	14	14	10	14	22	17	30		471
KC23	L	7.5	14	7.5	17	55	22	30	1405	449
KC24	R	40	150	150	90	90	-	-	1450	456
KC26	R	40	-	-	90	10	-	-	1425	422
	L	22	-	-	40	22	-	-		358
KC27	R	3	10	4.4	10	4.4	4.4	4.4	1455	436
	L	2	2	5	14	14	4.4	4.4		440
KC28	L	2	4.4	3	22	22	4.4	6	1430	536
KC29	R	150	8	4.4	2	40	90	90	1530	356
	L	22	22	10	17	40	22	40		516
KC30	R	90	30	17	90	90	90	90	1500	438
	L	55	40	17	40	55	90	90		431

KEY: Corneal Touch Threshold (CTT)
C .... Central
N .... Nasal
T .... Temporal
I .... Inferior
S .... Superior

Lid Touch Threshold (LTT)
I .... Inferior
S .... Superior

/Cont'd...

APPENDIX 4

KERATOCONIC GROUP WITHOUT CONTACT LENSES

CODE		TOUCH THRESHOLD (mg/mm²) CORNEA C	N	T	I	S	LID I	S	CORNEAL THICKNESS Time	Central (um)
KS01	R	2	2	2	2	8	10	6	1550	444
	L	2	3	2	8	10	14	8		418
KS02	R	5	6	8	14	14	–	–	1440	418
	L	4.4	22	10	12	30	–	–		440
KS03	R	5	4.4	7.5	7.5	14	5	–	1630	422
	L	8	5	8	10	10	5	–		433
KS04	R	30	40	30	40	55	10	10	1550	489
	L	22	40	22	30	30	22	22		542
KS05	R	55	22	17	22	30	30	22	1630	431
	L	55	30	22	40	40	55	40		373
KS06	R	17	22	22	55	40	–	90	1630	544
	L	30	90	150	150	40	–	90		547
KS07	R	8	14	14	30	–	22	10	1135	556
	L	30	14	12	22	14	17	40		533
KS08	R	40	12	10	14	55	22	14	1455	476
	L	40	90	17	12	40	30	40		442
KS09	R	12	10	17	14	40	14	17	1450	453
	L	10	40	22	14	55	10	12		458
KS10	R	5	55	10	14	17	–	–	1515	498
	L	8	14	17	12	90	–	–		498

KEY: Corneal Touch Threshold (CTT)
C .... Central
N .... Nasal
T .... Temporal
I .... Inferior
S .... Superior

Lid Touch Threshold (LTT)
I .... Inferior
S .... Superior

/Cont'd...

APPENDIX 4 continued ...

CODE		TOUCH THRESHOLD (mg/mm²) CORNEA C	N	T	I	S	LID I	S	CORNEAL THICKNESS Time	Central (um)
KS11	R	4.4	-	-	30	-	-	-	1430	431
	L	5	-	-	17	-	-	-		489
KS12	R	22	150	150	90	90	14	-	1700	467
	L	90	150	150	90	40	14	-		429
KS13	R	3	5	3	8	4.4	40	3	1700	524
	L	4.4	4.4	3	6	5	22	4.5		451
KS14	R	3	5	4.4	6	40	10	90	1510	498
	L	3	5	5	6	30	9	10		436
KS15	R	2	22	8	14	14	8	6	1735	484
	L	12	14	30	55	12	8	8		482
KS16	R	4.4	30	5	9	12	10	5	1445	482
	L	9	6	17	30	17	10	6		476
KS17	R	17	40	40	40	150	90	90	1100	498
	L	40	40	90	90	90	90	90		464
KS18	R	6	14	5	7.5	14	10	22	1410	453
	L	10	40	4.4	14	10	10	40		460
KS19	R	14	22	14	30	40	90	22	1330	460
	L	22	12	14	40	40	22	22		400
KS20	R	5	30	5	55	40	-	-	1325	500
	L	5	10	6	8	40	-	-		442

KEY: Corneal Touch Threshold (CTT)
C .... Central
N .... Nasal
T .... Temporal
I .... Inferior
S .... Superior

Lid Touch Threshold (LTT)
I .... Inferior
S .... Superior

/Cont'd...

APPENDIX 4 continued

CODE		TOUCH THRESHOLD (mg/mm²) CORNEA C	N	T	I	S	LID I	S	CORNEAL THICKNESS Time	Central (um)
KS21	R	40	90	30	90	40	22	90	1350	467
	L	7.5	22	12	22	55	30	90		513
KC01	L	4.4	5	4.4	4.4	10	-	-	1450	402
KC06	L	3	5	10	8	14	5	-	1705	438
KC14	R	5	8	4.4	5	5	4.4	5	1445	502
KC15	R	30	90	8	40	90	7.5	40	1415	431
	L	40	40	8	30	40	22	22		447
KC21	R	7.5	14	6	10	14	14	14	1605	504
	L	7	14	10	8	12	10	10		476
KC23	R	5	7.5	6	40	55	10	22	1505	420
KC24	L	55	150	90	150	150	-	-	1450	482
KC28	R	2	5	2	8	40	6	4.4	1430	502

KEY: Corneal Touch Threshold (CTT)
C .... Central
N .... Nasal
T .... Temporal
I .... Inferior
S .... Superior

Lid Touch Threshold (LTT)
I .... Inferior
S .... Superior

/Cont'd...

APPENDIX 4 CONTROL GROUP WITH & WITHOUT CONTACT LENSES

CODE		TOUCH THRESHOLD (mg/mm²) CORNEA C	N	T	I	S	LID I	S	CORNEAL THICKNESS Time	Central (um)
CS01	R	8	12	14	40	90	150	10	1750	527
	L	17	14	8	55	90	150	14		549
CS02	R	4.4	6	5	8	40	22	22	1700	514
	L	4.4	12	12	22	55	10	22		531
CS03	R	5	8	8	22	22	22	22	1730	547
	L	7.5	12	8	9	40	40	22		527
CS04	R	8	40	8	90	40	8	14	1630	516
	L	14	90	14	55	30	14	14		520
CS06	R	4.4	8	6	5	10	6	90	1610	493
	L	8	22	8	40	8	10	90		502
CS07	R	2	30	5	40	14	3	6	1650	540
	L	2	17	4.4	6	10	6	10		542
CC08	R	17	30	22	14	40	40	40	1620	516
	L	55	17	22	22	40	40	40		533
CS09	R	2	6	5	3	30	10	14	1440	502
	L	2	8	4.4	10	30	12	30		504
CC10	R	2	2	2	10	10	–	–	1825	500
	L	2	2	2	10	14	–	–		500
CS11	R	2	40	5	5	14	6	22	1355	524
	L	3	22	4.4	6	10	6	6		527

KEY: Corneal Touch Threshold (CTT)
- C .... Central
- N .... Nasal
- T .... Temporal
- I .... Inferior
- S .... Superior

Lid Touch Threshold (LTT)
- I .... Inferior
- S .... Superior

/Cont'd...

