CORPUSCULAR NERVE ENDINGS IN THE HUMAN CONJUNCTIVA.

Relationship to Conjunctival Touch Sensitivity and Sensitivity Changes in Contact Lens Wear.

A Thesis submitted by

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DECLARATION

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ABSTRACT

This study sought to investigate the structure, distribution, and functional significance of complex (corpuscular) nerve endings in the conjunctiva of the human eye. Although these were found to be widely distributed throughout the conjunctiva they were particularly common at the corneo-conjunctival junction and at the eyelid margin.

Corpuscular nerve endings were round or oval encapsulated structures which varied in size (mean greatest diameter 40μm, range 20-78μm). Each corpuscle was generally served by a single myelinated nerve fibre. The myelin sheath was lost soon after entering the body of the structure. The terminal axon was characterised by multiple beads or varicosities which contained an accumulation of mitochondria. Neural elements were surrounded by cytoplasmic processes of Schwann like cells. The capsule was variable in thickness and typically consisted of fibrocyte processes. In contrast, some corpuscles at the eyelid margin displayed a capsule which contained perineural cells.

An intra-vital staining technique using methylene blue was used on the living conjunctiva (N=6). Compared with histological technique this method appears to considerably underestimate the number of complex nerve endings.

In an attempt to correlate the position of sensory corpuscles with points of enhanced touch sensitivity an investigation of the sensitivity of the limbal conjunctiva was carried out using a Cochet-Bonnet aesthesiometer. Subjects consisted of both non-contact lens wearers (N=28) and contact lens wearers (N=22) (Hydrogel N=10, RGP N=12). Limbal touch thresholds (LTT) were determined at 18 test locations which were divided equally between the temporal and inferior limbus. Regional variations in LTT were apparent. Thresholds increased with increasing distance from the cornea. Sensitivity was greatest in the region of the conjunctiva corresponding to the palisade zone. This was found histologically to contain a greater concentration of putative receptors. Large inter-subject variations in sensitivity were recorded, and there was an overall reduction with age. A comparison of LTT between lens wearers and non-wearers failed to show any significant differences.

The variations in LTT apparent in this study may correlate with the variations in the incidence and local distribution of sensory corpuscles.

ABBREVIATIONS

A	Asian
C	Caucasian
CTT	Corneal touch threshold
CNE	Corpuscular nerve ending
FNE	Free nerve ending
GLUT	Glutaraldehyde
LTT	Limbal touch threshold
M	Male
mm	Millimeter
μm	Micrometer
nm	Nanometer
N	Negro
PARA	Paraformaldehyde
PMMA	Polymethylmethacrylate
RA	Rapidly adapting
SA	Slowly adapting
VIP	Vasoactive intestinal polypeptide
PPG	Pterygopalatine ganglion
RGP	Rigid gas permeable
SCG	Superior cervical ganglion

CHAPTER 1

INTRODUCTION

1.1. Conjunctival Topography and Aspects of Structure.

The conjunctiva is a thin transparent mucous membrane which extends from the eyelid margins anteriorly, providing a lining to the lids, and then turns sharply upon itself to form the fornices and is reflected onto the globe where it covers the sclera up to its junction with the cornea. It thus forms a sac opening anteriorly through the palpebral fissure. The conjunctiva is conventionally divided into six regions (Fig.1): marginal, tarsal, and orbital (which collectively form the palpebral conjunctiva), fornix, bulbar, and limbal.

The marginal conjunctiva extends from a line immediately posterior to the tarsal gland duct openings, located on the posterior aspect of occlusal surface of the lids, and passes around the marginal angle, and continues along the inner surface of the lids as far as the subtarsal fold which is located opposite the base of the tarsal plate. The marginal conjunctiva possesses a thick stratified squamous epithelium containing sparse goblet cells, with an underlying fibrous connective tissue layer.

In the tarsal zone the epithelium is reduced in thickness, the superficial cells become cuboidal, and goblet cell density increases. In this region the substantia propria is thin and highly vascular, and the tissue is tightly adherent to the underlying tarsal plate.

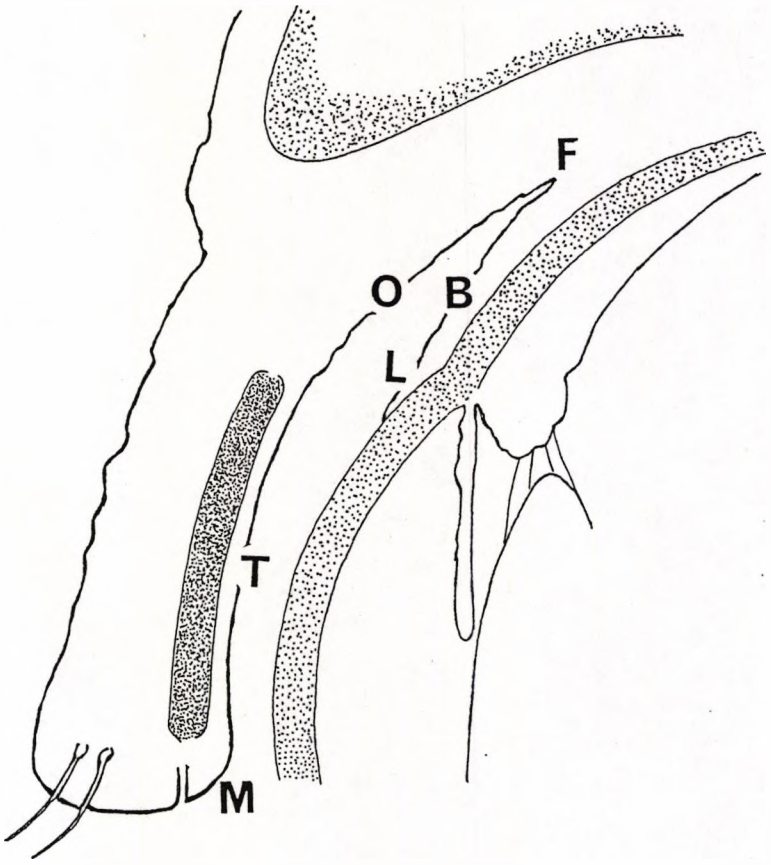


Figure 1. A sagittal section through the upper eyelid and anterior orbital structures showing conjunctival sac topography. M= marginal, T= tarsal, O= orbital, F= fornix, B= bulbar, L= limbal regions.

From the convex borders of the tarsal plates the orbital conjunctiva extends as far as the fornix. Here the conjunctiva is much more loosely attached to the underlying

tissues and tends to fold. The epithelium of both the orbital and fornix regions is thicker than in the tarsal zone, and the cells more columnar with numerous interposed goblet cells. The substantia propria is variable in thickness and can be divided into a superficial adenoid layer and a deep fibrous layer. It is in the region of the fornix that accessory lacrimal glands are commonly found, located deep in subconjunctival connective tissue.

The transparency of the bulbar conjunctiva readily permits the visualisation of the conjunctival and episcleral vasculature. The epithelial arrangement is similar to that of the fornix, and there is a regional variation in goblet cell density (Kessing 1968, Rivas et al 1991). The bulbar conjunctiva is freely movable due to its loose attachment to the underlying episclera and Tenon's capsule.

As the bulbar conjunctiva approaches the cornea the epithelium thickens and superficial cells become more squamous, with a gradual reduction in goblet cell density until they become lost completely. The limbal conjunctiva extends for approximately 1mm from the termination of Bowman's membrane, and when it is viewed grossly with a slit-lamp microscope it can be seen to be richly vascular containing a plexus of small calibre looped vessels which extend as far as the junction with the cornea. This junction is not well defined as a result of the conjunctiva and anterior sclera overlying the peripheral cornea. This overlap is greater in the vertical meridian and so the

cornea has a slightly oval appearance with its long axis in the horizontal meridian. Another feature of the limbal zone in man is the palisades of Vogt (Vogt 1921). These consist of a series of connective tissue ridges within the conjunctiva which form a radial pattern adjacent to the corneal margin. In histological section these are visible as stromal elevations projecting into the overlying epithelium. Palisades are most marked in the vertical meridian and they often display associated pigmentation which enhances their visibility (Ruskell 1991). The palisades often act as conduits for nerves, blood vessels, and lymphatics. The true functional significance of these structures however remains speculative. There has recently been a strong body of evidence which suggests that a proportion of limbal conjunctival basal cells act as stem cells for the provision of corneal epithelium (Tseng 1989), and one hypothesis, first proposed by Davanger and Evensen (1971), is that the palisades are a modification to enhance the local concentration of these limbal stem cells.

1.2. Innervation of the Conjunctiva.

Compared to the many studies on the neurology of other ocular tissues, the conjunctiva has been surprisingly neglected. The majority of the early investigators, as we shall see, concerned themselves mainly with the morphology of complex nerve terminals within the conjunctiva, although certain authors did make some more generalised observations

on the overall pattern of its innervation. It is worthwile in the first instance to consider the various sources of nerves which supply the conjunctiva.

1.2.1. Nerves supplying the conjunctiva.

It has now been established that the conjunctiva receives sensory, sympathetic and parasympathetic nerves.

The afferent nerves are trigeminal in origin and reach the conjunctiva principally via branches of the ophthalmic nerve. The traditional view is that the inferior conjunctiva is served, in addition, by the infra-orbital branch of the maxillary nerve (Duke-Elder and Wybar 1961). However, recent work by Oduntan (1989) tracing Wallerian degeneration following maxillary neurectomy in monkeys, questioned the constancy of a maxillary contribution, which when present was found to be small. There is some evidence that the limbal conjunctiva receives its sensory supply via the ciliary nerves which penetrate the eye close to the optic nerve and follow an intra-ocular course (Warwick 1976), However, a broader distribution of these nerves, including the bulbar conjunctiva, has been described by Ruskell and Simons (1990) in monkeys. This result indicates that nervous activity arising from stimulation of the bulbar conjunctiva could be transmitted, in part, through the eye.

Evidence for a sympathetic innervation to the conjunctiva was given by Ehinger (1971). Using the formaldehyde

induced flourescence method of Falck and Hillarp for catecholaminergic fibres, he was able to demonstrate such fibres in relation to conjunctival blood vessels. The classical notion that catecholaminergic fibres are exclusively sympathetic has been challenged in recent years (Landis et al 1987), and therefore in order to be more confident of sympathetic identity such techniques need to be supported by degeneration or tracer methods. These data are available for the conjunctiva. A sympathetic innervation, issuing from the superior cervical ganglion (SCG), has been confirmed using degeneration studies in monkeys (Macintosh 1974), from tracer experiments in rabbits (Ten Tusscher et al 1988), and combined immunohistochemical and denervation studies in rats (Luhtala et al 1991).

Parasympathetic nerve fibres of facial nerve origin are now known to innervate the eye and orbital structures (Ruskell 1965, 1970). Such fibres, which synapse in the pterygopalatine ganglion (PPG), were first shown to project to the conjunctiva by Macintosh (1974) who noted degeneration in nerve fibres supplying conjunctival vessels following PPG lesion in monkeys. Confirmation of Macintosh's claim of a parasympathetic innervation to the conjunctival vasculature has come from various sources. Ruskell (1985) supplied indirect evidence for a parasympathetic input by describing cholinergic fibres, as defined by the histochemical demonstration of acetylcholinesterase, surrounding conjunctival vessels in monkeys. The retrograde tracing study of Ten Tusscher et al (1988) reaffirmed

the facial nerve source of parasympathetic fibres in the conjunctiva by finding labelled neurones in the PPG of rabbits following sub-conjunctival injection of the tracer wheat-germ agglutinin coupled to horseradish peroxidase. Ocular parasympathetic nerves of PPG origin were shown to contain the neuropeptide vasoactive intestinal polypeptide (VIP) (Uddman et al 1980), which is known to be a potent vasodilator in the eye (Nilsson and Bill 1984). Although the conjunctiva was not examined in this study, later work by Butler and coworkers (1984) demonstrated VIP-ergic nerves in the conjunctiva of the rabbit, and the concentration of the neuropeptide was found to be reduced following PPG lesion. Surprisingly, however, conjunctival nerves displaying VIP immunoreactivity have not been identified so far in man (Miller et al 1983).

1.2.2. Distribution and morphology of conjunctival nerves.

The early writers on the subject of conjunctival innervation (Krause 1859, Ciaccio 1874, and Poncet 1875) generally agreed that conjunctival nerves formed, through a series of dichotomous branches, a rich plexus comprising deep and superficial components. The deeper plexus consisted of a coarse net-like arrangement of larger calibre nerve fibre bundles, which was linked to a more superficial layer of a finer mesh, containing smaller bundles. The most superficial layer comprised an interlacing network of single or small

groups of axons immediately underlying the epithelium. Axons tended to first lose their connective tissue investment and myelinated fibres subsequently shed their myelin sheaths on passing into the superficial layers. Although nerves were noted to follow vessels for much of their course, an input to the conjunctival vasculature was not described until the observations of Ciaccio (1874) on the vasomotor supply to conjunctival vessels.

The ultrastructure of conjunctival nerves has been described in monkeys (Mackintosh 1974, Ruskell 1985, Oduntan 1989) and to a lesser extent in man (Ruskell 1985). Small nerves approximately 7-10μm in diameter are present within the deep fibrous layer of the conjunctiva. These nerves are invested with a perineurium and contain predominantly unmyelinated axons. In addition, single and small groups of fibres (with or without a perineurium) are widely distributed throughout the substantia propria and there is a particular concentration immediately underlying the epithelium. Relatively few fibres are found within the epithelium itself, and when present these are largely confined to the basal cell layers. Vascular nerve fibre bundles are most frequently observed within the walls of arterioles where they associate with vascular smooth muscle. In addition, nerve fibre terminals are found in the walls of conjunctival capillaries.

1.3. Mode of Nerve Termination.

Since this study will be concerned specifically with nerve termination within the conjunctiva, the previous literature on the subject will be reviewed at length. Conjunctival nerves terminate in two ways: either as "free nerve endings", which are morphologically unspecialised , or as more complex (specialised) forms which fall within the general category "corpuscular nerve endings" and these will be considered first.

1.3.1. Corpuscular nerve endings

The description of the Pacinian (Pacini 1840) and Meissner (Wagner and Meissner 1852) corpuscles in the skin, provided an impetus for a period of intense investigation into the mode of nerve termination throughout the skin and mucosal surfaces of the body. The conjunctiva, because of its accessibility, transparency, and richness of its nerve supply, represented a convenient structure for such investigation.

In 1859 the German histologist Krause described nerve terminations within the human bulbar conjunctiva. He maintained that all myelinated nerve fibres terminated as small corpuscular structures which he called endkolben or endbulbs *(corpuscula nervorum terminalia bulboidea)* In order to visualise these structures tissues were first placed in dilute acetic acid for several days and the epithelium was

gently scraped away. Observation of the nerve ending revealed a round or oval structure possessing a thin connective tissue capsule which enclosed a granular substance. In favourable preparations he was able to observe fine unmyelinated nerve fibres within the interior of the corpuscle with small swellings at their terminal. Usually 1 or 2 myelinated axons served each corpuscle and the myelin sheath was lost upon entry, although in some cases the axon underwent numerous twists to form a dense entanglement on the surface just prior to entering. In the conjunctiva of lower mammals, for example in calves, terminals of a different form were observed. These were more cylindrical and simpler in appearance, resembling small Pacinian corpuscles. In addition to the conjunctiva, Krause also described similar bodies in other mucosa, for example the lip, buccal cavity, and external genitalia. Their structure and distribution left him in no doubt that wherever these nerve endings were found they were responsible for the general sensibility of the particular region.

Krause's observations were initially unchallenged, but Arnold (1862) after conducting a comprehensive search of the conjunctiva , using similar methods, found no trace of endbulbs, and furthermore maintained that all axons ended without modification. Arnold suggested that the structures which Krause observed at the ends of myelinated axons were the result of the mechanical trauma to which the tissues were subjected.

Although there were other sceptics, support for the existence of endbulbs came from various sources (Lightbody 1867, Mauchle 1867, Rouget 1868). Mauchle using both the recently available gold chloride method, in addition to treating tissues with acetic acid, described terminal structures in the conjunctiva of both man and calves which he considered synonymous with Krause's endkolben. Mauchle had to disagree with the assertion that all myelinated fibres terminated in this way since he was additionally able to observe nerves ending freely within the tissue.

Rouget (1868) questioned the observations of earlier workers with regard to the presence of a distinct connective tissue capsule enveloping the corpuscle. He maintained that the "capsule" was in fact the perineurial sheath of encircling myelinated axons.

In 1874 the Italian anatomist Ciaccio published a comprehensive account of the structure of the human conjunctiva. Using tissues impregnated with gold chloride or osmic acid he described the general pattern of conjunctival innervation. Nerves were observed to terminate as both free endings and Krause's endbulbs. The latter were variable in size and distribution. In some areas these corpuscles were absent whereas in others they were numerous, being especially common in the superior temporal quadrant of the conjunctiva. The Krause corpuscle, according to Ciaccio, was enveloped by a connective tissue capsule continuous with the "neurilemma" of the serving axon. The interior of the

structure contained several unmyelinated nerve fibres, surrounded by a finely granular substance, and each corpuscle was supplied by 1-4 myelinated axons.In addition to the Krause corpuscle Ciaccio also described another distinct type of corpuscular ending which he termed "fiochetti". These were similarly derived from myelinated nerve fibres and comprised a bush-like arrangement of unmyelinated fibres surrounded by a thin, almost transparent capsule.

Poncet (1875) was familiar with Ciaccio's work and based on his own observations was able to confirm the Italian author's main conclusions, particularly with regard to the preponderance of Krause corpuscles in the superior temporal quadrant.

Variation in both incidence and distribution of Krause corpuscles between eyes and local differences within the same eye was noted by Longworth (1875). He subjected the whole bulbar conjunctiva from one human eye to a complete analysis. Dividing the tissue into 5 equal segments he found no corpuscles in 2 segments and between 30 and 60 in the remainder. However he could not relate an increased concentration with a particular region as Ciaccio and Poncet. Observation of terminals in both man and calf led him to conclude that the human endbulb shared features common to Meissner's touch corpuscle whereas those found in calves were more akin to the Pacinian corpuscle. In his

preparations Longworth was unable to demonstrate the fiochetti described by Ciaccio.

By this time the Krause corpuscle (or endbulb) had become well established in the literature. The textbook of Key and Retzius published in 1876 included a review of previous work on the subject together with the results of their own observations. In the human conjunctiva the majority of corpuscles were found within an annular zone extending approximately 5mm from the corneal margin , and it was common to find them grouped closely together. Their illustrations, which were more detailed than those of earlier workers, showed structures which varied in size and complexity. All were encapsulated with a perineurial sheath derived from the serving myelinated axon. In some preparations the axon formed a convoluted arrangement at the base of the corpuscle before entry. The body of the corpuscle displayed cellular elements and unmyelinated fibres embedded in a granular matrix. Although Key and Retzius (1876) were able to demonstrate morphological variation, they similarly were unable to observe structures corresponding to Ciaccio's fiochetti.

Krause (1881) added further comments in a general review of nerve termination within the skin and mucosa. As with previous work he differentiated endbulbs in man, which he termed kulige (spherical) endkolben, from those found in lower mammals, cylindrische (cylindrical) endkolben. Human endbulbs, although structurally distinct from Meissner touch

corpuscles, were thought to be functionally analogous. On the incidence of endbulbs, Krause argued that any estimate of incidence could easily be biased by an insufficient sampling protocol. For example in a small area of conjunctiva 0.28mm^2 he found 11 endbulbs which on extrapolation would be equivalent to an incidence of 40 per mm^2. However, analysis of the whole conjunctiva only gave a yield of between 76 and 87, an incidence of approximately 2 per 5mm^2. Furthermore, Krause argued that although they were locally abundant there was no obvious pattern to their distribution and explained the results of Ciaccio (1874), Poncet (1875) and others as due to limited sampling.