APPENDIX 4 CONTROL GROUP CONTINUED

CODE		TOUCH THRESHOLD (mg/mm²) CORNEA C	N	T	I	S	LID I	S	CORNEAL THICKNESS Time	Central (um)
CS12	R	10	40	10	22	14	10	8	1550	540
	L	10	40	14	22	22	10	10		536
CS13	R	5	150	8	22	90	–	–	1715	518
	L	8	40	6	40	22	–	–		516
CS14	R	2	90	8	5	40	–	–	1800	522
	L	2	8	4.4	14	40	–	–		509
CC15	L	3	55	17	90	90	–	–	1600	529
CS16	R	2	8	8	4.4	10	6	4.4	1800	522
	L	2	40	4.4	8	10	6	6		520
CC17	R	14	22	22	10	40	–	–	1415	542
	L	10	14	14	14	90	–	–		551
CS18	R	4.4	30	22	14	150	6	6	1725	524
	L	4.4	30	14	30	90	4.4	10		518
CS19	R	4.4	12	6	6	10	–	–	1725	536
	L	2	14	10	6	14	–	–		540
CS20	R	8	5	8	9	22	10	14	1530	516
	L	7.5	12	12	5	22	5	14		518
CS21	R	2	5	4.4	3	8	6	–	1750	553
	L	2	7.5	2	6	8	6	–		553

KEY:

Corneal Touch Threshold (CTT)
- C .... Central
- N .... Nasal
- T .... Temporal
- I .... Inferior
- S .... Superior

Lid Touch Threshold (LTT)
- I .... Inferior
- S .... Superior

APPENDIX 5

KERATOCONIC CORNEAS EXAMINED FOR SENSITIVITY AND INNERVATION.

		SLITLAMP EXAMINATION				KERATOMETRY		
CODE		F-R x/360°	STRIAE	SCAR	N-VIS	FLAT	STEEP	CODE
K1	L	0	2	3	A-1	6.00 @ 170	5.86 @ 100	5
K3	L	260	0	6	A-1	5.60 @ 180	4.80 @ 90	5
K4	L	320	3	6	ABC-3	4.90 @ 60	4.50 @ 26	5
K5	L	120	2	0	ABC-2	5.98 @ 160	5.30 @ 75	3
K6	R	320	0	6	A-1	5.80 @ 110	?4.80 @ ?	5

		TOUCH THRESHOLD (mg/mm²)							CORNEAL THICKNESS	
CODE		CORNEA					LID			
		C	N	T	I	S	I	S	TIME	Central (um)
K1	L	8	6	8	8	8	10	-	1720	318
K3	L	90	150	150	55	14	90	90	1230	367
K4	L	12	17	12	14	30	-	-	1705	460
K5	L	8	5	5	5	-	-	-	0905	331
K6	R	150	90	40	30	55	-	-	0900	606

KEY:

N-VIS ..... (nervefibre) (visibility)
- A. peripheral
- B. mid-periphery
- C. central
- 1. just visible
- 2. visible
- 3. very visible

Keratometry .. (code)
1. clear image, no distortion
2. slightly irregular image, good alignment
3. irregular image, not at right angles
4. very irregular image, reading possible
5. reading not reliable

F-R ..... Fleischer's ring in degrees of arc

Striae .... 0. none; 1. 1-3 faint; 2. 4-10; 3. >10 marked

Scarring .. 0. none; 1. 1-10%; 2. 11-25%; 3. 26-50%; 4. 51-75%; 6. >75%

Touch threshold
- C .... Central
- N .... Nasal
- T .... Temporal
- I .... Inferior
- S .... Superior

Corneal thickness
- C ... Central
- N ... Nasal approx 1 mm from limbus
- T ... Temporal at midperiphery
- I ... Inferior approx 2 mm from limbus
- S ... Superior approx 2 mm from limbus

APPENDIX 6 CALIBRATION OF THE AMERICAN OPTICAL KERATOMETER.

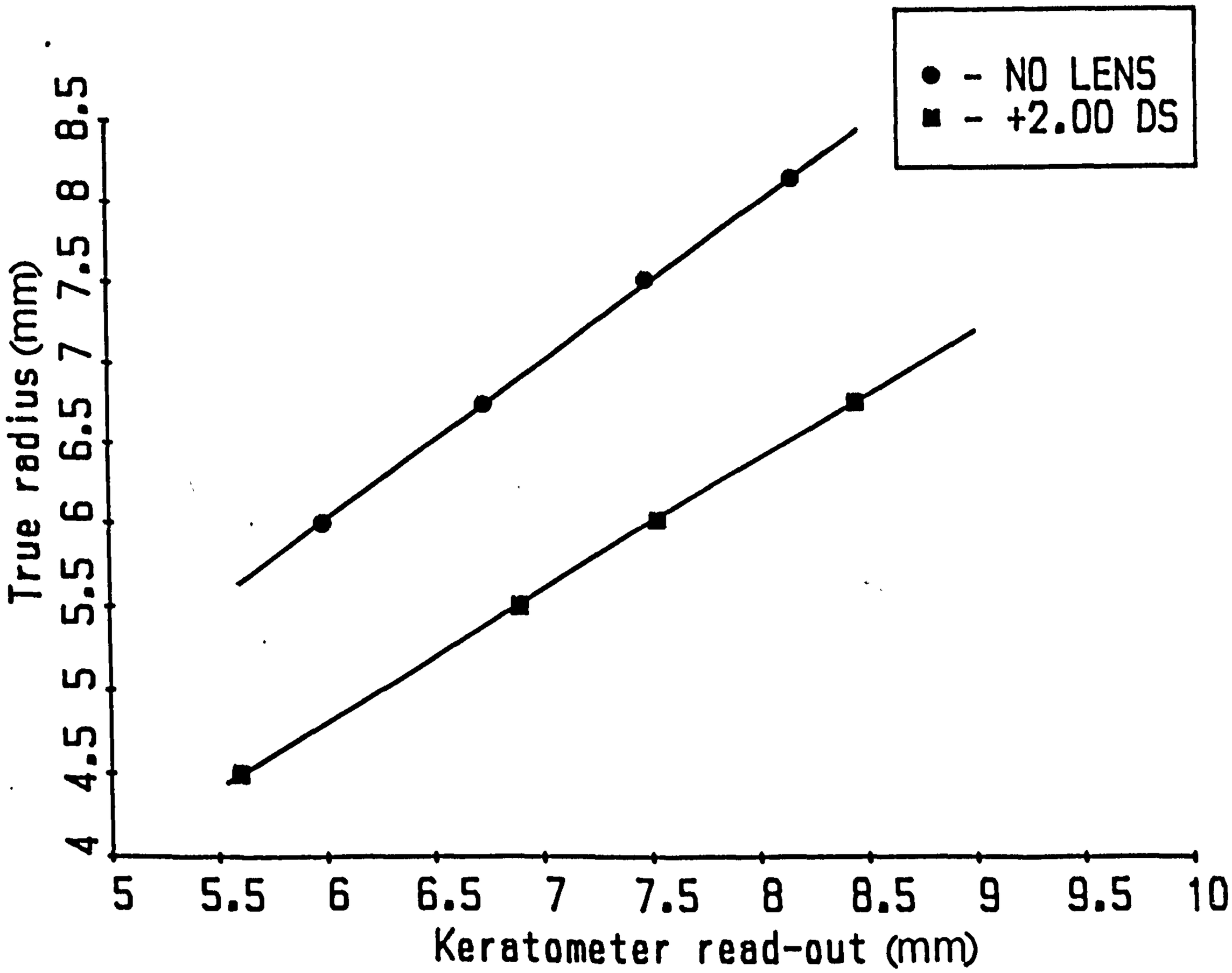
The measuring range of the American Optical keratometer was extended by introducing a +2.00 D spherical lens in front of the keratometer objective. This made it possible to measure very steep radii, and a series of steel ball bearings were used for calibration as indicated below.

Ball bearing radius (mm)	Unmodified keratometer readout (mm)	Modified keratometer (+2.00 D) readout (mm)
8.130	8.175	N/A
7.500	7.496	N/A
6.745	6.743	8.46
6.000	5.987	7.54
5.500	N/A	6.90
4.500	N/A	5.60

The steel balls were measured with a micrometer and all values represent the mean of ten readings. Calibration was repeated at regular intervals throughout the duration of the experimental period and did not show significant variations from the values seen above. The modified keratometer readout was plotted against the steel ball values as seen in figure A-6.

FIGURE A-6

CALIBRATION OF AO KERATOMETER

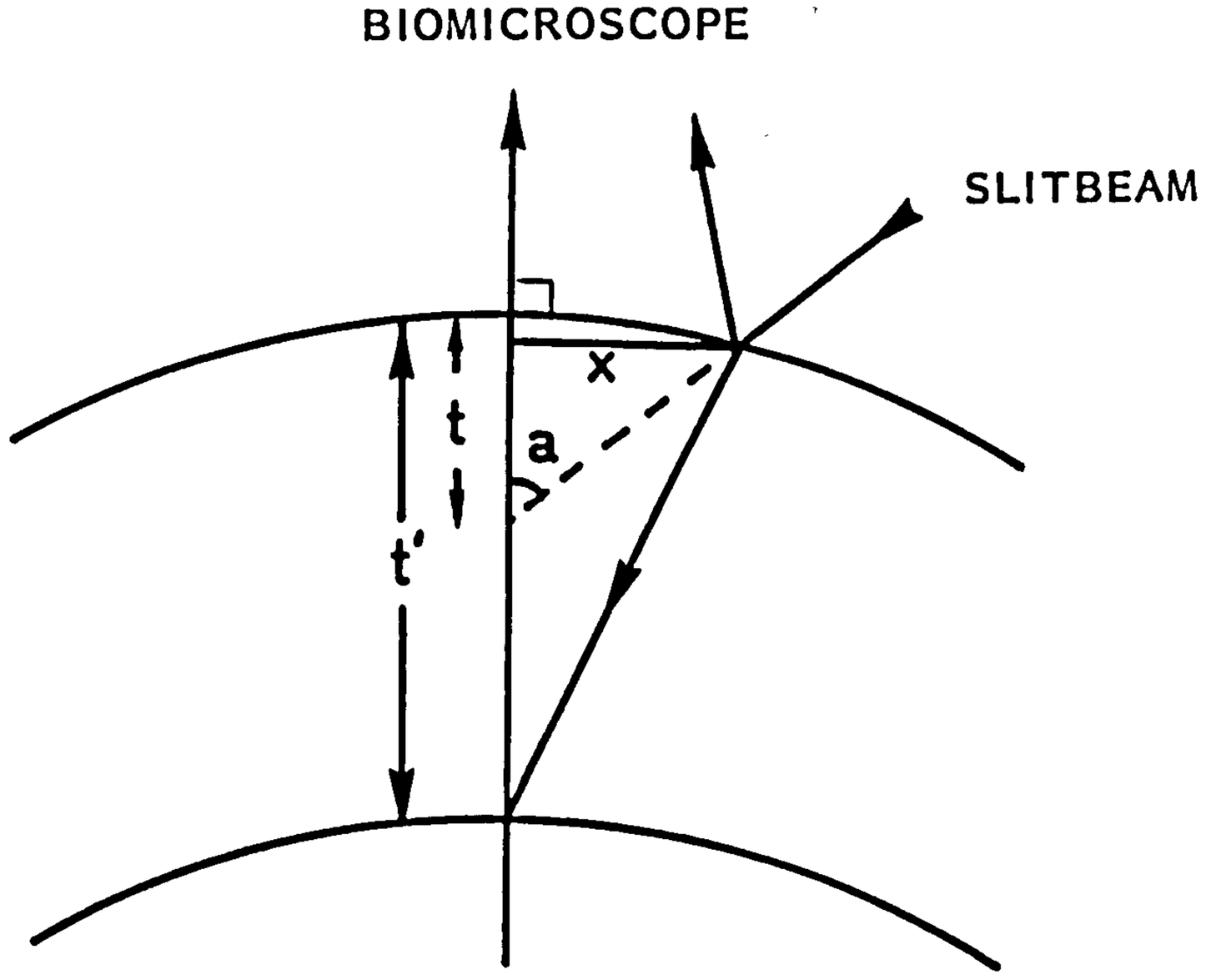


The measuring range of the American Optical keratometer was extented by using a +2.00 DS lens in front of the objective. Calibration curves without and with the spherical lens were produced.