A significant development came with the introduction of methylene blue as a means of staining nerve axons intra-vitally. This was first used on the eye by Dogiel (1891) who described numerous terminal corpuscles, which he identified as Krause's endkolben, in the conjunctiva adjacent to the cornea, and furthermore claimed to have also observed similar structures underlying Bowman's membrane in the outer 1-2mm of the cornea. Each corpuscle, according to Dogiel, was enclosed by a thin connective tissue capsule, which was revealed by utilising a counterstain. The serving nerve fibre, having shed its myelin , underwent several divisions to form a dense tangle of fine varicose threads. Although flattened nuclei were observed within the capsule , no trace of cell or nucleus was seen inside the corpuscle, and the space between nerve fibres was filled with a finely granular substance. It was

not unusual for one or more nerve fibres to emerge from one corpuscle to enter a similar complex entanglement. Although these structures were observed at conjunctival sites distant from the cornea the majority were found in a 2-3mm broad area of pericorneal conjunctiva, to emphasise this, in one 0.5mm circumferential stretch over 20 were observed.

Up to this point the majority of investigators confined their observations to the bulbar and limbal conjunctiva, but in 1894 Dogiel, using the methylene blue technique, studied the innervation of the palpebral conjunctiva and eyelid margin. Corpuscular nerve endings, like those previously described in the bulbar conjunctiva, were observed in both locations. They were more numerous in the region of the margin were they were usually located within stromal papillae.

Crevatin (1903) studied nerve termination in both the conjunctiva and digital skin. The conjunctiva displayed a variety of complex nerve terminals whose incidence was subject to some variability, although they were generally more numerous close to the cornea. The Krause corpuscle was the most commonly encountered, and Crevatin's description was similar to that of earlier workers. Furthermore, he also observed structures corresponding to Ciaccio's fiochetti, and in addition described several novel forms of nerve ending termed "striscette" "plessicini" and "reticelle". The latter group were devoid of a capsule,

and could be distinguished by the arrangement of the terminal nerve fibre.

A novel application of methylene blue was used by Knüsel and Vonwiller (1922) who applied the dye to the living conjunctiva of the human eye and were able to intra-vitally stain corpuscular nerve endings which they interpreted as Krause's endkolben. These varied in size and distribution, generally the greatest number were found in areas of bulbar conjunctiva covered by the upper lid, their incidence decreasing in the interpalpebral zone, with fewest in areas covered by the lower lid. Additionally, the limbus was noted to have a particular concentration. In an especially well stained eye as many as 50 corpuscles were observed. These were round or oval in shape and were typically 40-50μm in diameter. Each corpuscle was supplied by 1 to 3 axons. During observation through a slit-lamp microscope Knüsel and Vonwiller attempted to stimulate stained corpuscles directly but could not illicit a sensation of touch or pain.

This in vivo staining method was utilised by Strughold and Karbe (1925a, 1925b, 1925c) to correlate the position of Krause corpuscles in the conjunctiva to points of enhanced cold sense. They were able to illicit a repeatable cold response at conjunctival locations previously shown to contain vitally stained corpuscles.

Winkelman (1957) described discrete nerve endings displaying the morphology of Krause corpuscles, occuring in concentrated numbers in the transition zone between hairy skin and mucous membrane at various locations, for example the eyelid margin, glans penis, lip, and perianal region of primates. He introduced the term "mucocutaneous end-organ" to describe these structures.

In 1958 Oppenheimer and coworkers published a comprehensive study of nerve termination in the conjunctiva. This included the results of both histological observations and in-vivo staining with methylene blue. In the human bulbar conjunctiva histological technique revealed corpuscular nerve endings displaying a variety of morphological forms, many falling neatly into the categories proposed by Crevatin (1903), but additionally they were able to observe corpuscles which were considered "intermediate" in form. This diversity led them to believe that any classification of these complex nerve endings is an artificial one. Furthermore, they were in disagreement with previous investigators regarding the presence of a distinct connective tissue capsule, of which they saw no trace. Furthermore, they considered the existance of beading or varicosity of the terminal fibre, as demonstrated by Crevatin (1903) and others, to be staining artifacts. In-vivo staining showed large variations in the number of corpuscular nerve endings between eyes in addition to regional differences within the same eye. The superior temporal quadrant of the conjunctiva was found to contain

the largest number. An irregular distribution, together with large morphological variations, led Oppehheimer to conclude that rather than representing distinct receptors, complex nerve endings in the conjunctiva were formed as a result of degeneration and regeneration of certain peripheral nerve fibres.

Riisager (1962), using the methylene blue technique, added support to this hypothesis by the demonstration that there is a statistically significant increase in the number of corpuscular nerve endings in the conjunctiva with age. Moreover, he was able to confirm the findings of Oppenheimer et al regarding the preponderance of these endings in the upper temporal quadrant. In each case approximately half of the corpuscles were found in this region.

Based on the histological appearance of complex nerve endings in the human limbal conjunctiva Wolter (1964) identified them as Krause corpuscles. These often possessed a fine calibre unmyelinated nerve supply in addition to the large myelinated axon which supplies the corpuscle. This confirmed the earlier findings of Lightbody (1867). Wolter considered this accessory supply to be autonomic in origin and was able to follow the fine calibre axons, which often derived from the same nerve fibre bundle as the myelinated axon, to the surface of the corpuscle.

More recently Munger and Halata (1984) in a study of the sensory innervation of the human eyelid described various

corpuscular nerve endings which were concentrated in the occlusal zone and marginal conjunctiva (greater in the upper than the lower lid). Both Meissner and "simple" corpuscles (vide infra) were identified and these were found to be

especially common at the angle of the occlusal/marginal conjunctiva zone. On passing into the conjunctiva away from the margin corpuscles were absent.

1.3.2. Free nerve endings

The term "free nerve ending" describes terminals which show a minimal degree of structural specialisation, where an axon ends blindly with few cellular wrappings. The existence of free nerve endings in the human conjunctiva is well documented (Arnold 1862, Ciaccio 1874, Oppenheimer, Palmer, and Weddell 1958, Ruskell 1985). They arise from both myelinated and unmyelinated axons, and account for the mode of termination of all autonomic and the majority of sensory fibres. At the light microscopic level, traditionally using either heavy metal stains or methylene blue, free endings were observed as fine tapering threads or alternatively displaying a small bead like expansion at their tip (Ciaccio 1874). Conjunctival nerves terminate in close proximity to vascular smooth muscle , or near to the walls of capillaries (Macintosh 1974). Furthermore, there is a concentration of terminals within the substantia propria close to the basal epithelial layers (Ruskell 1985). Intra-epithelial terminals

have been described (Ciaccio 1874, Oppenheimer, Palmer, and Weddel 1958, Macintosh 1974), but their distribution would appear to be irregular.

Ultrastructural observations of conjunctival terminal form have been confined largely to monkey (Macintosh 1974, Oduntan 1989) and to a lesser extent in man (Ruskell 1985). Pre-terminal axons characteristically display beads or varicosities, and the Schwann cell sheath is retained until close to their termination. In contrast, axons passing into the epithelium lose their sheath at the point of entry (Macintosh 1974). Varicosity content can give a crude guide as to terminal type: for example, sensory varicosities contain aggregated mitochondria with few vesicles, whereas autonomic varicosites display abundant vesicles and less frequent mitochondria.

The characterisation of terminal type with more certainty has been possible due to the advent of histochemical techniques for the demonstration of specific neuropeptides (Burnstock 1986, Stone et al 1987, Unger and Butler 1988). Sensory fibres characteristically display immunoreactivity to substance P and calcitonin gene related peptide, and both have been identified in the conjunctiva (Stone and Kuwayama 1985, Stone and McGlinn 1988, Luhtala et al 1991). Parasympathetic fibres of facial nerve origin are immunoreactive for VIP (Uddmann et al 1980), and the distribution of neuropeptide Y can be correlated with the

distribution of sympathetic fibres (Stone et al 1987, Luhtala et al 1991). More recent techniques allow the electron microscopic visualisation of specific histochemical markers permitting terminal characterisation at the ultrastructural level (Burnstock 1986) but such techniques have yet to be applied to the conjunctiva.

1.4. Sensitivity of the Cornea and Adnexa.

The sensory nerve supply to the front of the eye confers the ability to detect changes in its environment. Sensory receptors within the tissue are capable of registering a full range of sensory modalities including touch, pain, and temperature. The classical theory of cutaneous sensibility proposed by Von Frey (1884,1895), based on the concepts of Müller (1838) and Blix (1884), maintained that each sensory modality was subserved by a separate anatomically distinct terminal form. Free nerve endings were thought to be the exclusive receptors for pain, Meissner corpuscles for touch, Ruffini endings for warmth, and the Krause corpuscle for cold. The cornea and adnexa represented convenient structures to test this theory: their considerable sensitivity was well known, and the mode of nerve termination within these tissues was fairly well defined. Von Frey and his school thus attempted to quantify ocular sensation with refined technique (Strughold 1925, Strughold and Karbe 1925a, 1925b 1925c, Von Frey and Strughold 1926).

1.4.1.Mechanical sensitivity

The first aesthesiometer (Von Frey 1894) consisted of graded hairs of different calibres which were applied to the cornea or conjunctiva until a sensation was felt. This determined the mechanical threshold (Von Frey and Strughold 1926). In contrast to the skin, these early workers were unable to illicit a sensation of touch or pressure from either the cornea or the majority of the conjunctiva (with the exception of the lid margin). The sensation instead was percieved as one of pain. Such thresholds showed marked regional variation, being lowest at the corneal centre, and increasing towards the corneal periphery. On crossing the limbus there was a considerable decrease in sensitivity with thresholds as much as 175x higher that of the central cornea. Conjunctival thresholds were found to decrease towards the fornices and were lowest in the region of the margin. The method introduced by Von Frey still forms the basis of modern aesthesiometry, and the instrument described initially by Boberg-Ans (1955) and later improved by Cochet and Bonnet (1960) has become the standard in both clinical practice and research. The aesthesiometer incorporates a nylon monofilament which can be varied in length to produce a range of applied force. There are two versions of the Cochet-Bonnet aesthesiometer: the "standard" instrument with a filament diameter of 0.12mm enabling measurement in the range 11-200mg/0.0113mm2, and the more accurate 0.08mm filament diameter with a range in applied force of 2-90mg /0.005mm2.

Boberg-Ans (1955) confirmed the results of Von Frey with regard to the variation in corneal sensitivity. Conjunctival sensitivity was represented as a range without comment on regional variation, but interestingly threshold values were only 5-13x greater than typical values for the cornea.

There was still some debate at this time regarding whether the cornea and conjunctiva possessed the ability to differentiate touch from pain. Although, this was subsequently shown fairly conclusively for the cornea by Lele and Weddell (1956) using graded punctate stimuli. With reference to the conjunctiva, even though few studies are available, its touch sensitivity has been similarly confirmed (Norn 1973, Draeger 1984, Winter et al 1986).

Norn (1973) found little variation in touch sensitivity across the conjunctiva and found mean threshold values only 8x greater than the central cornea. In contrast, using an electronic-optical aesthesiomter, Draeger (1984) found large regional variations in sensitivity, recording mean thresholds ranging from only 2 to over 1400 times greater than central cornea. Least sensitive areas generally included those covered by the lids, with the most sensitive being an inter-palpebral zone close to the temporal limbus. Moreover, the distribution in threshold within small 2x2mm areas of bulbar conjunctiva displayed up to an 850 fold variation. Winter and coworkers (1986), using the same

aesthesiometer, confirmed a sensitivity profile showing marked variation across the conjunctiva, and similarly found the temporal region to have the highest sensitivity.

Investigators who have recorded the mechanical threshold of the lid margin have shown its heightened sensitivity (Von Frey and Strughold 1926, Dixon 1964, Norn 1973, Lowther and Hill 1968, Collins et al 1989, Bleshoy 1990). Although the upper of the two lid margins is generally found to be more sensitive, differences are small. Published values of the mechanical threshold of the margin show great variability, and depending on author, thresholds are found to exceed that of the central cornea by as much as 4x (Norn 1973), 2x (Von Frey and Strughold 1926) , or 1.5x (Collins et al 1989). In contrast Lowther and Hill (1968) found that the upper lid displayed touch thresholds which were often less than the central cornea. It could be argued however that there is a problem with using the central corneal touch threshold for such comparisons. The measurement has been found to be affected by apprehension factors (Bonnet and Millodot 1966), and the minimum applied force of many of the early aesthesiometers was in excess of the true central corneal threshold, and so a mid or peripheral corneal point would make a more reliable comparison. Dixon (1964) found that the touch threshold of conjunctiva close to the upper lid margin was 13x greater than a mid-peripheral corneal location. In contrast Collins et al (1989) found the threshold of the margin to be only 1.5x greater than the corneal periphery, and similarly Bleshoy (1990) recorded values equivalent to

the corneal periphery. Such variability relates to methodological differences and more importantly to differences in the position of the stimuli relative to the margin.

Much of the literature on the touch sensitivity of the anterior segment, not surprisingly, is concerned with the touch sensitivity of the cornea. Corneal touch thresholds (CTT) in addition to showing variation according to corneal eccentricity (Von Frey and Strughold 1926, Boberg-Ans 1955), also vary with time of day (Millodot 1972a), iris colour (Millodot 1975a), lid closure (Millodot and O'Leary 1979), the menstrual cyle (Millodot and Lamont 1974, Riss et al 1982), and during pregnancy (Millodot 1972b, Riss and Riss 1981). In addition, an increase in CTT with age is well documented (Boberg-Ans 1955, Millodot 1977, Draeger 1984).

The effect of contact lenses on CTT has been studed by many workers (Boberg-Ans 1955, Schirmer 1963, Dixon 1964, Millodot 1974, Millodot 1975b, Millodot 1976, Millodot 1978). PMMA lenses cause a marked reduction in CTT (Millodot 1975b) especially when worn for many years (Millodot 1978). The effect is less marked with hydrogel lenses (Millodot 1974) even when worn on an extended wear basis (Millodot 1984). A study of the loss of corneal sensitivity with oxygen deprivation (Millodot and O'Leary 1980) suggests the possibility that the aetiology of contact lens induced reduction in sensitivity could have a hypoxic in addition to a mechanical component.

Changes in conjunctival or lid margin sensitivity have been little studied. Both Norn (1973) and Winter and coworkers (1986) have observed a reduction in bulbar conjunctival sensitivity with age. Lowther and Hill (1968) studied the sensitivity of the lower lid margin during adaptation to hard contact lens wear. They observed a decline in sensitivity which was greatest during the period from 7-14 days following the commencement of wear.

1.4.2. Thermal sensitivity

Strughold and Karbe (1925a,1925b, 1925c) investigated the cold sensitivity of the eye. Their apparatus consisted of a thin copper wire with a small sphere at its tip. Since the copper was a good conducter of heat, they assumed that by applying the sphere to the eye it would conduct heat away from the point of contact, thus making it relatively cooler than the surround. By increasing the size of the sphere they believed that more heat would be extracted. The threshold for "cold" was therfore defined as the smallest sphere diameter that resulted in a cold sensation. Using this unsatisfactory methodology these workers found numerous "cold spots" throughout the conjunctiva, and, as described earlier, by combining this technique with intra-vital staining they claimed to show a correlation between the location of Krause corpuscles, as stained by this technique, and points of enhanced cold sensitivity. In contrast, the cornea showed no such sensitivity. Although they were able to illicit a cold sensation at the corneal

periphery which they attributed to Krause corpuscles described at this location (Dogiel 1891, Knüsel and Vonwiller 1922).

According to the Von Frey theory, although the eyelid and caruncle had the capacity to detect warmth, by virtue of their Ruffini corpuscles, the cornea and conjunctiva were incapable of such sensitivity (Strughold and Karbe 1925a).

The capacity of the cornea to detect a thermal stimulus has been the subject of intense debate (Lele and Weddell 1956, Kenshalo 1960, Beuerman et al 1977, Beurman and Tanelian 1979). However, where attempts have been made to utilise stimuli which can be precisely confined to the cornea, and which have a reduced mechanosensory component, the sensation illicited is one of irritation or pain (Kenshalo 1960, Beuerman et al 1977).

Kenshalo (1960) found, in contrast, that the conjunctiva was capable of accurately differentiating a cool, from a warm or hot stimulus. In terms of its sensitivity to warmth the conjunctiva was equivalent to the lip or forehead, and its cold sensitivity was slightly lower.

1.4.3. Electrophysiological investigations

Although electrophysiological recording techniques have not

been applied to the conjunctiva specifically, some of the data obtained for the cornea have some relevance.

Recording the activity of corneal nerve fibres to mechanical and thermal stimuli in the cat (Giraldez et al 1979 and Belmonte and Giraldez 1981) has demonstrated that these fibres, belong to the A-delta and C-sensory group, based on their conduction velocities, and they can be classified functionally as nociceptors. The majority of these were found to be polymodal, responding to mechanical thermal and chemical stimuli, others were exclusive mechanonociceptors, mechano-heat nociceptors, and "cold" nociceptors. Receptive fields were large and overlapping and interestingly many were found to extend beyond the limbus to include the adjacent conjunctiva. Recently Pozo et al (1990) described a distinct population of cold thermoreceptors which were localised in the episclera 1-5mm behind the limbal border. These have small receptive fields and have an exclusive cold sensitivity.

CHAPTER 2

MATERIALS

2.1. Histology

Three whole anterior eye segments (HW22, HL2, HL4) and samples from 4 others were sectioned completely. Three further complete anterior segments (HL5, HL6, HL8) were processed for whole-mount gold chloride impregnation.

Several pieces of human bulbar conjunctiva were taken from two eyes (HW19, HG9). Material from HG9 consisted of three pieces of conjunctiva (E1,E2,E3) from near the fornix. Although the exact location of tissues (B1,B2,B3) obtained from HW19 was unknown, the observation of an accessory lacrimal gland in one sample would suggest that these adjacent pieces (B1, B2, B3) were also taken from the fornix region.

Four pieces of upper eyelid obtained from 2 eyes (HG9, HG16), and two small pieces of lower lid tissue (HG19C,HG16A1) were used to study the innervation of the eyelid margin and palpebral conjunctiva.

Further details of all specimens used are given in appendix A.

2.2. Intra-vital Staining of the Conjunctiva with Methylene Blue.

Preliminary investigations consisted of qualitative observations on 4 subjects (CC,JL,TS,GR), comprising 3 males and 1 female aged 21, 31, 36, 59yrs. A quantitative study of the distribution of corpuscular nerve endings was performed on 3 subjects (SD,JL,AH). These consisted of 2 males, 1 female aged 21, 31, 31yrs).

Further subject details are given in appendix B.

2.3. Limbal Touch Sensitivity.

Subjects for this part of the study consisted of 28 non-contact lens wearers, 16 females and 12 males, with a mean age of 42.0 yrs (range= 15-81yrs, SD= 17.8).

The sensitivity of 22 contact lens wearers was also investigated. These were divided into rigid gas permeable (RGP) wearers (N=12), and hydrogel wearers (N=10). RGP wearers comprised 8 females, 4 males with a mean age of 34.3yrs (range= 21-59yrs, SD= 10.6). Mean length of lens wear was 10.0 yrs (range= 0.5-20yrs, SD= 5.3).