APPENDIX 7 CALIBRATION OF THE PACHOMETER.

The pachometer was used as described by Donaldson (1966) and calibrated after the method described by Mandell & Polse (1969). Contact lenses of known radii and thicknesses were used (Table A-7) and a correction made for the difference in refractive indices from PMMA to cornea (Fig. A-7.1).

Figure A-7.1 (After Mandell & Polse 1969)



x = apparent thickness of the slitlamp section.
t = true corneal or contact lens thickness.
t'= paraxial image of apparent corneal or contact lens thickness.
t' is proportional to x.
a = fixed angle between slitlamp illumination and observation systems.

Assuming the two curved surfaces are coaxial with the measurement position, then the paraxial formula for a single refracting surface may be used :

$$\frac{n'}{l'} - \frac{n}{l} = \frac{n' - n}{r}$$

Where
l = object distance
l'= image distance
n = refractive index of object media
n'= refractive index of image media
r = radius of curvature for refractive surface

For cornea :

$l' = t'$ & $l = t$
$n' = 1$ & $n = 1.376$
r = anterior corneal radius
t_{COR} = true corneal thickness

$$\frac{1}{t'} - \frac{1.376}{t} = \frac{-0.376}{r}$$

$$t_{COR} = \frac{1.376\ t'\ r}{0.376\ t' + r}$$

For PMMA contact lenses :

$l' = t'$ & $l = t$
$n' = 1$ & $n = 1.490$
r = front optic radius
t_{CL} = true contact lens thickness

$$\frac{1}{t'} - \frac{1.490}{t} = \frac{-0.490}{r}$$

$$t_{CL} = \frac{1.490\ t'\ r}{0.490\ t' + r}$$

then the correction factor from contact lens thickness to corneal thickness will be :

$$\frac{t_{COR}}{t_{CL}} = \frac{1.376\ (0.490\ t' + r)}{1.490\ (0.376\ t' + r)}$$

Assuming an apparant thickness (t') as Mandell & Polse of 0.45 mm and an average curvature for :

A. Control group : r=7.80 mm
B. Keratoconus group : r=7.00 mm

we obtain correction factors :

A. Control group ..... $t_{COR} = 0.929 \times t_{CL}$
B. Keratoconus group ... $t_{COR} = 0.930 \times t_{CL}$

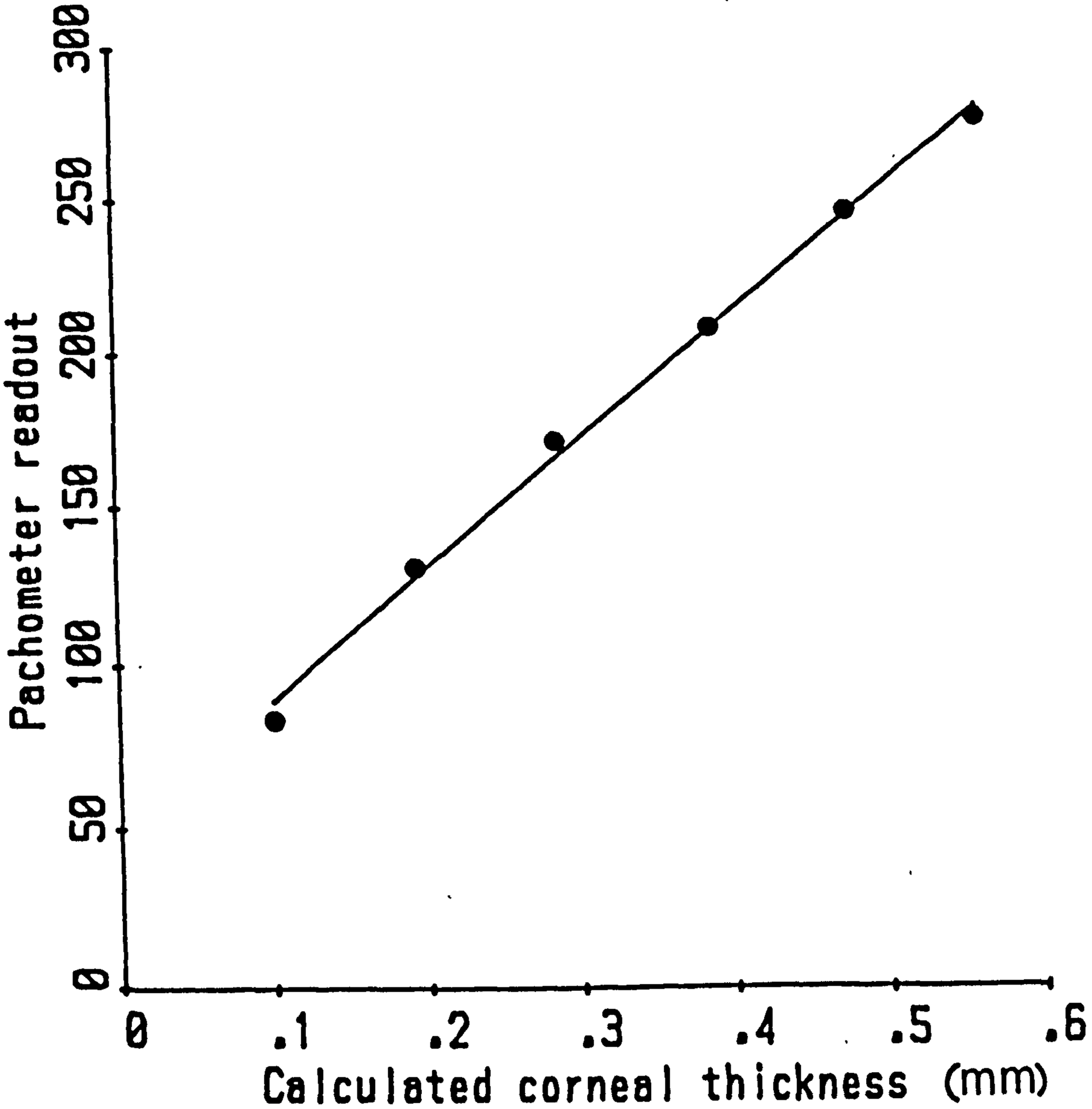
The difference in correction factor caused by different corneal curvatures for the control group and the keratoconus group is insignificant, and one factor (0.930) can be used in Table A-7.

Table A-7 Calibration of the pachometer.