Hydrogel wearers were all female with a mean age of 33.0yrs (range= 18-59yrs, SD= 11.4). Mean length of wear was 7.5yrs (range= 0.5-13yrs, SD= 4.0).

Prior to the study all contact lens wearers had worn their lenses for at least six months on a daily basis for a minumum of 8hrs per day .

Further details of subjects are given in appendix C.

CHAPTER 3

METHODS

3.1. Dissection and Preparation of Tissues for Histology

3.1.1. Light and electron microscopy

Anterior halves of cadaver eyes were first orientated by noting the positions of either muscle insertions or the extent of conjunctival/scleral overlap to mark the vertical meridian. These were then cut into 12-14 roughly equal radial segments from which most of the cornea and uvea was trimmed away. Other specimens consisting of large pieces of bulbar or palpebral conjunctiva were cut into smaller units. Eyelids were sectioned vertically into narrow strips which were bisected and the skin and sub-cutaneous connective tissue removed.

All of the above specimens were fixed by immersion in 2% glutaraldehyde and 3% paraformaldehyde buffered to pH 7.4 with sodium cacodylate. Prior to the embedding procedure tissues were stored overnight in buffered sucrose, washed briefly in distilled water, and post-fixed in 1% osmium tetroxide for one hour. They were then dehydrated through graded ethanol dilutions (30%, 70%, 90%) for 20 minutes each, and in absolute ethanol for one hour with a solution change. Specimens were immersed in xylene for one hour (with

agitation and one change of solution), transferred to a 1:1 xylene \Araldite mixture for 30 minutes, and rotated overnight in Araldite before embedding in Araldite-filled trays or capsules. These were then incubated at 60°C for 48 hours and allowed to cool to room temperature.

Trays were were especially useful for large specimens, and for certain samples of bulbar conjunctiva which were spread to reduce folding and facilitate subsequent tangential sectioning.

For light microscopy semi-thin sections (0.75-1.00μm) were cut with glass knives on a Reichert-Jung Ultracut-E microtome, then mounted on microscope slides, and stained with 1% toluidine blue in 2.5% sodium carbonate. A combination of full serial and interrupted serial sectioning was utilised using intervals ranging from 10-30μm. Transverse, radial, and tangential cuts were made although limbal conjunctiva was sectioned predominantly tranversely to the palisades of Vogt beginning on the corneal side.

At intervals in each series tissue blocks were trimmed and ultra-thin sections (70-100nm) were cut with a diamond knife and mounted on unfilmed copper grids. These were stained in a saturated solution of uranyl acetate in 70% ethanol for 20 minutes, washed in distilled water, followed by 0.4% lead citrate in 0.1M sodium hydroxide for 20 minutes, and a final

wash with distilled water. Sections were examined with a JEOL- 100B electron microsope.

3.1.2. Whole-mount gold chloride impregnation

Four unfixed anterior eyes were subjected to gold chloride impregnation following the method of Schimmelpfennig (1982). The specimens were placed in fresh filtered lemon juice for 15 minutes, washed rapidly in distilled water, and transferred to a 1% solution of gold chloride at room temperature for 20 minutes. They were then placed in a solution containing 50ml of distilled water to which 5 drops of glacial acetic acid had been added and left for 14-18 hours. Tissues having been prepared in this way display a very dense staining pattern, and in order to visualise conjunctival nerves they were first cut into radial segments and the majority of the underlying sclera removed using a Graefe's knive or razor blade, and then the epithelium gently scaped away. Specimens were subsequently fixed in 10% formal saline, dehydrated through graded ethanol dilutions, cleared in xylene, before mounting on glass slides for examination.

3.2. Intra-vital Staining with Methylene Blue

Vital staining was performed on the living conjunctiva of human subjects. Prior to the staining procedure the eye was

first anaesthetised by the instillation of three drops of oxybuprocaine 0.4% (Smith and Nephew Pharmaceuticals) into the lower fornix, each drop separated by intervals of one minute. This was followed by the application of three drops of methylene blue 1% (Martindale Pharmaceuticals) with an interval between each application of five minutes. After each drop the subject was requested to roll the eye under a closed lid to aid spreading of the stain. Following the last application, the eye was irrigated with saline 0.9% to remove excess stain. Repeated applications of oxybuprocaine were necessary to overcome the marked irritation associated with the technique. The progress of staining was followed using a Nikon FS-2 photo slit-lamp microscope and results were recorded photographically on Ektachrome 200 ASA colour transparency or Kodak Technical-Pan monochrome film .For higher power observation and photography a Nikon AS-1 non-contact endothelial camera was used which allows magnifications of up to 60x to be obtained. Immediately following each staining session, corneal and conjunctival integrity was verified by the application of sodium fluorescein (Smith and Nephew pharmaceuticals), and this was repeated the next morning.

An attempt was made to quantify the number of stained nerve endings in three subjects. Prior to conjunctival staining taking place a detailed photographic montage was made of the anterior eye surface, including areas of bulbar conjunctiva which would be covered by the lids in normal positions of gaze. The eye was then subjected to methylene blue staining

and corpuscular nerve endings were plotted onto the photograph using the conjunctival vascular pattern to aid localisation.

3.3. Determination of Limbal Touch Thresholds using a Cochet-Bonnet Aesthesiometer

3.3.1. Description of apparatus

Touch thresholds were determined using a Cochet-Bonnet asthesiometer which incorporated a 0.12mm nylon monofilament (Fig 2). The instrument was first calibrated. The specified filament diameter was verified by mounting a piece of spare nylon filament under a cover slip and its diameter measured using a microscope eyepiece graticule. The aesthesiometer was then clamped onto a laboratory stand and was suspended above the pan of a sensitive digital analytical balance (capable of measuring to 1mg). The filament, which was viewed through a binocular viewing system, was then lowered towards the pan smoothly. The criterion of first visible bending was taken to generate the applied pressure. A total of 20 readings were taken for 6 filament lengths. The mean balance readings are tabulated in appendix D.

For measurement of touch thresholds the asthesiometer was mounted onto a slit lamp microscope using a modified Bleshoy (1986) applicator (Fig.3). This apparatus allows fine

control of filament orientation. Furthermore, the filament tip can be viewed under magnification through the slit lamp observation system. Thus, the nylon monofilament can approach the conjunctival surface both smoothly and perpendicularly and allows for accurate placement of the stimulus.

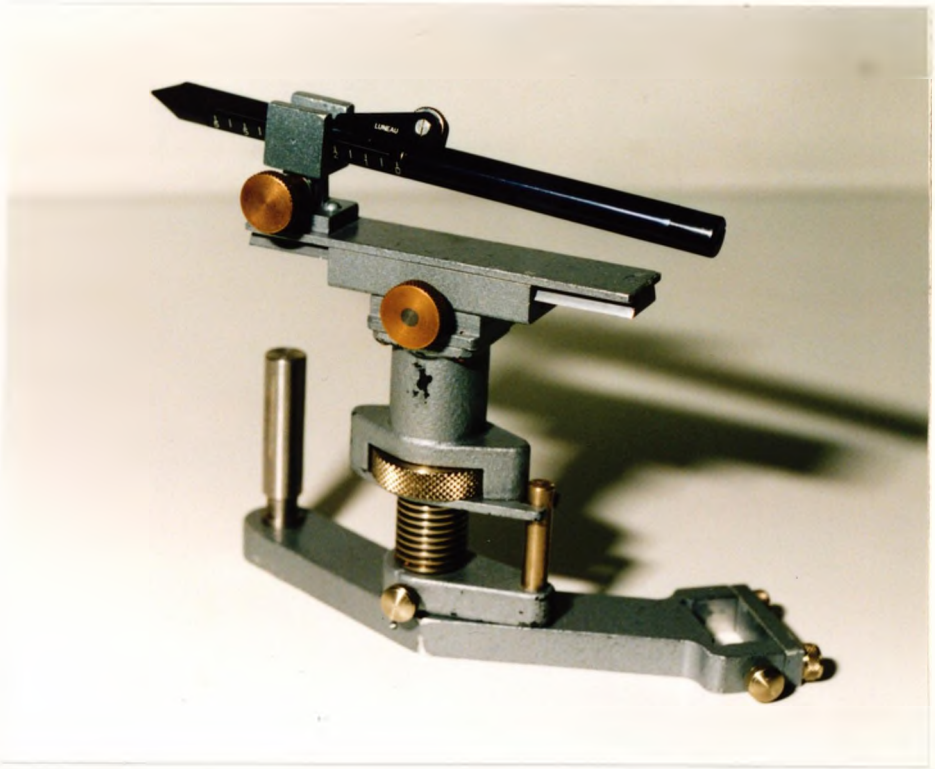


Figure 2. A Cochet-Bonnet aesthesiometer attached to an applicator which allows precise manipulation of the orientation of the aesthesiometer.

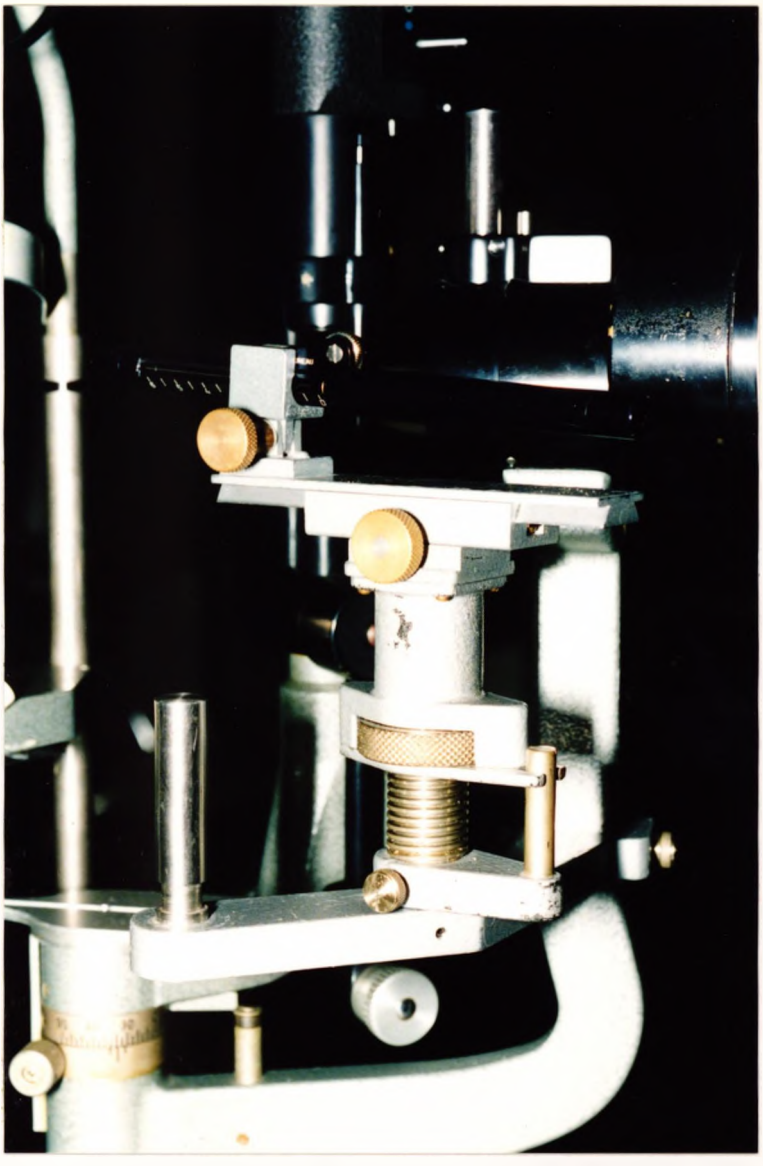


Figure 3. The apparatus attached to a slit-lamp microscope.

3.3.2. Measurement of touch threshold

The limbal touch threshold was determined at 18 locations within a 1.5mm wide peri-corneal area of conjunctiva which was divided into three 0.5mm wide concentric zones beginning at the anterior termination of the palisades. Thus, test locations within zone 1 corresponded approximately to a mid-palisade position. Stimuli were divided equally between zones and between temporal and inferior limbus (Fig. 4).

Since the aim was to ensure an accurate repeat testing of the same conjunctival location, in order to minimize mechanical trauma it was decided to limit testing to three filament lengths (4cm, 3cm, and 2cm). The procedure was first explained to the subject who was instructed to indicate when the stimulus was felt. The longer length was employed first and the criterion of first visible bending was used to generate the applied force (Millodot 1969), and the threshold criterion consisted of a 50% positive response from 4 stimulus applications. If threshold was not achieved at this length then the progressively shorter lengths were used. The pattern of limbal vessels was utilised as a guide to ensure repeated stimulation at the same test location. A number of "dummy runs", where the monofilament did not contact, were included to test subject reliability.

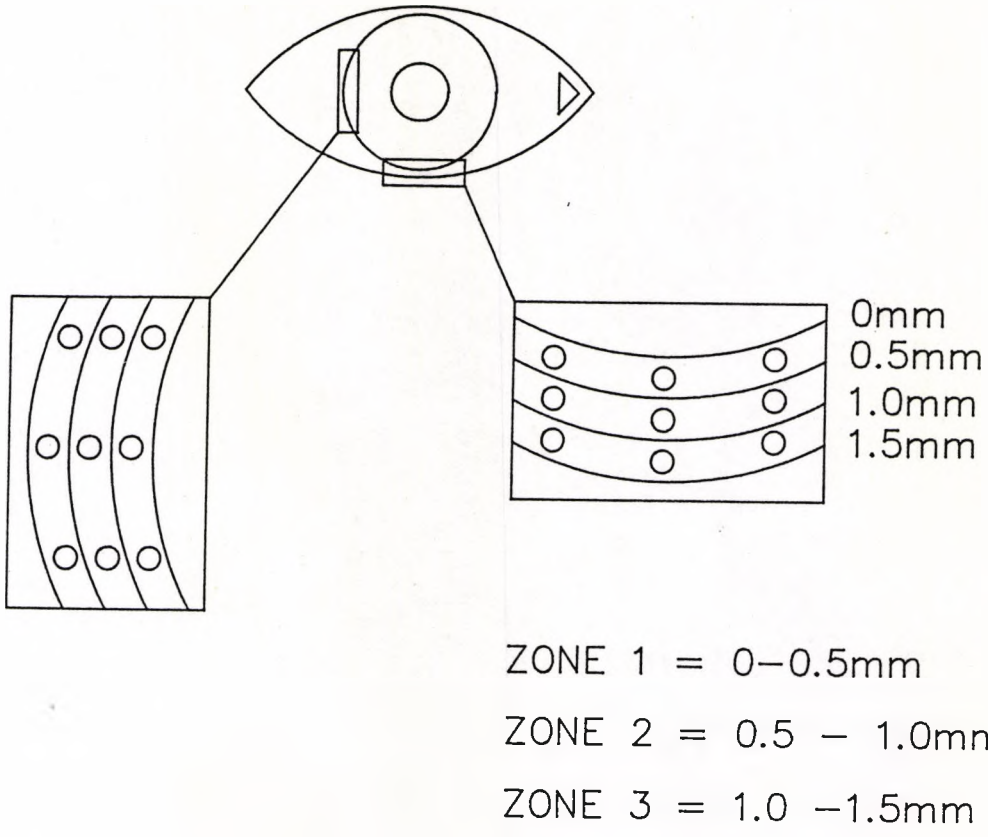


Figure 4. Stimulus test locations within three 0.5mm wide concentric zones of limbal conjunctiva. A total of 18 points are divided equally between inferior and temporal limbus.

3.3.3. Sensitivity grading

Using this method any limbal test location can have a threshold in terms of filament length of >=4, >=3, >=2 or <2cm. To make these values more amenable to analysis these values were converted to an arbitrary sensitivity scale as shown in the table below.

THRESHOLD (cm)	SENSITIVITY SCALING (arbitrary units)
<2	1
>=2	2
>=3	3
>=4	4

Table 1. Sensitivity conversion scale.

3.3.4. Repeatability

The above procedure was carried out on 5 subjects and stimulus test locations were noted on a photograph of their limbal conjunctiva. Thresholds were determined at these same locations on a separate occasion to assess repeatability.

3.3.5. Contact lens wearers

Contact lens wearing subjects were instructed to wear their lenses for a minimum of three hours prior to testing, and thresholds were determined immediately on removal of their lenses.

3.3.6. Estimation of iris colour

The iris colour classification system of Seddon et al (1990) was employed. The iris colour is compared with photographs of four standard irides. An iris is thus given a grade which relates to its predominant colour. Blue or grey irides are classified as grade 1 or 2 , a green iris as grade 2 or 3. A predominantly brown iris is graded as 3, 4, or 5 according to the density of pigment.

CHAPTER 4

RESULTS

HISTOLOGICAL OBSERVATIONS

4.1. Limbal Conjunctiva

4.1.1. Gross and light microscopic observations.

A prominent feature of the limbal conjunctiva are the palisades of Vogt. These consist of a closely packed radial array of connective tissue ridges within the conjunctiva adjacent to the corneal margin. They are most prominent in the vertical meridian where conjunctival/scleral overlap of peripheral cornea is most marked. Palisades show a variety of form, and furthermore vary in the degree of associated pigmentation (Figs.5,6). Figure 5 shows the appearance of a pigmented conjunctiva viewed with a slit-lamp microscope displaying the more common linear pattern. The post-mortem specimen in Figure 6 is similarly from a pigmented eye, but in contrast has a less regular appearance with a number of interspersed papillae. Palisades are generally between 0.5 and 1.0mm in length and usually 5-10 are present per mm of circumference. They terminate anteriorly largely in register 0.3-0.5mm from commencement of Bowman's membrane. The origin of the anterior limiting membrane of the cornea makes a

convenient histological reference point, and this was used subsequently for measurement purposes.

Since the majority of segments were sectioned parallel to the limbus the palisades were cut in transverse section, revealing the characteristic series of connective tissue elevations projecting into the epithelium (Fig.7). A flat conjunctival surface is retained by the epithelial cells filling the troughs between adjacent palisades. Cell thickness varies from 3-4 cells thick overlying the palisade crests to 10-15 between palisades. The disposition of pigment in a pigmented epithelium is largely confined to the basal cells which display compound melanosomes within their cytoplasm.

Sectioned material indicated that the arrangement of conjunctival nerve fibres was largely similar to that described in monkey (Macintosh 1974, Ruskell 1985, Oduntan 1989). Nerves of varying calibre (10-30μm) where distributed throughout the conjunctival stroma. Larger calibre bundles tended to be localised deep in the stroma where they frequently lay adjacent to blood vessels. These nerves contained both myelinated and unmyelinated fibres and were invested by a perineurium. Smaller bundles, often lacking a perineurium, were observed at all levels of the stroma, and in contrast to monkey (Macintosh 1974, Oduntan 1989) myelinated axons were commonly seen within the superficial layers up to the level of the epithelium. Many of these fibres were subsequently observed to terminate as

corpuscular endings (vide infra). Whole-mount gold chloride impregnated specimens showed many fine calibre nerve fibres which followed the course of conjunctival vessels (Fig.8). A proportion of these fibres were presumably vasomotor and often demonstrated beading or varicosities along their length. Although many fine calibre fibres, independent of vessels were found lying immediately below the epithelium, few actually penetrated the epithelium itself and such fibres were largely located at basal cell level (Fig.9).

Corpuscular nerve endings were often located within the stromal elevations which form the palisades (Figs. 7,10,11), they were additionally observed in the inter-palisade zone (Fig.12), and in areas of limbal conjunctiva lacking prominent palisades (Fig.13). These structures were rarely located more than 25μm from the epithelium, and were typically found within 5-10μm of the basal cell layer. Corpuscles were round to oval in shape, and were variable in size with a mean greater diameter of 30μm (range 20-60μm). They were most often served by a single myelinated nerve fibre (mean greater diameter 4μm, range 2-6μm) derived from deeper lying nerves (Figs.10,14). In addition, using a whole mount gold chloride impregnation technique 1 or more fine calibre axons, which appeared to terminate close to or within the corpuscle, were occasionally observed (Fig.15). The whole structure was demarcated from the surrounding connective tissue by a capsule comprising flattened cells with extended processes. In semi-thin section the capsule appears thin and often

incomplete (Figs.10,11), whereas in whole-mount preparations it was more substantial, usually appearing to enclose the whole of the perimeter (Fig.14). The body of the corpuscle contained neural elements which were recognised in semi-thin section as either pale or darkly stained circular profiles, surrounded by non-neural accessory cells (Figs. 10,11). The latter were subsequently found to have the ultrastructural characteristics of Schwann cells and will be referred to by this name. The large oval nuclei of these cells varied in number and location but tended to be located peripherally. In addition to staining neural elements, gold-chloride stained a variety of non-neural cells including capsular cells, and the dense staining pattern within the interior of the corpuscle (Fig.14) probably results from the staining of Schwann cells. The density of the Schwann cell packing showed some variation. Although often tightly packed (Fig. 10), others had a looser arrangement with connective tissue channels partitioning the interior (Fig. 11) In some cases two or more distinct "lobes" were present.

4.1.2. Electron microscopic observations.

Conjunctival nerves were typically surrounded by 2-4 layers of concentrically arranged perineural cells and fibrocyte processes. These enclosed usually 1-3 myelinated axons, 2 to 6μm in diameter, and more numerous fine calibre

unmyelinated axons. Several of the latter shared a common Schwann cell (Fig.16).

Smaller bundles of unmyelinated fibres, lacking a perineurium, were commonly found close to, or within, the walls of blood vessels (Fig.17). Pre-terminal axons where characterised by periodic beading or varicosities (Fig.18). Many such fibres where found immediately underlying the epithelium (Fig.19), although rarely within it (Fig.20). These features are consistent with the light microscopic observations using gold chloride.

At the ultrastructural level, features of limbal corpuscular nerve endings common to similar endings found elsewhere in the body were recognised (Figs.21,22,23). The afferent myelinated axon of the corpuscle (Fig.24), having lost its perineural sheath, shed its myelin sheath immediately before entering the corpuscle, and then divided several times (Fig.25) to give rise to a variable number of axon terminals. Axon terminals consisted of a series of varicosities, which were predominantly sectioned transversely, presenting round or oval profiles in cross section (Fig.26). The varicosities contained irregularly arranged neurofilaments, scattered microtubules, clear vesicles approximately 30nm in diameter, and aggregates of mitochondria; the latter accounted for the increase in local volume (Fig.27) The inter-varicose region of the nerve fibres, in contrast, contained a parallel array of neurofilaments with microtubules and sparse mitochondria

(Figs. 27,28). A particular morphological variant displayed axon terminals in which the axoplasm contained an electron dense material which hindered organelle visibility; however, within varicosities mitochondrial profiles could usually be made out, apparently in good order (Fig.29). Small unmyelinated nerve fibres, either single or in small groups, were occasionally seen outside the capsule (Fig. 30), and also near to the point of entry of the serving myelinated fibre, but it was not possible to follow these fibres to their termination.

Schwann cell cytoplasm contained predominantly microfilaments with microtubules and sparse mitochondria (Figs.31,32). The cell border was characterised by numerous pinocytotic vesicles which were visible as membrane invaginations or as cytoplasmic enclosures. A basal lamina was present which distinguished Schwann cells from neighbouring connective tissue or capsular cells. Away from the main body of the cell, which housed a large heterochromatic nucleus, the cytoplasm was drawn into a number of thin processes or lamellae which subsequently divided several times.(Fig. 31). Two or more layers of these lamellae invested both pre-terminal and terminal axons. These were either concentrically arranged arround the axon terminal or disposed in parallel stacks. The innermost layer lacked a basal lamina at the interface with its axon, with a narrow space of approximately 20nm separating the plasma membranes (Figs.26,33). Often the lamellae did not make a complete investment and the axon directly abutted the

basal lamina. Junctional complexes were occasionally seen between axon and lamellae which resembled attachment plaques (punctae adhaerentes) (Fig.33). These consisted of paired dense membrane plaques with an interposed lighter material within the intercellular space. They could be differentiated from desmosomes since they lacked an organised arrangement of microfilaments inserting into these densities. Although the Schwann cell lamellae were often in apposition no junctional complexes were observed between the membranes.

Extracellular material consisted of a fine filamentous substance, similar to that found in basal laminae, which was interspersed with collagen fibres.

As with the light microscopic observations the delicate capsule, was frequently found to be incomplete. It comprised fibrocyte-like cells (Fig.21,30) which gave rise to fine extended processes enclosing the greatest part of the perimeter of the corpuscle. There was no consistent sub-capsular space. The number of layers of capsular cells was variable, sometimes only a single layer was present although 3 or 4 were occasionally seen due to overlapping of adjacent processes (Fig.30). It was not uncommon for these processes to extend into the main body of the structure. Fibrocytes were occasionally encountered within the body of the structure which did not contribute to the capsule. The processes which derived from these cells acted as septa partitioning the interior of the corpuscle (Fig.34). Both capsular and non-capsular fibrocytes lacked a basal lamina,

and the cytoplasm of these cells contained pinocytotic vesicles, as well as rough endoplasmic reticulum and free ribosomes.

4.1.3. Distribution of sensory corpuscles.

Observation of the limbal conjunctiva indicated that corpuscular nerve endings were locally abundant. Often several corpuscles were found in adjacent palisades (Fig. 7), and in whole mount preparations of limbal conjunctiva as many as 6 corpuscles were present in a low power objective field. In order to determine the total number of corpuscular nerve endings in a particular eye the gold chloride technique was found to be unsatisfactory in this respect. Staining of non-neural elements, particularly epithelial cells, resulted in reduced tissue transparency, and although the epithelium was routinely removed the process was not always complete and sometimes caused damage to the underlying stroma. Therefore, for more accurate counts an interupted serial technique was preferred and the results for three complete anterior eye segments are given in Figures 35-37. Corpuscular nerve endings were found to predominate in a narrow annular zone of limbal conjunctiva, 1.00mm wide, located approximately 0.5mm from the corneo-conjunctival junction as defined by the termination of Bowmans' membrane. Corpuscular nerve endings appeared not to be uniformly distributed around the limbus, and equivalent sized segments often displayed large differences in the number of corpuscles which they contained. No distinct

pattern was apparent however with regard to incidence according to limbal location. From these data a mean incidence equivalent to 2-3 corpuscles per mm of corneal circumference was estimated.

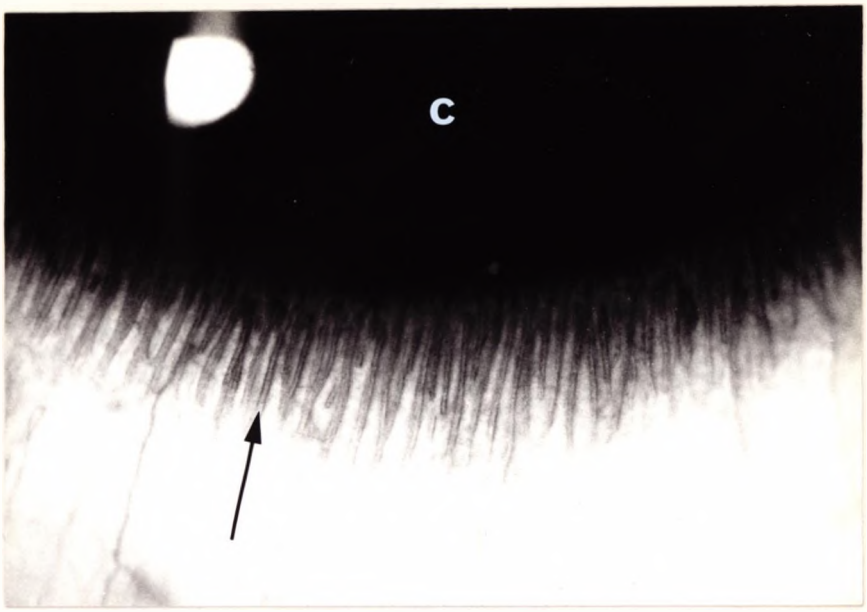


Figure 5. A slit-lamp photograph showing a pigmented example of the palisades of Vogt at the conjunctival limbus. The crests of the palisades are represented by the narrow clear interval (arrow) between each pair of pigment lines. The wider clear intervals separating pairs of pigment lines indicate the interpalisade zones. c= cornea. (40X)

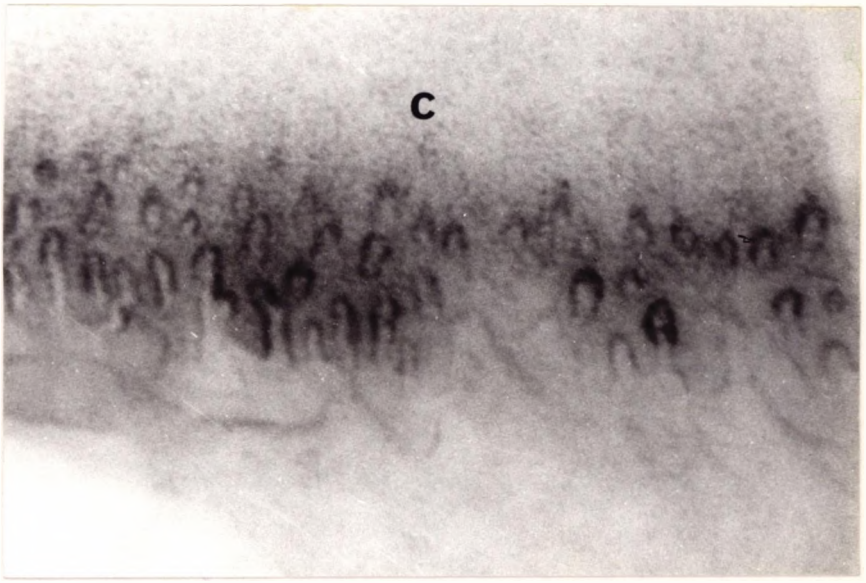


Figure 6. Palisades from a post mortem specimen. This example, in contrast, shows an irregular palisade pattern displaying numerous papillae in addition to linear forms. Each palisade is outlined by dense pigment, and there is a diffuse pigment migration into the cornea (c). (36X)

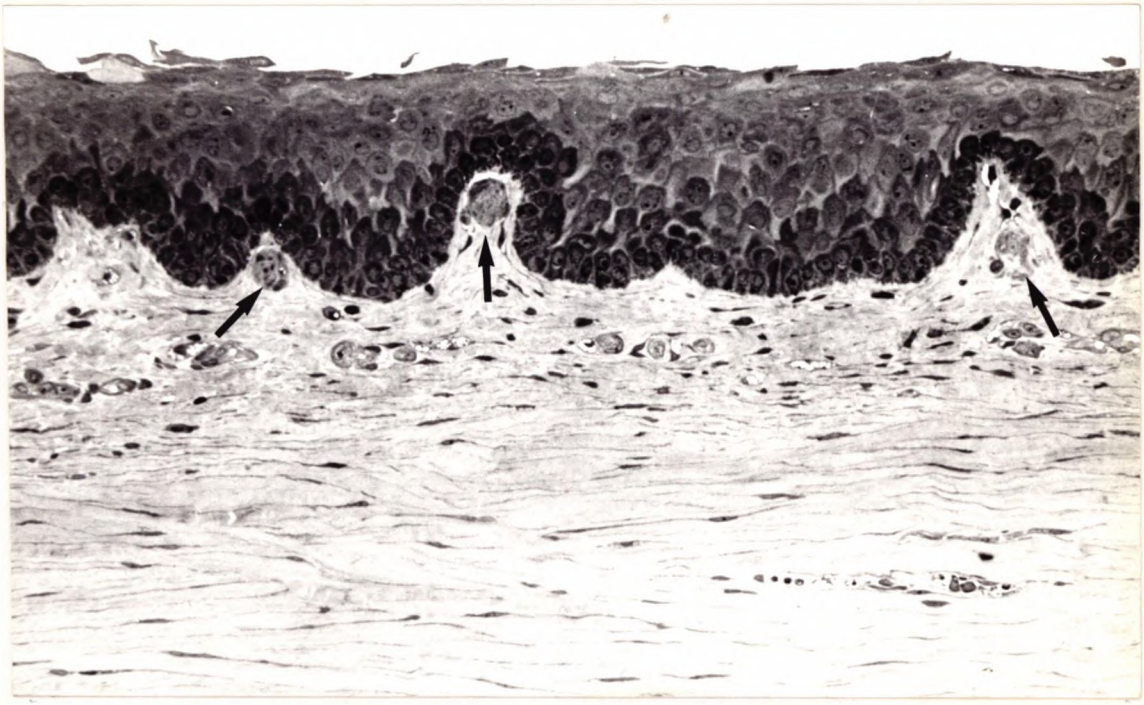


Figure 7. Transverse section through the palisade zone showing the connective tissue ridges projecting into the epithelium. Three adjacent palisades contain corpuscular nerve endings (arrowed). (279X)

Figure 8. Whole-mount gold chloride impregnated specimen staining the limbal vascular arcades and associated nerve fibres. These fibres show periodic beading (arrows). (629X)

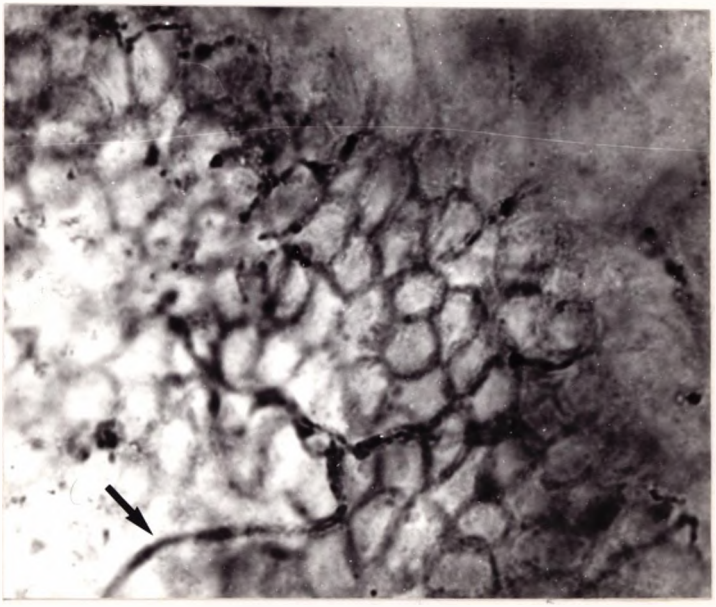


Figure 9. Whole-mount preparation of limbal conjunctival epithelium focussed at the level of the basal cell layer. The nerve fibre (arrowed) is ascending from conjunctival stroma and branches upon entering the epithelium. The varicosities seen at the top of the photograph derive from the same parent axon. (759X)

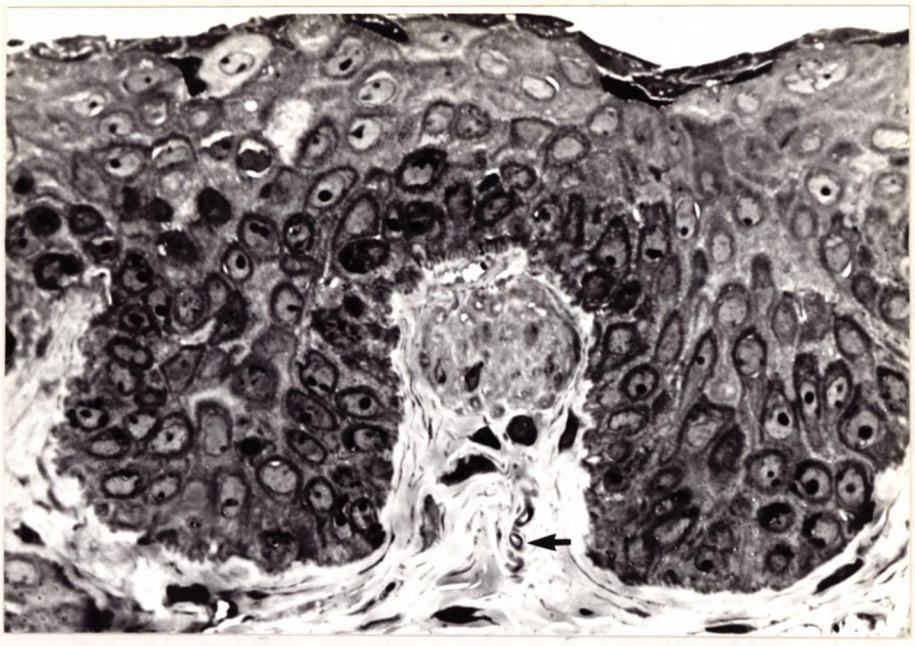


Figure 10. Corpuscular nerve ending located towards the apex of a palisade. The serving myelinated fibre is sectioned obliquely as it ascends the palisade (arrow). (589X)

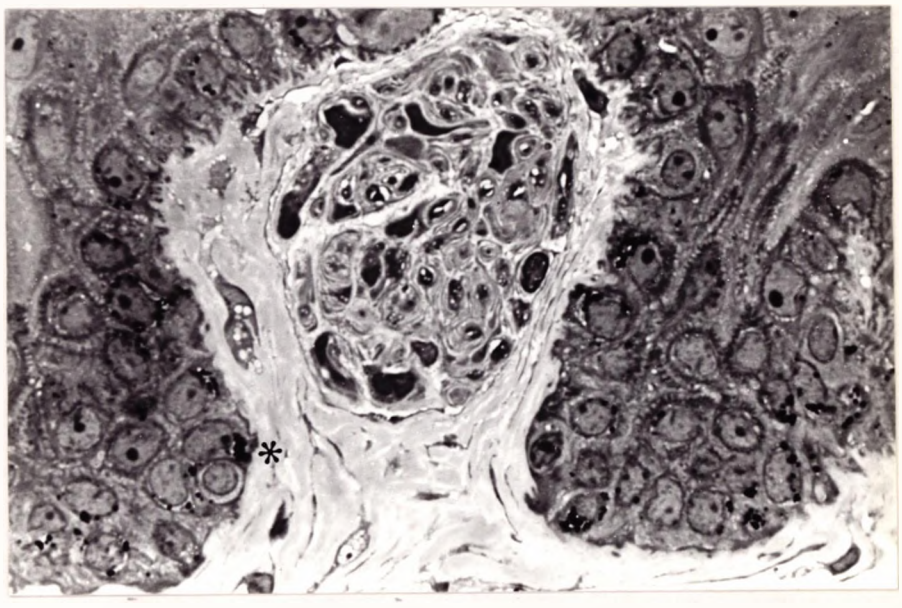


Figure 11. A limbal corpuscle showing detail within the body of the structure. Densely stained round or oval neural profiles are surrounded by the lightly stained cytoplasm of Schwann cells. A delicate connective tissue capsule is visible arround the perimeter of the corpuscle. Pigment is present within the basal cells lining the slopes of the palisade (asterisk). (963X)