Contact lens thickness (mm)	Calculated corenal thickness (mm)	Pachometer read-out (mm)
0.110	0.102	82.9
0.210	0.195	130.3
0.309	0.287	171.0
0.417	0.388	208.4
0.512	0.477	246.2
0.604	0.562	276.9

All values represent the mean of ten readings. The pachometer read-out was plottet against the calculated corneal thickness from Table A-7 resulting in the straight line seen in Figure A-7.2 with the correlation coefficient of r=0.998. Calibration was repeated at regular intervals throughout the duration of the testing period.

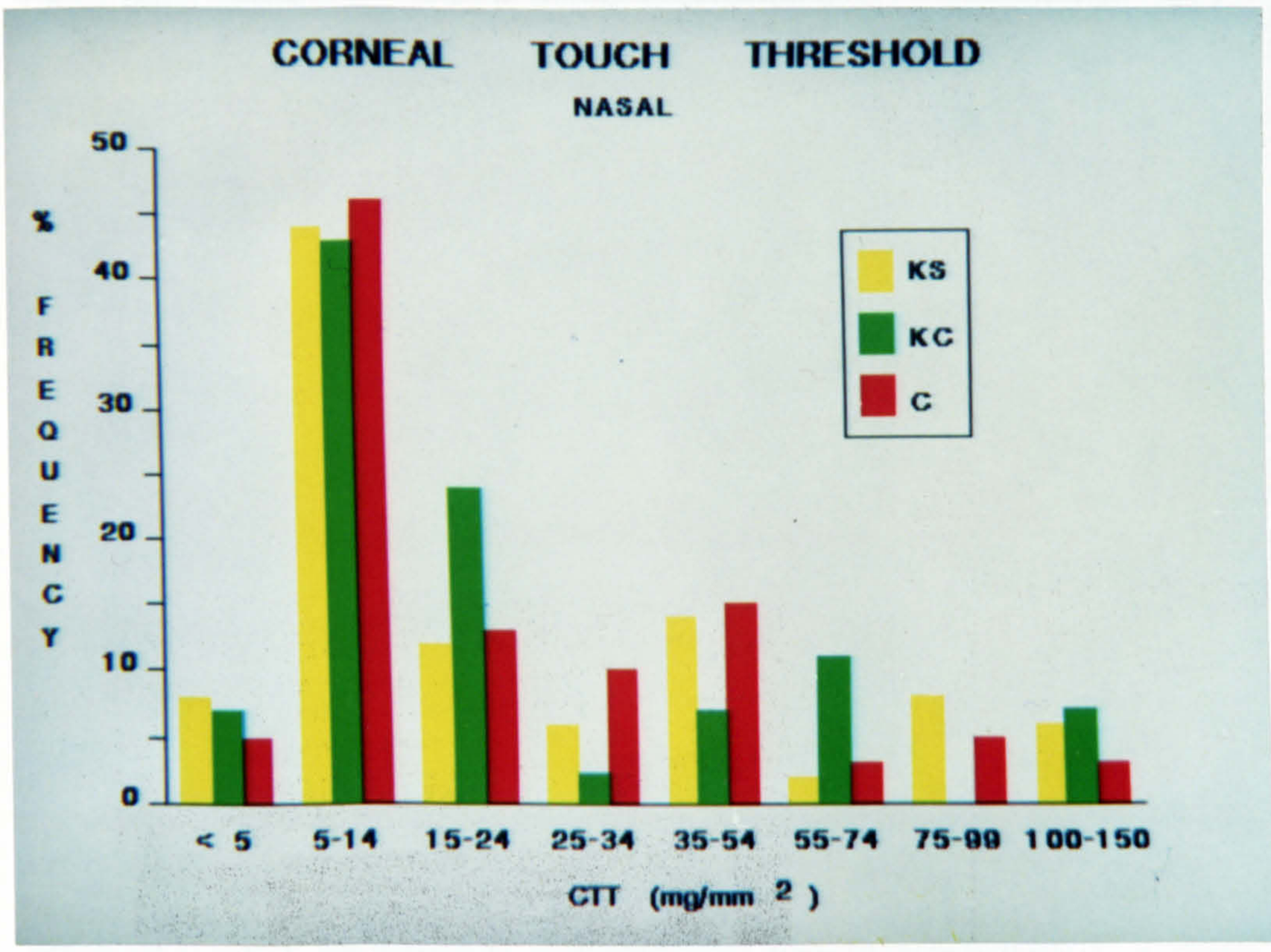
FIGURE A-7.2 Calibration of the Haag-Streit pachometer.