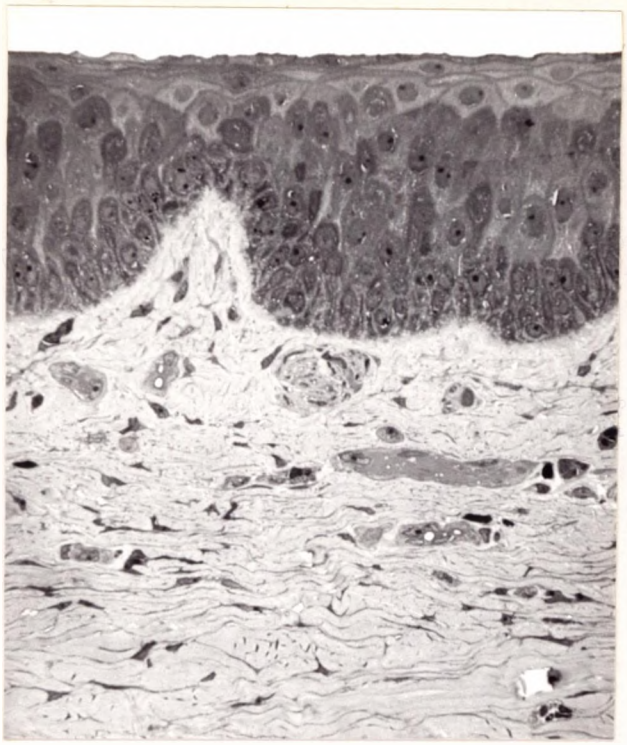


Figure 12 . Corpuscular ending underlying the epithelium in the interpalisade zone. (250X)

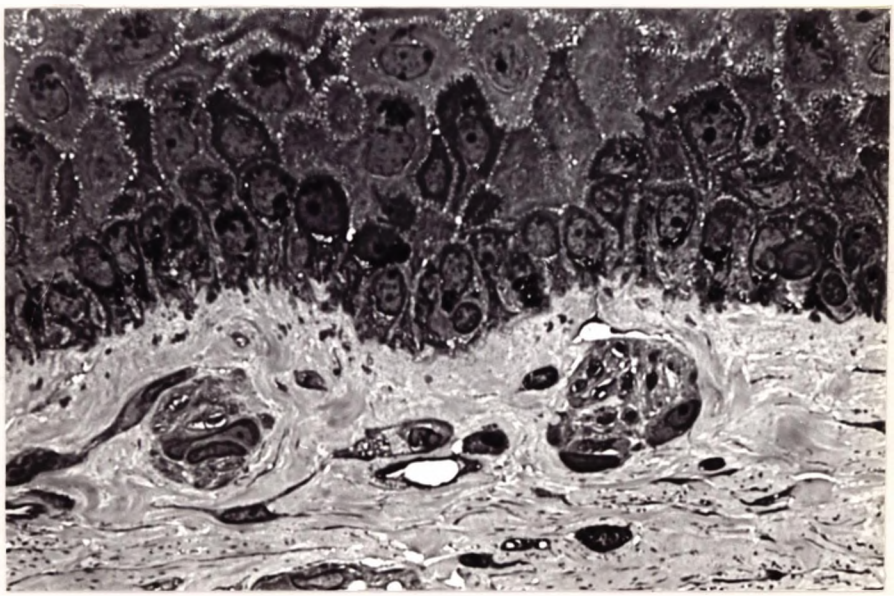


Figure 13. Two corpuscles located in limbal conjunctiva lacking prominant palisades. (917X)

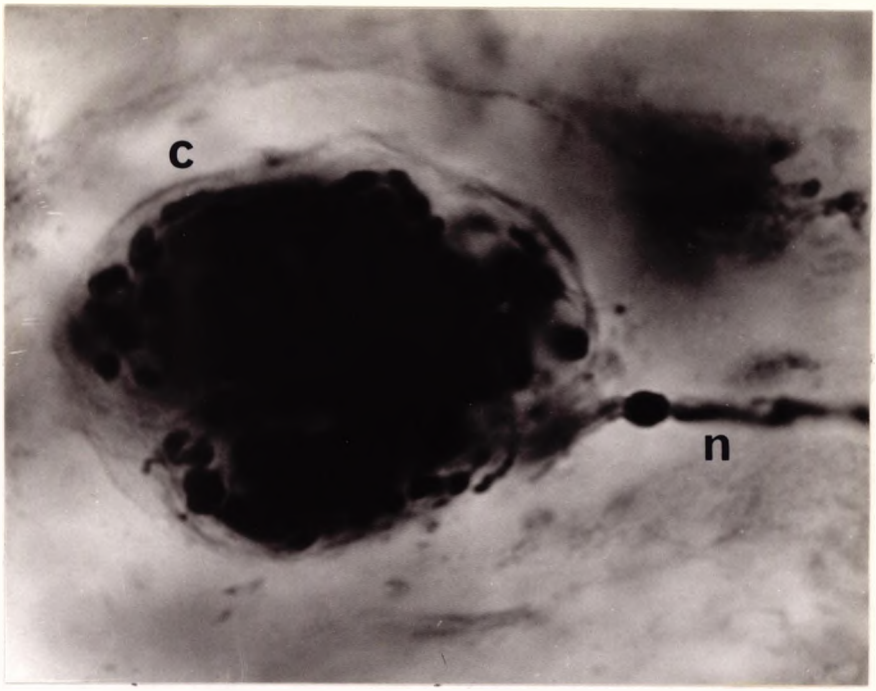


Figure 14. Whole mount gold chloride preparation showing the afferent nerve fibre (n) and the capsule (c) which appears complete when examined at different levels of focus. Dense staining obliterates most of the detail within the corpuscle. (2,102X)

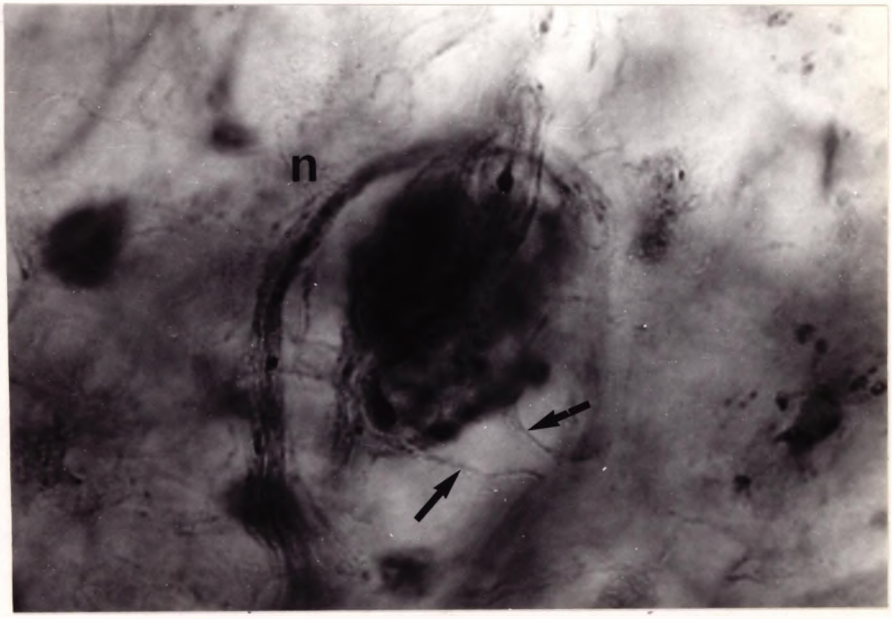


Figure 15. This gold chloride stained example shows a possible accessory nerve supply. At this plane of focus the parent myelinated axon (n) is seen to arch around the corpuscle. In addition fine calibre nerve fibres (arrowed) are visible which were followed and appeared to terminate on or within the corpuscle. (1,375X)

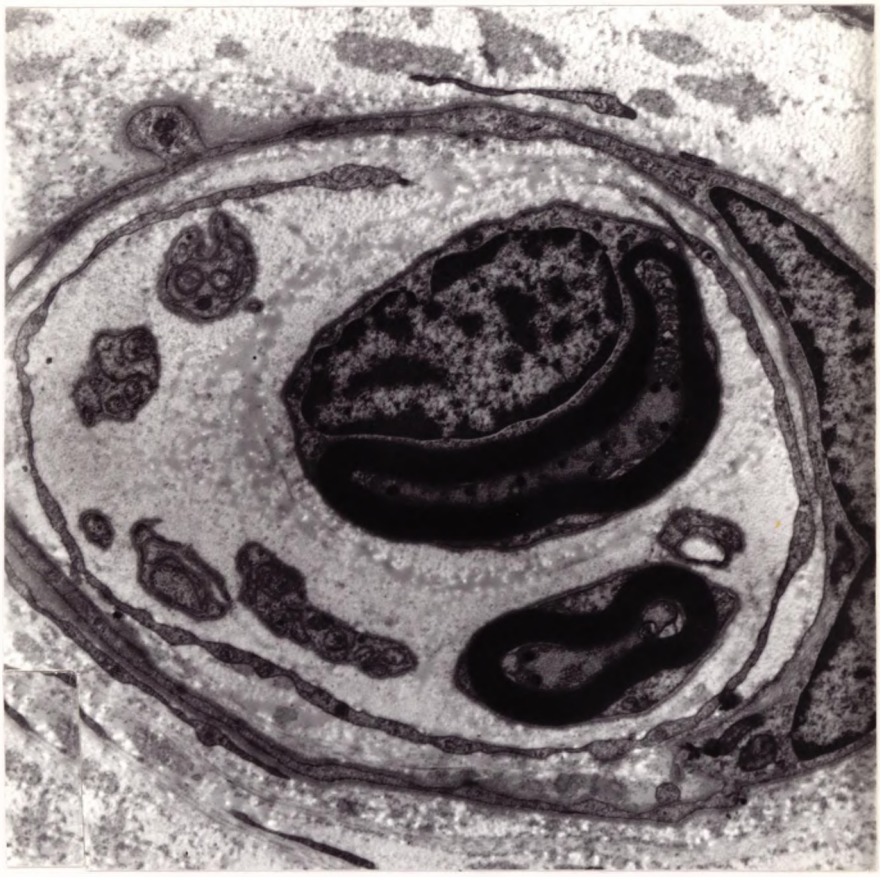


Figure 16. Conjunctival nerve fibre from deep in conjunctival stroma. A perineural sheath and concentrically arranged fibrocyte processes enclose two myelinated nerve fibres, one with the nucleus of its Schwann cell, and several unmyelinated fibres. (10,138X)

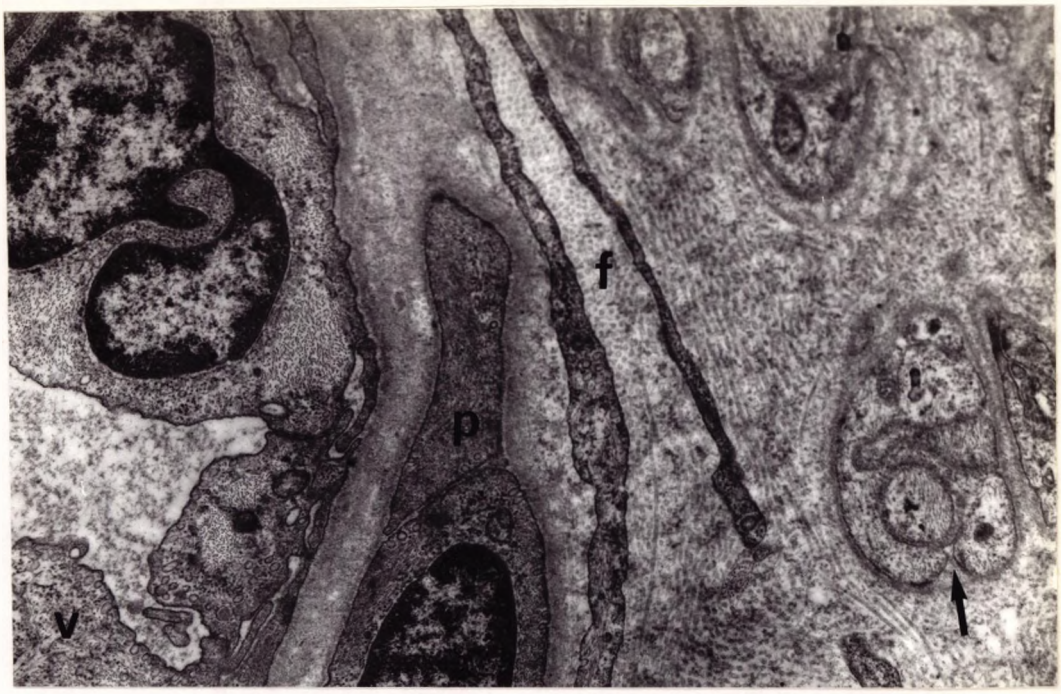


Figure 17. Unmyelinated nerve fibre bundles (arrowed) adjacent to a conjunctival capillary. v= vascular endothelium, p= pericyte, f=fibrocyte process. (14,954X)

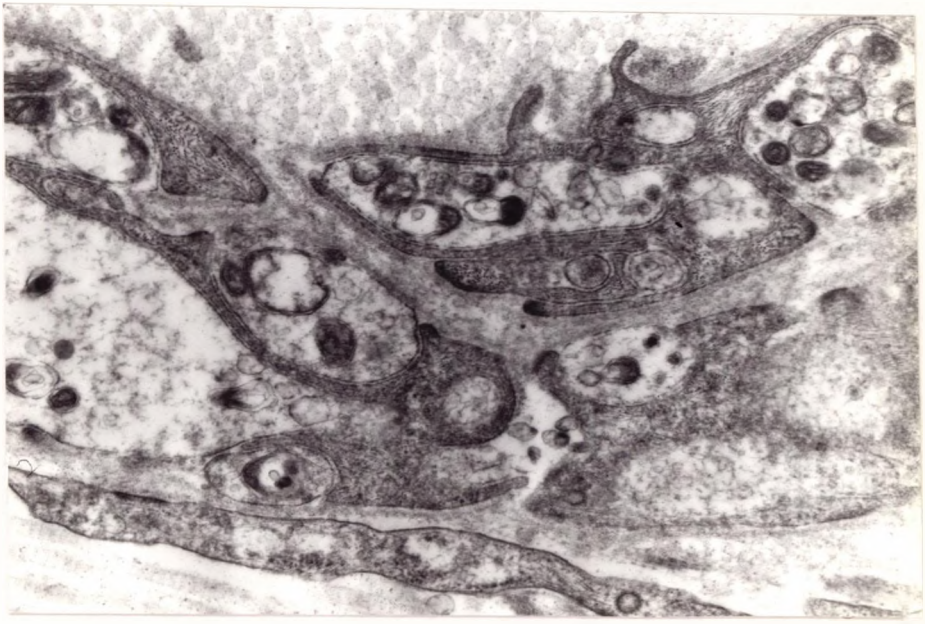


Figure 18. Axon terminal varicosities from conjunctival stroma. Several axons are seen enveloped by each Schwann cell. (23,328X)

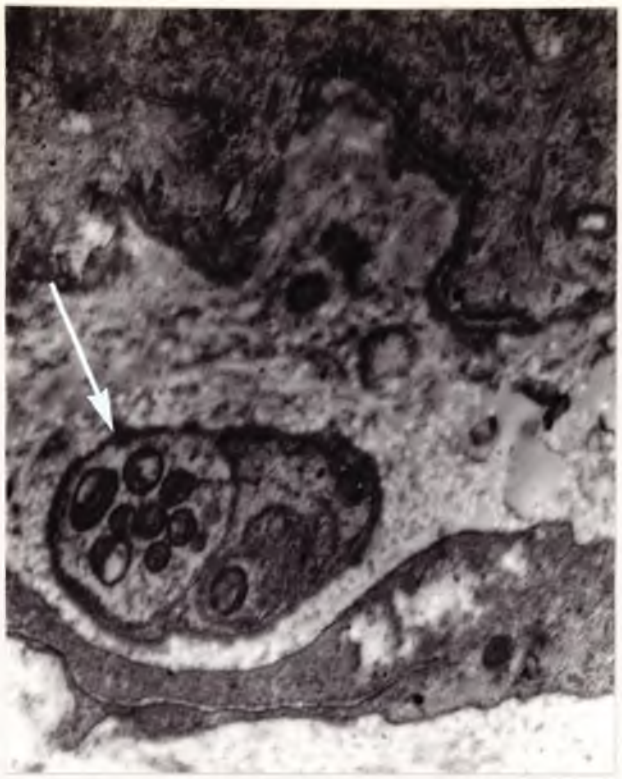


Figure 19. A sub-epithelial nerve fibre bundle including a large axon terminal varicosity (arrowed) underlying limbal conjunctival epithelium. (20,755X)

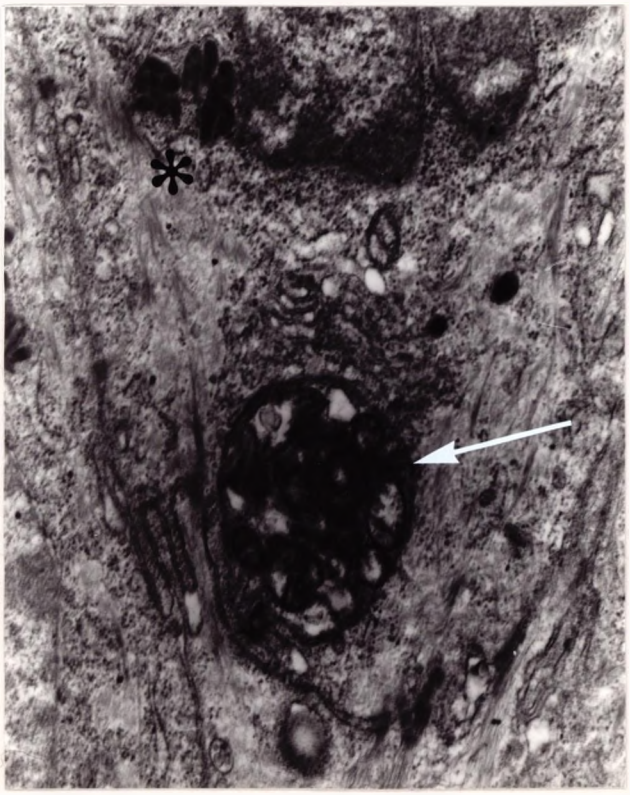


Figure 20. Intra-epithelial axon terminal varicosity (arrowed) enclosed by a basal epithelial cell. Note the compound melanosomes (asterisk) within an adjacent epithelial cell. (20,584X)