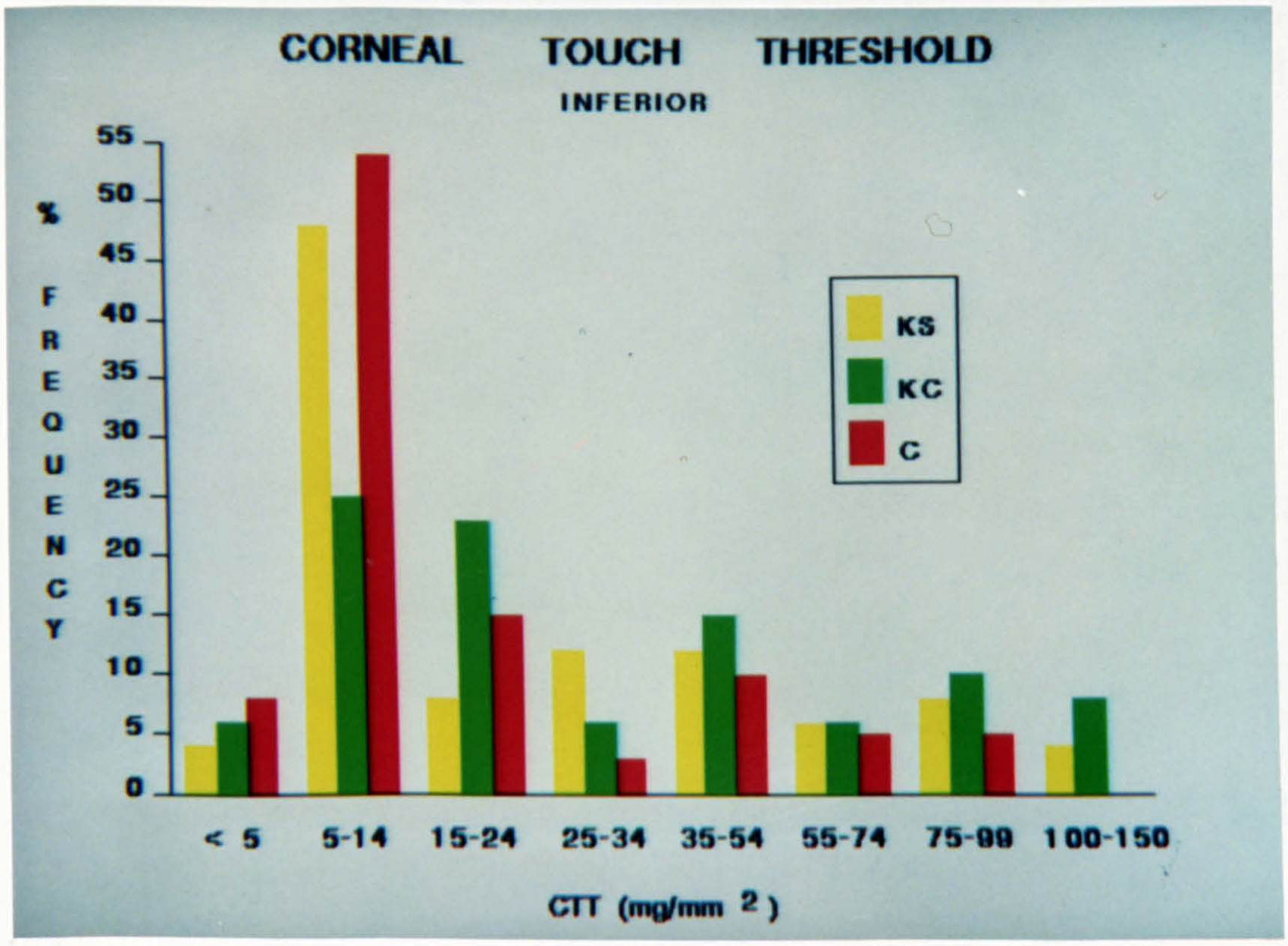


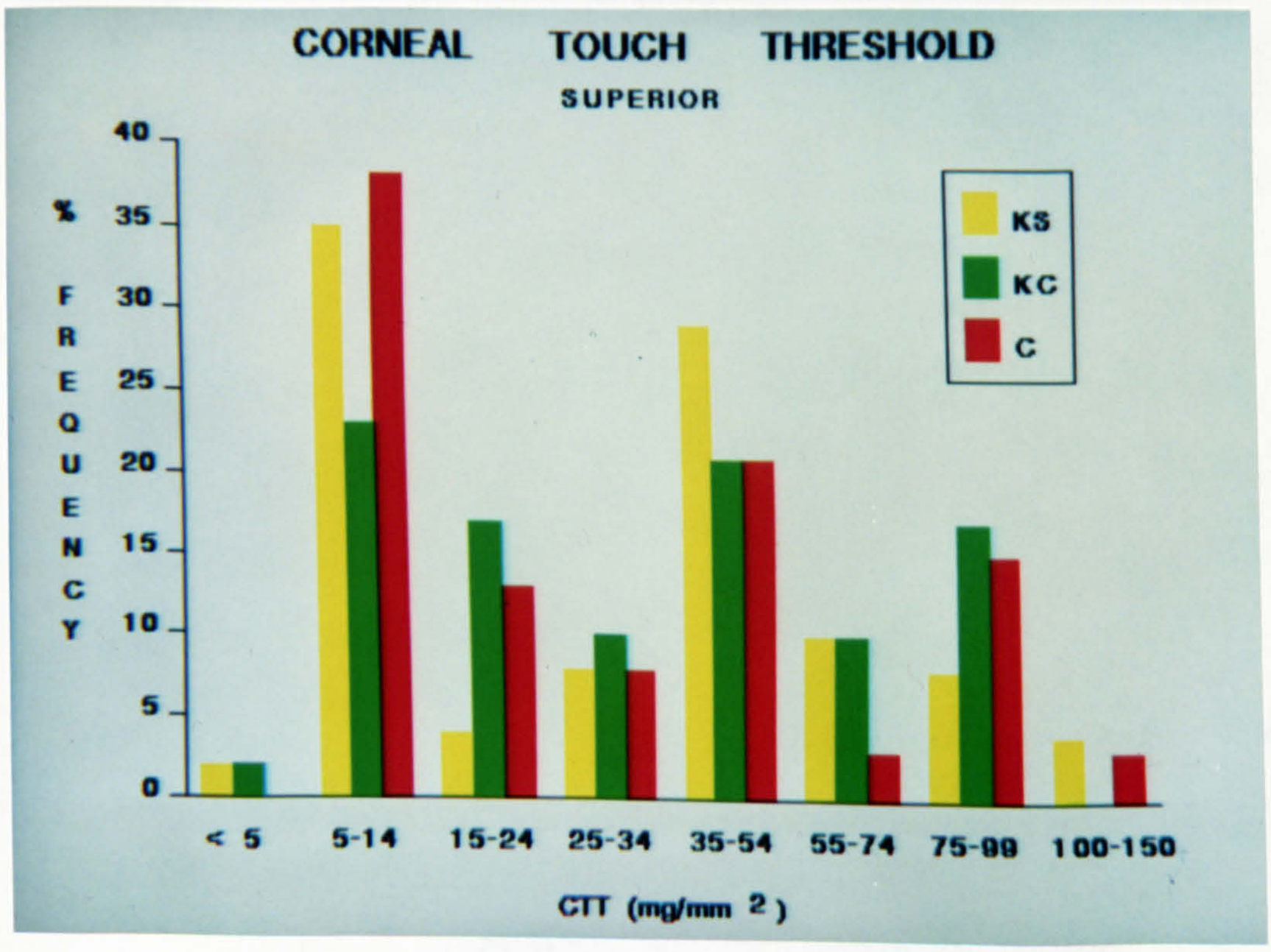
Calculated corneal thickness was plotted against pachometer read-out (correlation coefficient r = 0.998).

APPENDIX 8

Frequency distribution of CTT for nasal, temporal, inferior and superior corneal positions.



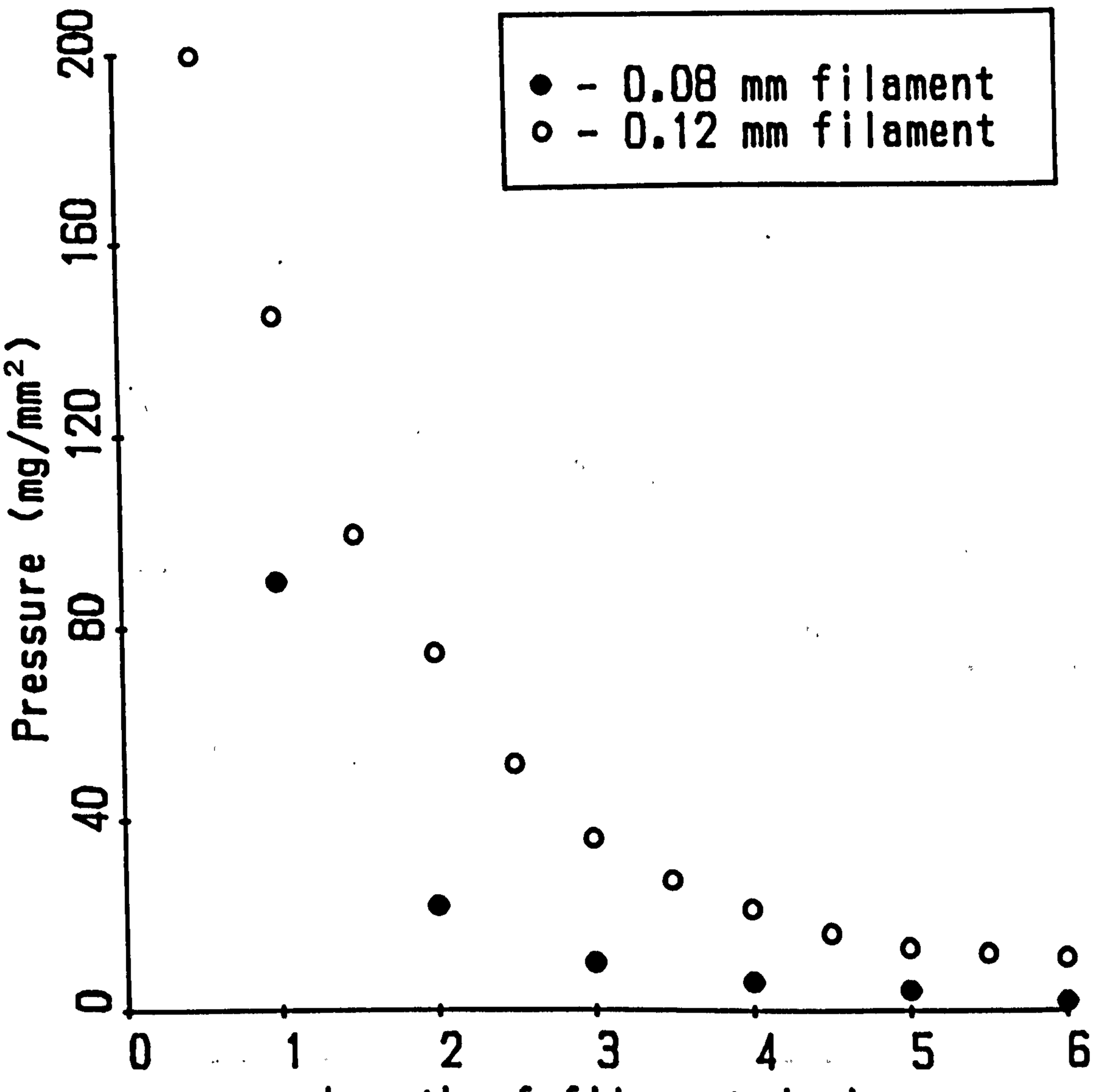

CORNEAL TOUCH THRESHOLD
INFERIOR
% FREQUENCY
55
50
45
40
35
30
25
20
15
10
5
0
KS
KC
C
< 5
5-14
15-24
25-34
35-54
55-74
75-99
100-150
CTT (mg/mm 2)


CORNEAL TOUCH THRESHOLD
SUPERIOR
% FREQUENCY
40
35
30
25
20
15
10
5
0
KS
KC
C
< 5
5-14
15-24
25-34
35-54
55-74
75-99
100-150
CTT (mg/mm 2)

APPENDIX 9

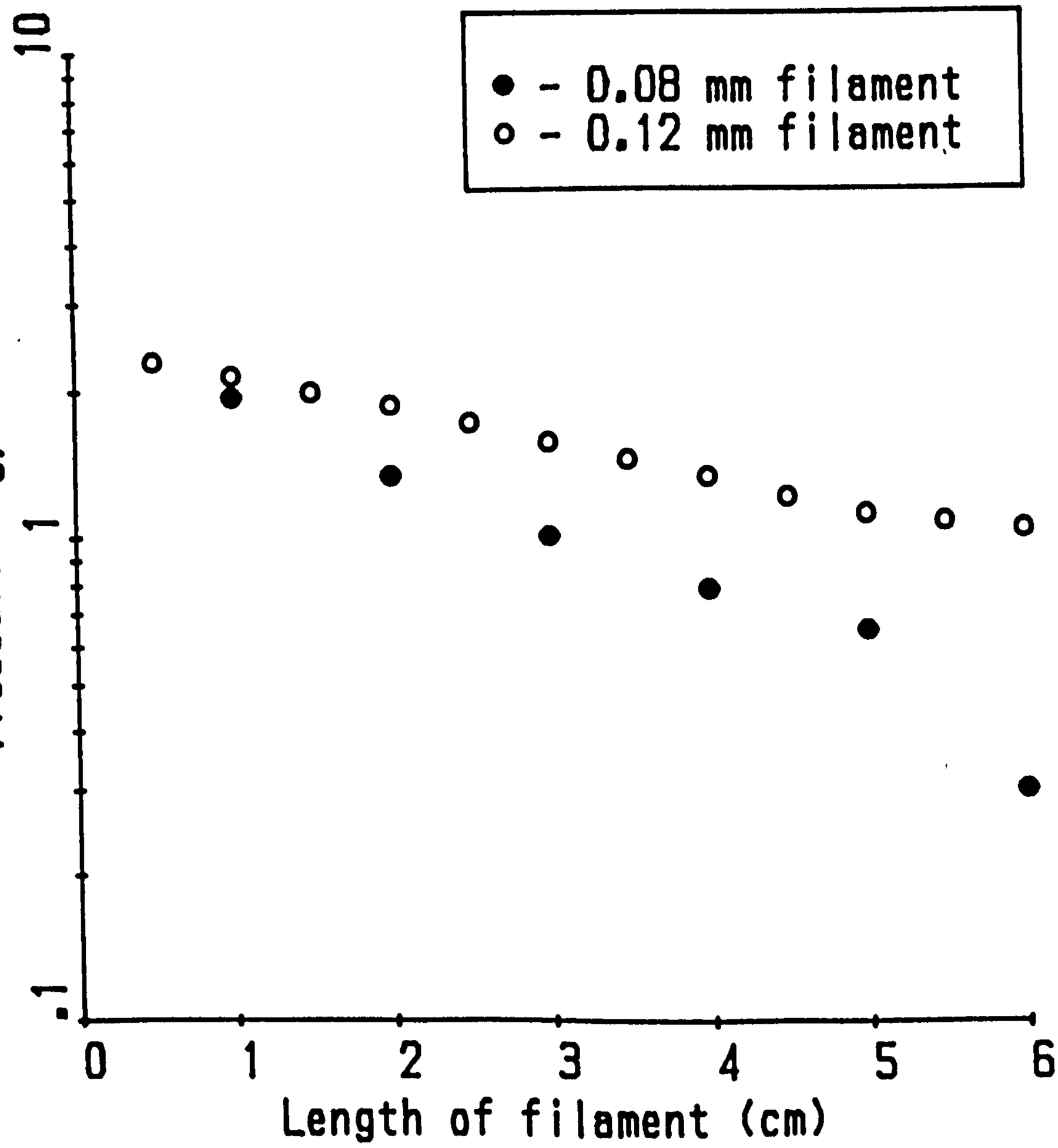
Calibration of the Cochet-Bonnet aesthesiometer. Data as provided with the instrument.