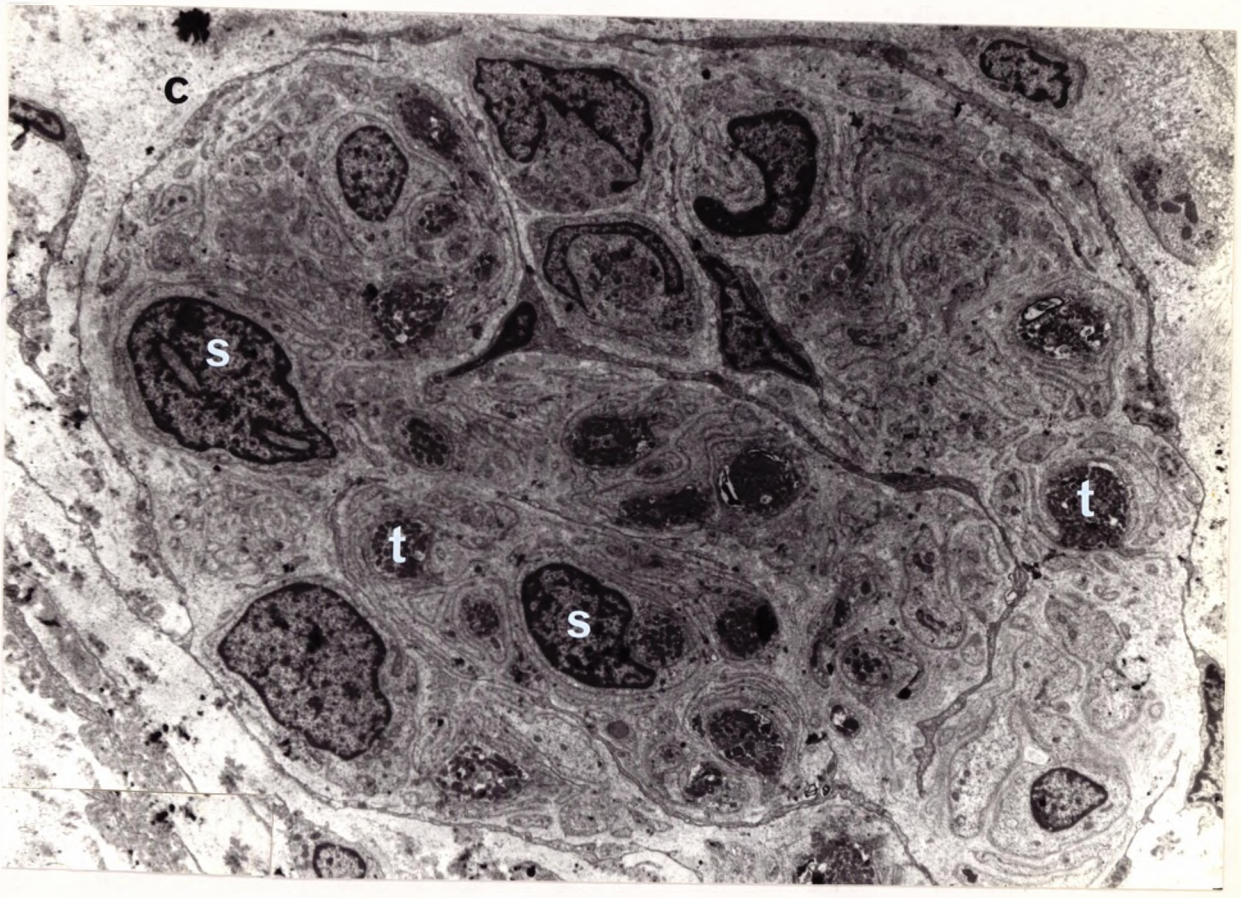


Figure 21. Limbal corpuscular nerve ending. The interior of the corpuscle contains several axon terminals (t) with Schwann-like accessory cells (s). A thin fibrocyte capsule (c) demarcates the perimeter of the structure. (3,701X)

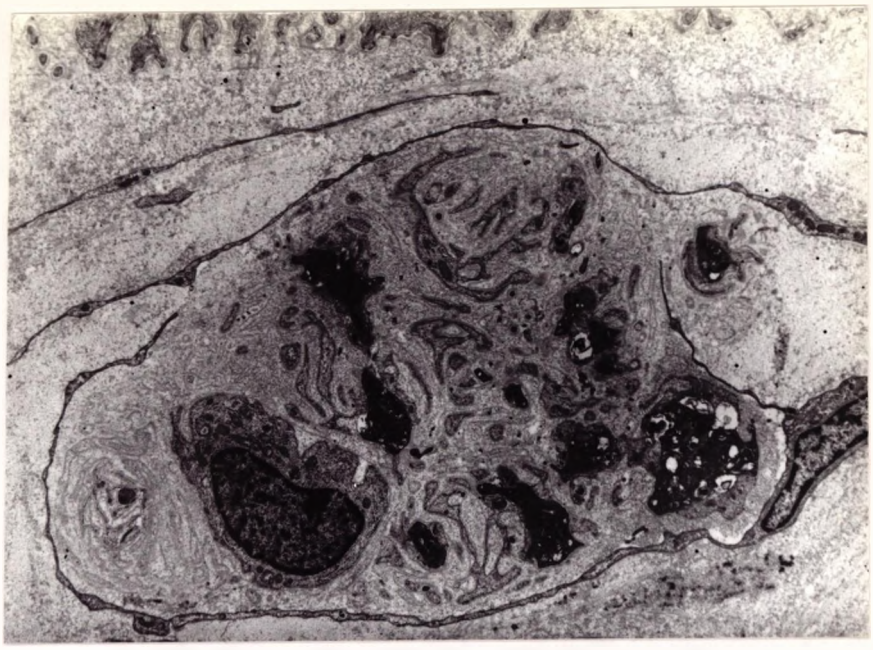


Figure 22. A small encapsulated nerve ending immediately underlying limbal conjunctival epithelium. This example shows a loose arrangement of densely stained terminal axons and Schwann cell lamellae. (4,350X)

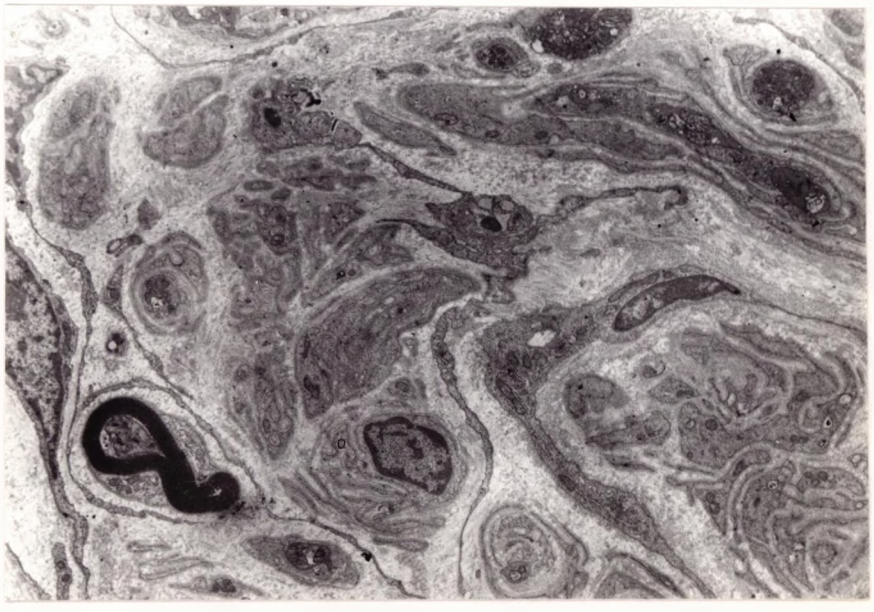


Figure 23. Detail showing axon terminal varicosities surrounded by an irregularly arranged network of Schwann cell lamellae. A myelinated axon is present at the periphery of the corpuscle. (4,290X)

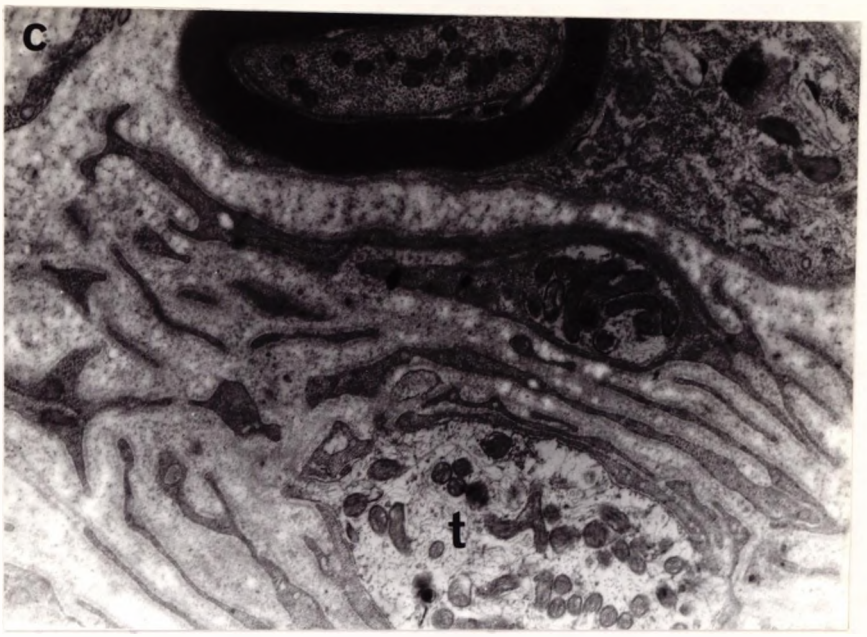


Figure 24. Detail showing a myelinated nerve fibre at the periphery of a corpuscle. c= capsule, t= terminal axon. (13,085X)

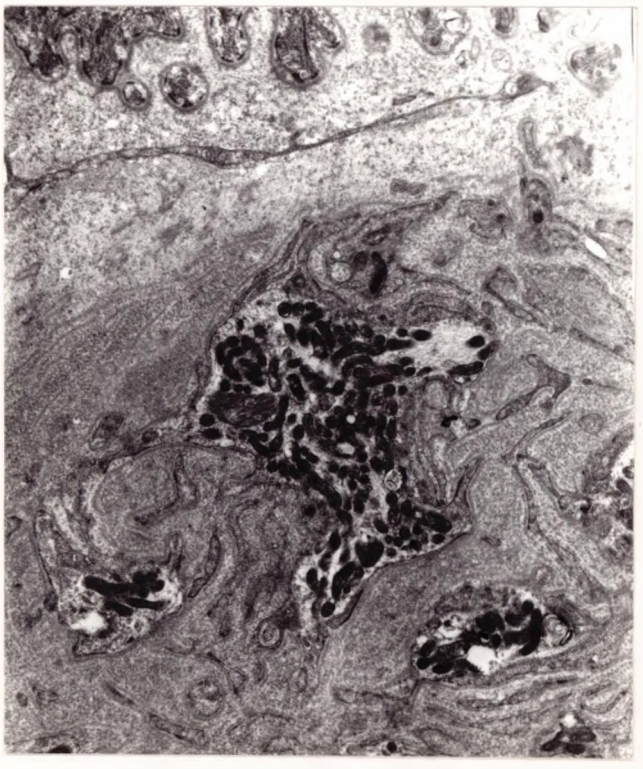


Figure 25. Branching of an axon terminal. (8,083X)

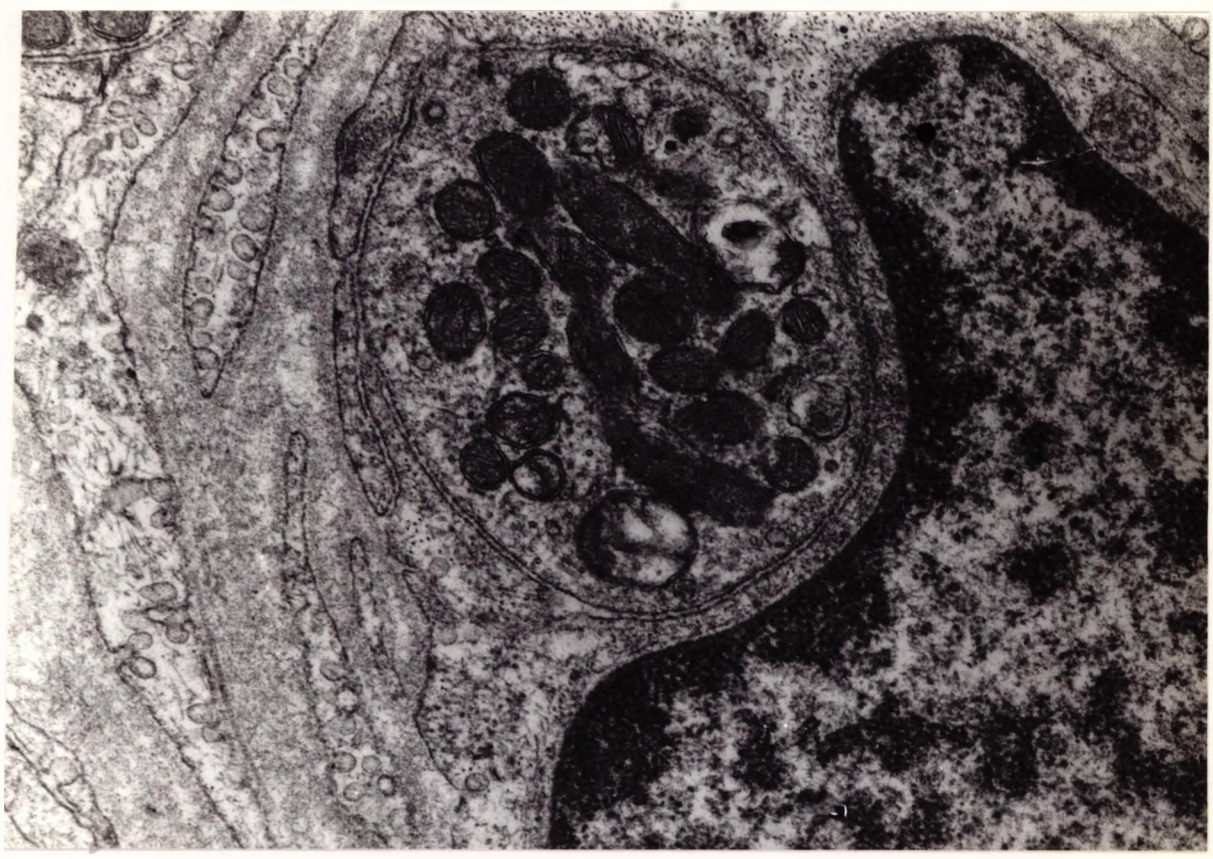


Figure 26. Detail of an axon terminal lying within an invagination of a Schwann cell. Accumulated small mitochondria, neurofilaments, neurotubules, and a few agranular vesicles are present in the axoplasm. (48,300X)

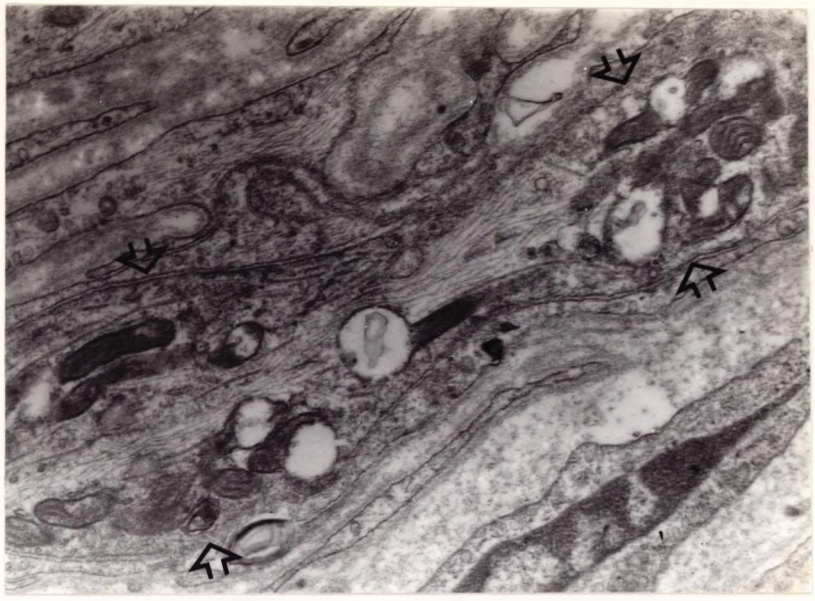


Figure 27. A longitudinal section through a terminal axon. The varicose nature of the terminal is apparent: mitochondria are largely confined to the varicose regions (between arrows) with the inter-varicose region containing predominantly neurofilaments. (21,207X)

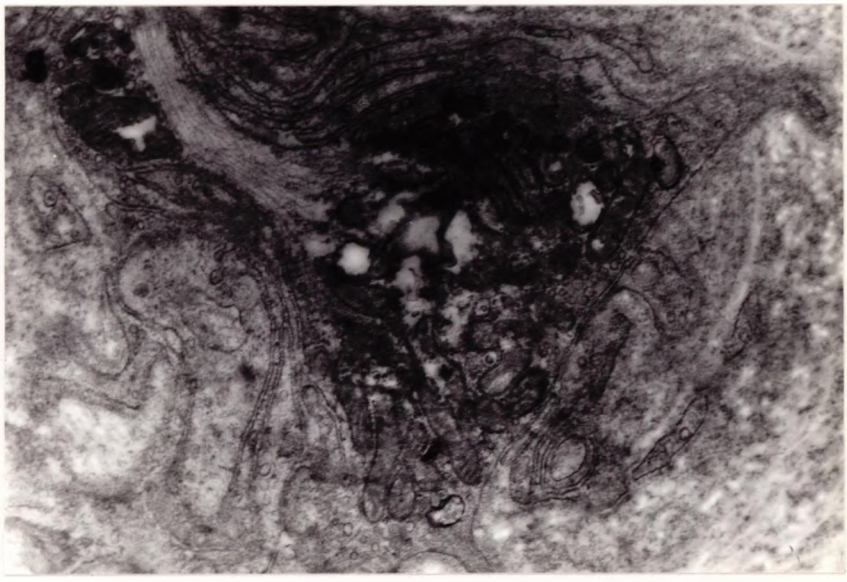


Figure 28. Another example showing a narrow inter-varicose region containing parallel neurofilaments connecting with a varicosity containing numerous mitochondria. Schwann cell lamellae show a close association with the terminal axon. (20,412X)

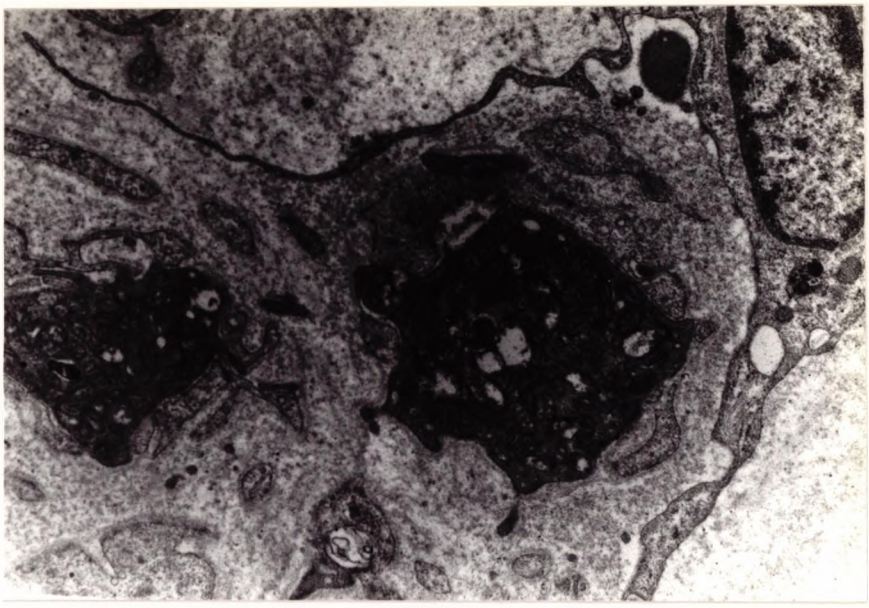


Figure 29. Axon terminals displaying a densely stained axoplasm. (16,414X)

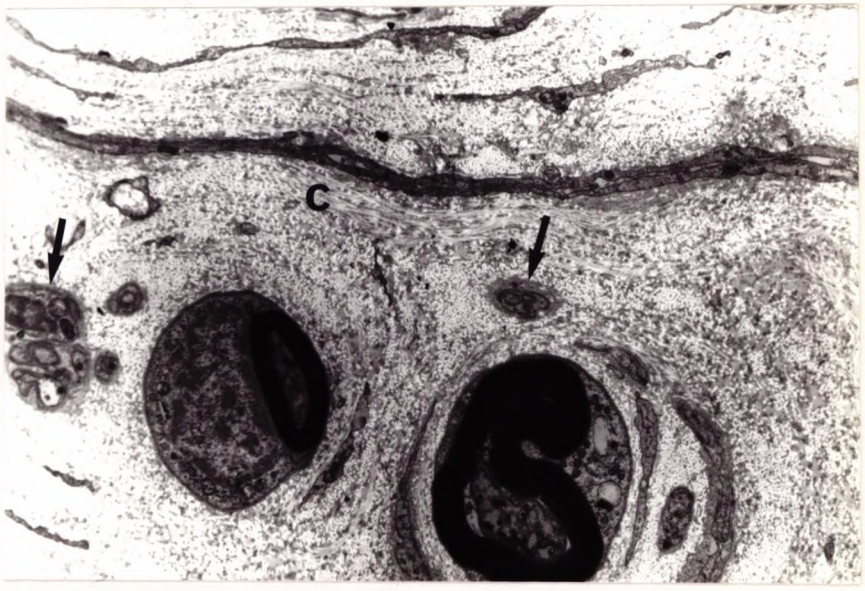


Figure 30. Two myelinated axons outside the capsule (c) of a corpuscular nerve ending. In addition several groups of small calibre unmyelinated fibres (arrowed) are present. (5,892X)

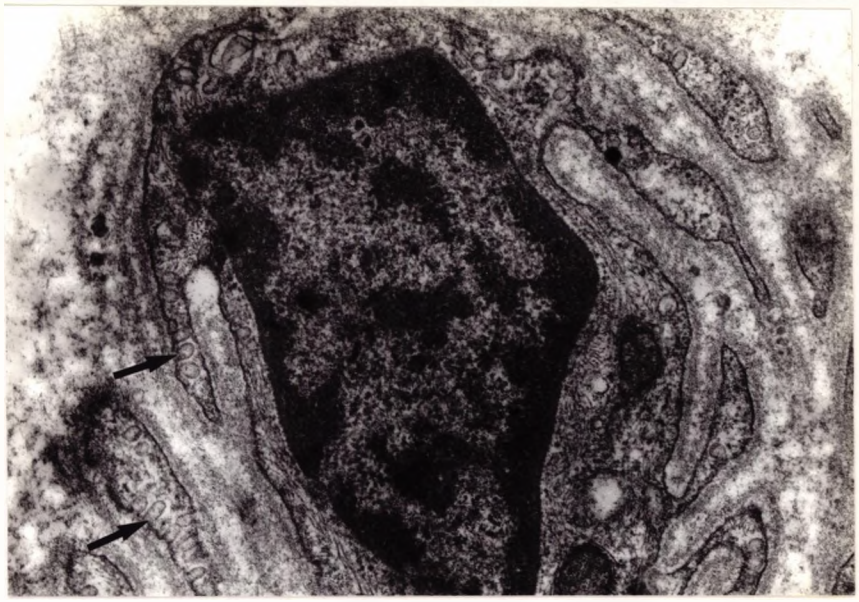


Figure 31. A Schwann-like accessory cell showing numerous processes each with a basal lamina, which contain many pinocytotic vesicles (arrowed). (26,509X)

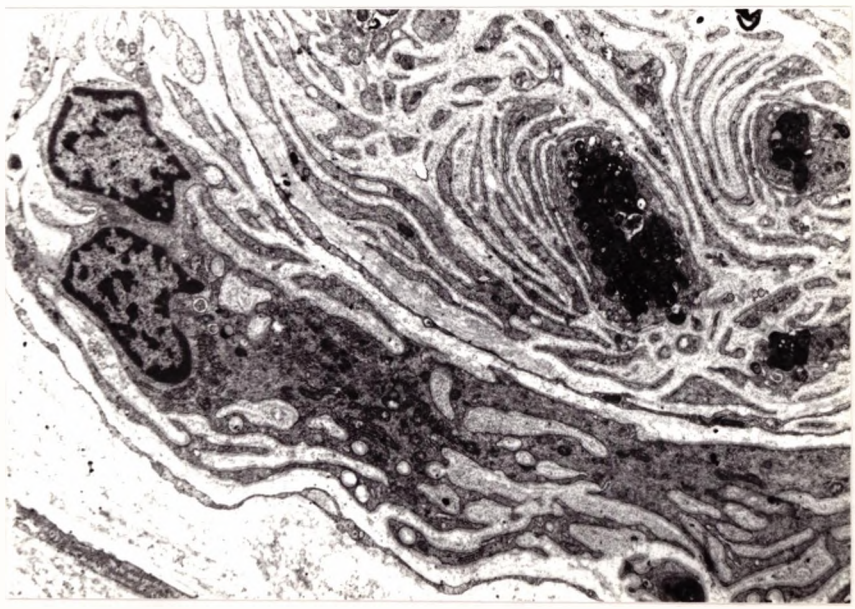


Figure 32. This micrograph shows a complex lamellar system produced by extensive branching of Schwann cell processes. These lamellae form an asymmetrical pattern with several lamellae surrounding each terminal axon. (6,568X)

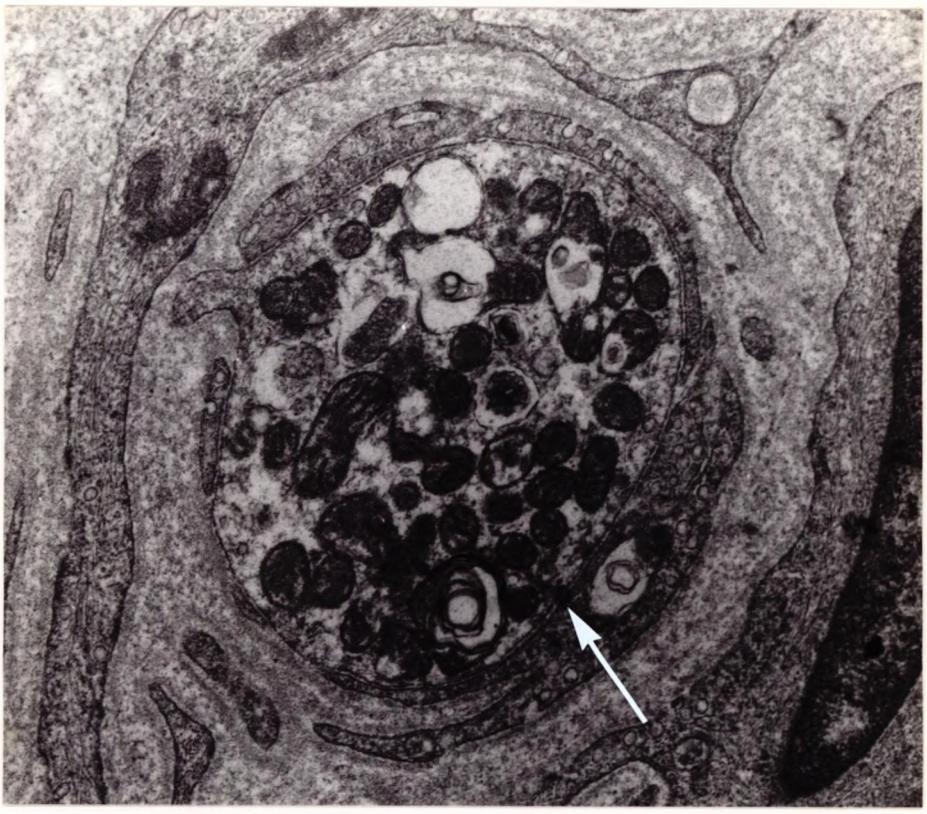


Figure 33. An axon terminal showing an attachment plaque (arrow) between axolemma and Schwann cell lamella. (29,161X)

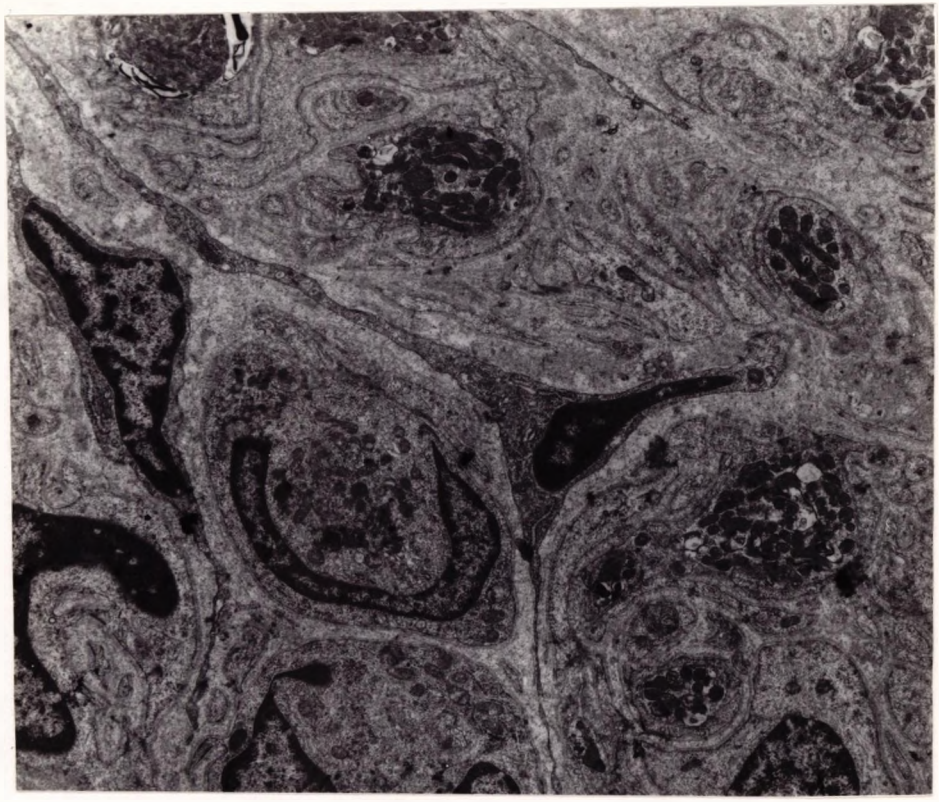
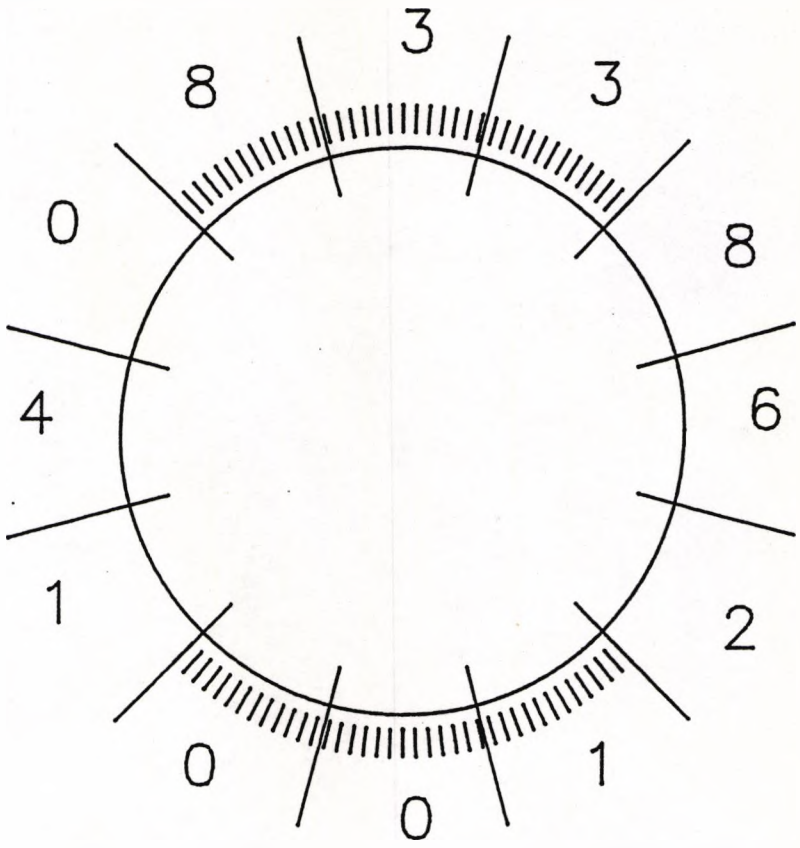
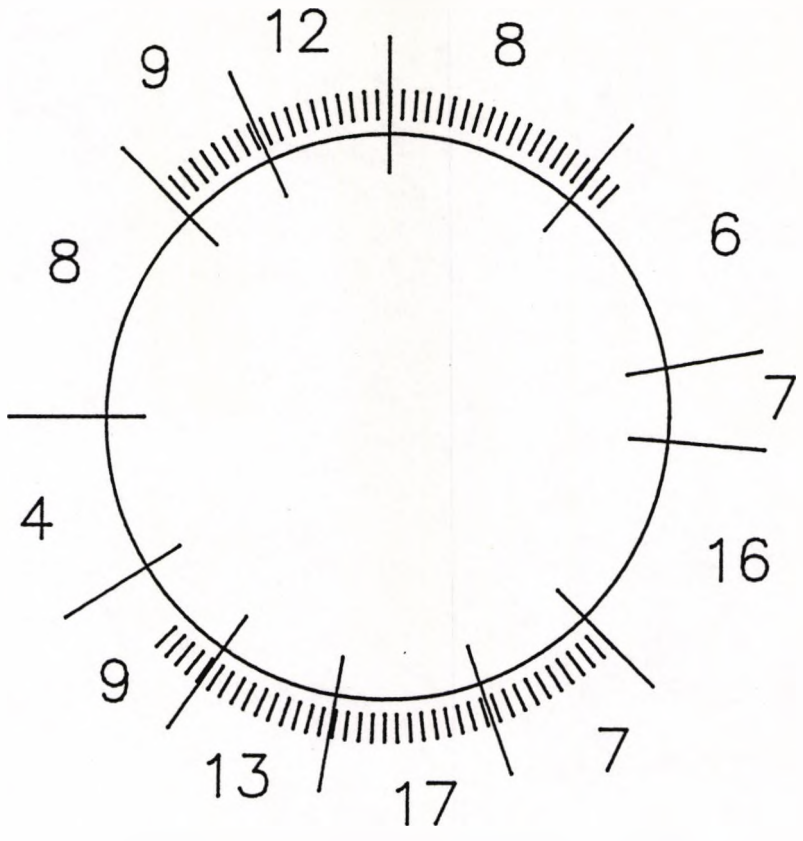


Figure 34. A non-capsular fibrocyte with its processes which partition the body of the corpuscle. (8,210X)



REF: HW22. TOTAL =36

Figure 35. Distribution of sensory corpuscles (HW22). The numbers refer to the number of corpuscles found within each radial segment. The relative sizes of each segment are drawn to scale.



REF: HL2. TOTAL = 122

Figure 36. Distribution of sensory corpuscles (HL2).

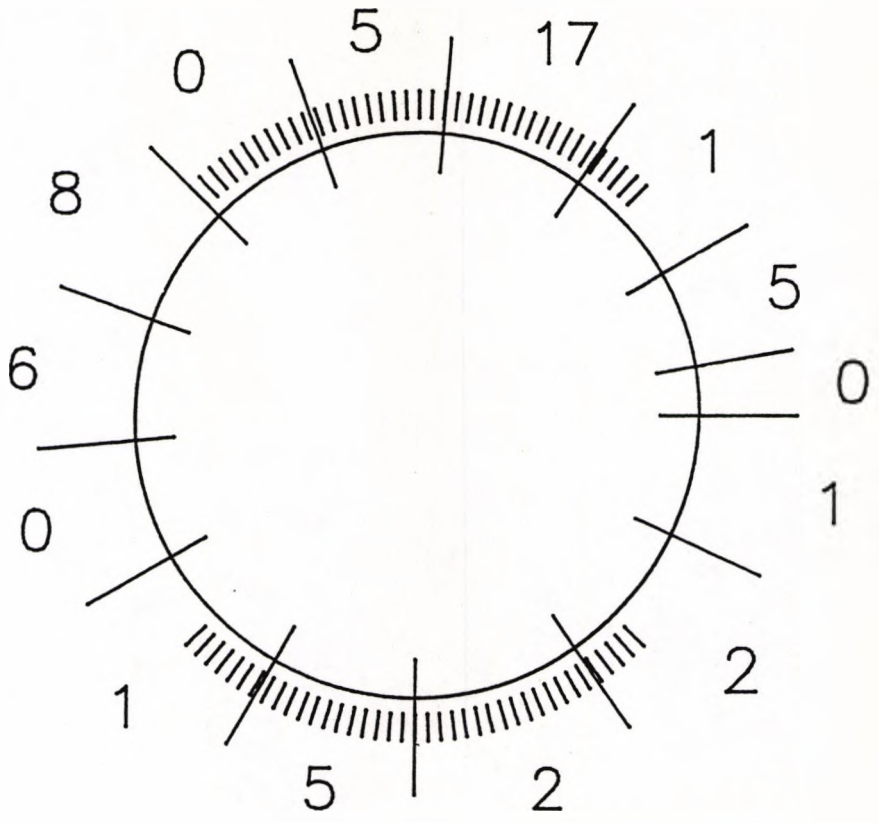


Figure 37. Distribution of sensory corpuscles (HL4).

4.2. Bulbar Conjunctiva.

4.2.1. Light microscopy (bulbar conjunctiva).

Histological sections through the bulbar conjunctiva revealed a goblet cell containing stratified epithelium of varying thickness, comprising 2-5 layers of cells (Fig.38). The stroma could be resolved into a superficial adenoid layer and a deep fibrous layer.

In terms of its general innervation the bulbar conjunctiva is similar to that described for the limbus, although nerve fibre bundles were of larger calibre, particularly those located in the deep fibrous layer which often exceeded 50μm in diameter. In the adenoid layer nerves were smaller with a thinner perineurium. As with the limbal conjunctiva myelinated axons were found in the superficial stroma (Fig.39).

Corpuscular nerve endings were located at varying distances from the epithelium (Figs.38,40,41) although always within the adenoid layer. These varied in size, and were generally larger than their limbal counterpart with a mean maximum diameter of 55μm (range 27-78μm). Bulbar corpuscles were thinly encapsulated and typically had a densely packed complement of Schwann cells which enclosed several neural profiles. Each corpuscle was supplied by 1-3 myelinated nerve fibres, which usually passed directly into the body of the structure, losing their myelin in the process. However,

in some corpuscles the parent axon appeared to form a complex series of convolutions before entry. This was manifest as numerous myelinated axon profiles, many sectioned obliquely, around the perimeter of the corpuscle (Fig.42). In addition, isolated myelinated profiles were observed within the body of the terminal , although these still tended to maintain a peripheral location. Larger corpuscles were often associated with small capillaries (Fig.42).