Figure A-9.1



Calibration of the Cochet-Bonnet aesthesiometer. Data supplied with the instrument and plotted on a logarithmic scale for the pressure data. Note the liniar relationship between filament length and logarithmic pressure.

Figure A-9.2



APPENDIX 10

Nerve fibre bundle and axon contents of individual specimens.

STROMA

Mid-peripheral cornea

Specimen	Bundles	Axons	Length in mm	Axons per bundle	Bundles per mm	Axons per mm
C1	31	265	10.6	8.6±5.1	2.9	24.9
C3	11	82	3.7	7.5±5.5	3.0	21.4
C4	7	137	6.2	19.6±22.5	1.1	22.3
C5	9	105	2.3	11.7±8.9	3.1	45.6
C7	16	119	6.9	7.4±4.3	2.3	17.2
C8	6	33	3.8	5.5±2.2	1.6	8.7
K1	6	110	1.9	18.3±12.9	3.2	58.2
K3	22	390	6.6	17.7±13.3	3.4	58.8
K4	9	97	5.7	10.8±3.6	1.6	16.9
K5	63	1482	8.8	23.5±16.4	7.2	168.3
K6	29	1027	10.1	35.4±26.6	2.9	102.2
K11	27	1334	8.6	49.4±30.9	3.2	155.7

Central cornea

Specimen	Bundles	Axons	Length in mm	Axons per bundle	Bundles per mm	Axons per mm
C1	15	136	6.0	9.1±7.6	2.5	22.6
C3	4	33	3.2	8.3±5.1	1.3	10.3
C4	9	93	4.2	10.3±7.3	2.1	22.2
C5	N/R	N/R	N/R	N/R	N/R	N/R
C7	3	9	4.1	3.0±1.7	0.7	2.2
C8	3	11	1.7	3.7±3.8	1.8	6.4
K1	4	8	2.8	2.0±0.8	1.4	2.9
K3	15	321	4.3	21.4±19.0	3.5	74.8
K4	7	74	3.5	10.6±4.8	2.0	21.2
K5	13	224	3.5	17.2±12.2	3.7	64.2
K6	24	1178	1.9	49.1±52.3	12.7	622.9
K11	10	324	4.7	32.4±25.0	2.1	68.5

Cont'd/...

APPENDIX 10 Continued...

EPITHELIUM

Mid-peripheral cornea

Specimen	Bundles	Axons	Length in mm	Axons per bundle	Bundles per mm	Axons per mm
C1	137	228	10.6	1.7±1.5	12.9	21.4
C3	18	29	3.0	1.6±1.1	6.0	9.8
C4	8	28	4.5	3.5±2.7	1.8	6.2
C5	12	37	2.4	2.0±19.9	8.7	17.4
C7	106	399	5.9	3.8±4.1	18.0	67.7
C8	6	17	3.0	2.8±1.6	2.0	5.8
K1	48	158	1.5	3.3±3.9	31.2	102.8
K3	136	354	5.8	2.6±3.1	22.8	59.3
K4	71	343	3.9	4.8±4.8	18.2	87.9
K5	149	961	9.2	6.5±10.2	16.3	104.9
K6	103	346	10.1	3.4±3.7	10.3	34.4
K11	N/R	N/R	N/R	N/R	N/R	N/R

Central cornea

Specimen	Bundles	Axons	Length in mm	Axons per bundle	Bundles per mm	Axons per mm
C1	104	185	6.0	1.8±1.4	17.3	30.7
C3	5	12	2.0	2.4±1.5	2.6	5.1
C4	25	120	3.3	4.8±3.7	7.9	36.3
C5	N/R	N/R	N/R	N/R	N/R	N/R
C7	38	94	3.3	2.5±2.0	11.7	29.8
C8	5	12	1.4	2.4±2.2	3.5	8.5
K1	51	279	2.5	5.5±5.4	20.7	113.5
K3	39	112	3.0	2.9±3.5	13.0	37.4
K4	66	336	2.9	5.1±4.2	22.8	115.9
K5	65	263	3.3	4.1±4.9	20.0	80.9
K6	N/R	N/R	N/R	N/R	N/R	N/R
K11	N/R	N/R	N/R	N/R	N/R	N/R

Appendix 11 TISSUE LENGTHS EXAMINED FOR AXON COUNT.

TISSUE	STROMA Mid-P mm	STROMA Central mm	EPITHELIUM Mid-P mm	EPITHELIUM Central mm
CONTROL				
C1	10.6	6.0	10.6	6.0
C3	3.7	3.2	3.0	2.0
C4	6.2	4.2	4.5	3.3
C5	2.3	N/R	2.3	N/R
C7	6.9	4.1	5.9	3.3
C8	3.8	1.7	3.0	1.4
Total (mm)	33.5	19.2	29.3	16.0
KERATOCONUS				
K1	1.9	2.8	1.5	2.5
K3	6.6	4.3	6.0	3.0
K4	5.7	3.5	3.9	2.9
K5	8.8	3.5	9.2	3.3
K6	10.5	1.9	10.1	N/R
Total (mm)	33.5	16.0	30.7	11.7
DESENSITISED KERATOCONUS				
Total (mm)	8.6	4.7	N/R	N/R

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