4.2.2. Electron microscopy (bulbar conjunctiva).

The general features of bulbar corpuscles at the ultrastructural level were largely consistent with those described for the limbal conjunctiva: comprising axon terminals with Schwann like cells and enclosed by a fibrocyte capsule (Figs.43,44). The myelinated profiles observed at the periphery of many corpuscles (Fig.44) were largely enclosed by the capsule, and although typically the myelin sheath was lost before or soon after entry, it was sometimes retained for some distance into the corpuscle (Fig.44).

Axon terminals were numerous. They were varicose and contained a similar complement of organelles to those in limbal corpuscles (Figs.45,46). Each was surrounded by multiple, thin Schwann cell lamellae which were closely spaced and often arranged in a roughly concentric manner

(Fig.47). The densely stained axon terminals which were present in some limbal corpuscles was not observed.

4.2.3. Incidence of bulbar corpuscles.

The incidence of corpuscular nerve endings in samples of bulbar conjunctiva from two eyes is shown in the Table.

REFERENCE		AREA mm2	NUMBER OF CORPUSCLES
HW19	B1	5	7
	B2	6	28
	B3	12	41
HG9	E1	17	11
	E2	18	11
	E3	3	4

Table 2. Incidence of bulbar corpuscles.

All samples contained corpuscular nerve endings although their incidence showed some variation from one sample to the next. In total 61mm^2 of bulbar conjunctiva was sectioned using an interrupted serial sectioning technique and 102 corpuscles were observed. This is equivalent to an incidence of 1-2 corpuscles per mm^2

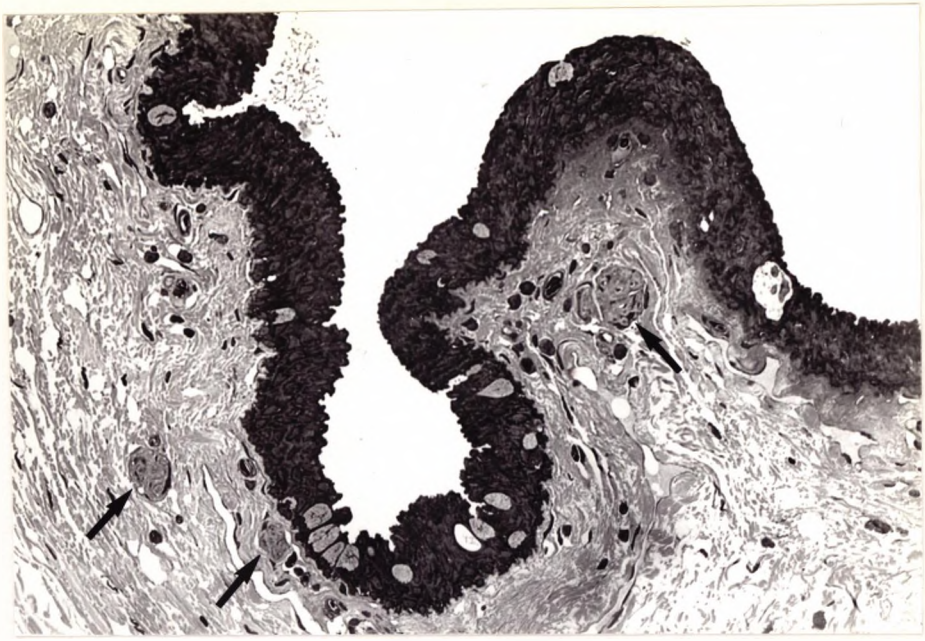


Figure 38. Three corpuscular nerve endings (arrowed) from the bulbar conjunctiva at varying distance from the goblet cell containing epithelium. (220X)

Figure 39. Myelinated nerve fibres (arrows) in the superficial conjunctival stroma. (572X)

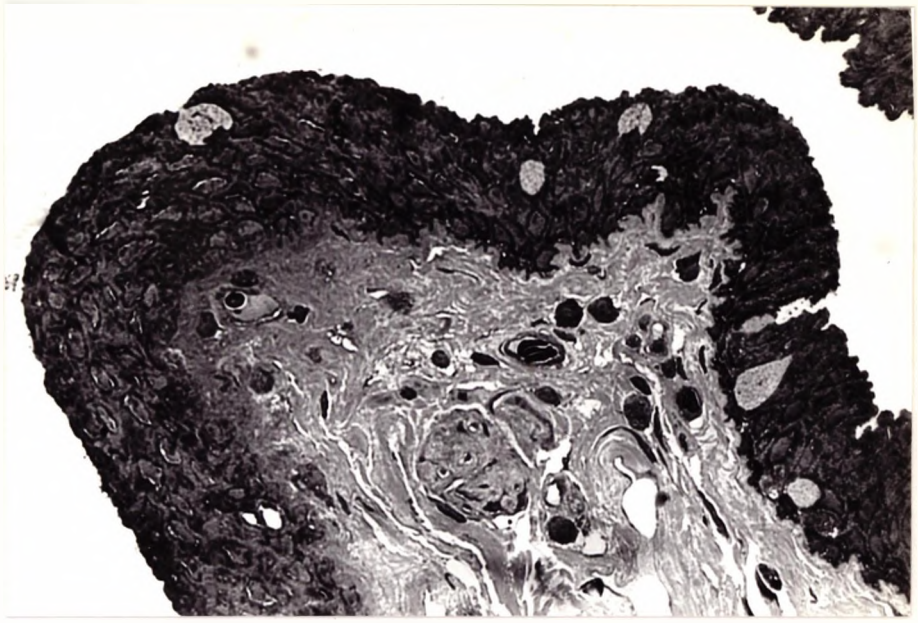


Figure 40. A corpuscle located within a fold of conjunctiva.(493X)

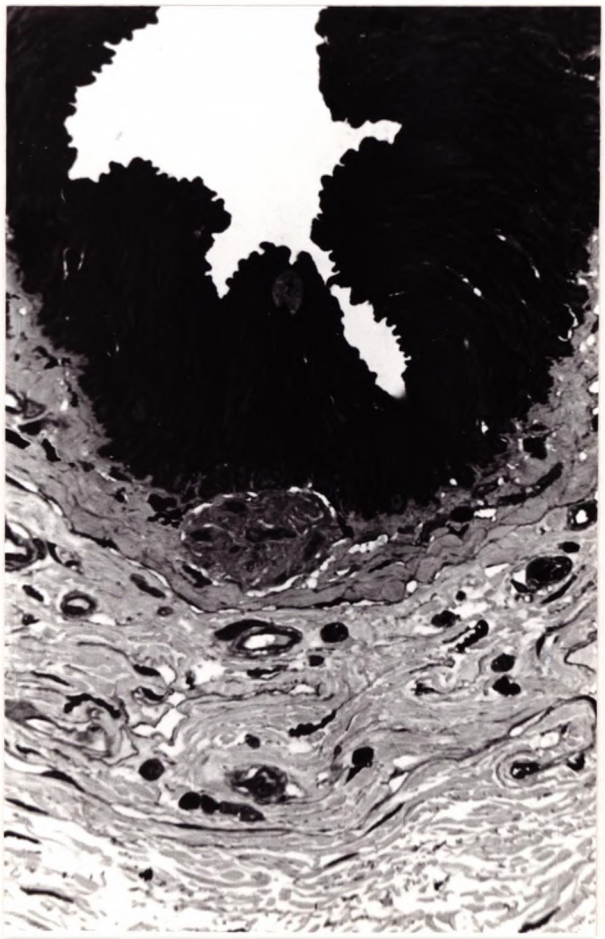


Figure 41. A corpuscle abutting the basal epithelial layer.(493X)

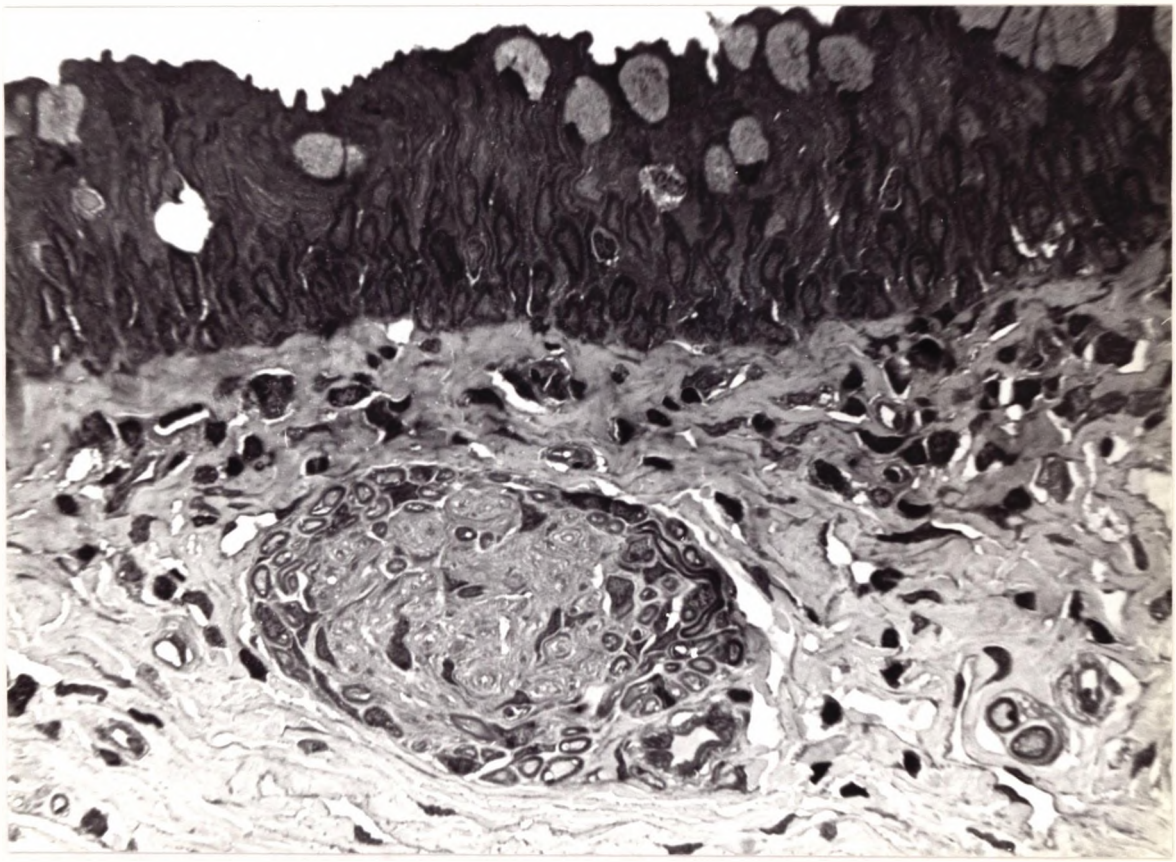


Figure 42. This example shows multiple myelinated axon profiles surrounding the perimeter of the corpuscle. In addition, isolated myelinated fibres penetrate deeper into the structure. A small calibre capillary is located in close proximity to the terminal. (634X)

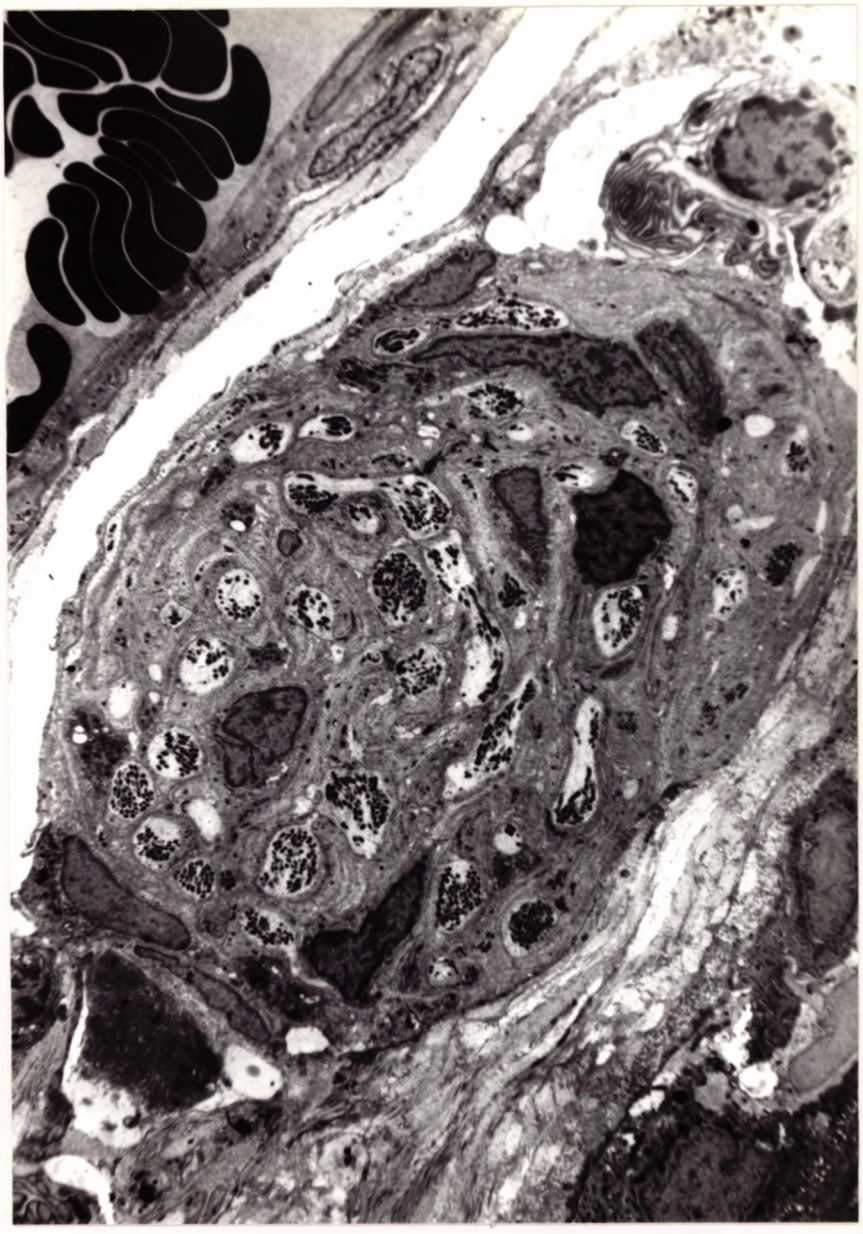


Figure 43. A low power electron micrograph of a corpuscular nerve ending from the bulbar conjunctiva. (4,578X)

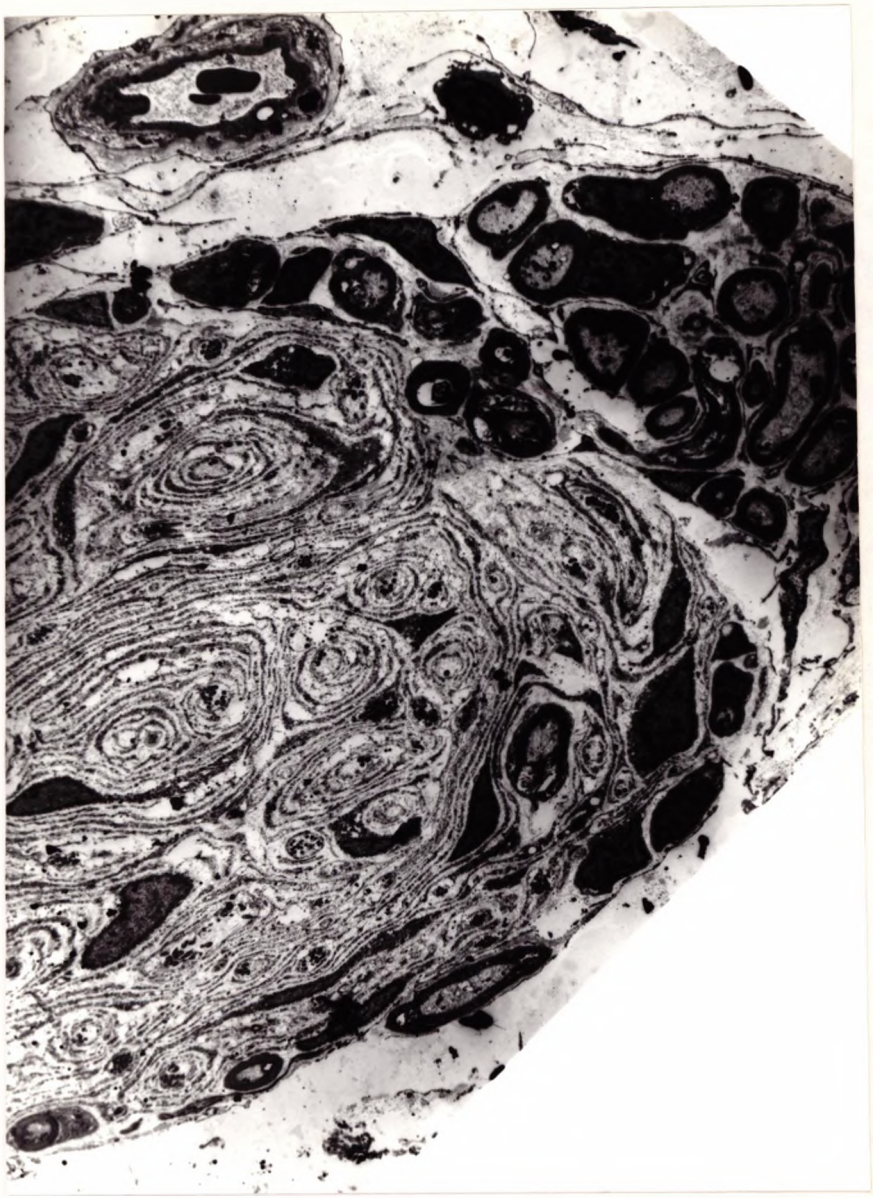


Figure 44. In this example myelinated fibres are present which are largely enclosed by the capsular cells. An asymetrical network of thin closely spaced lamellae derive from Schwann like cells. (2,346X)

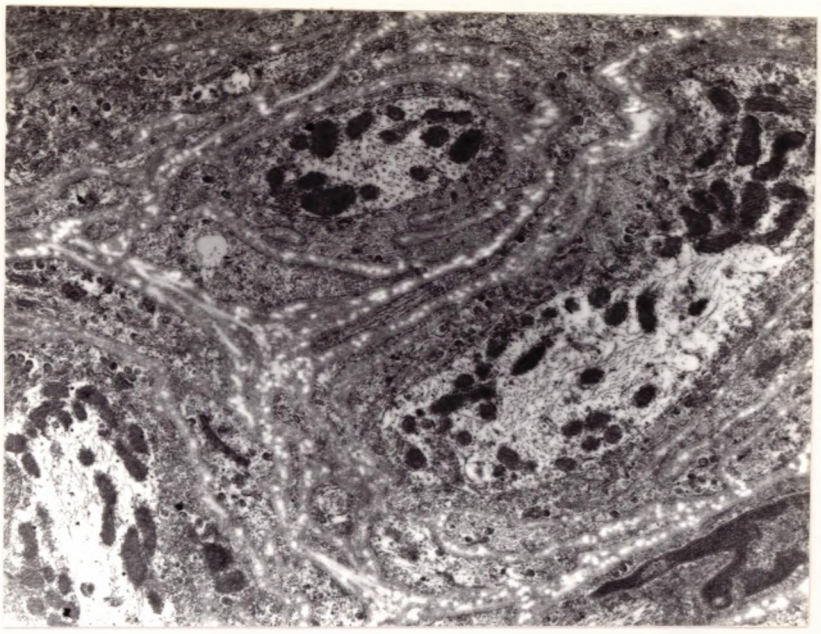


Figure 45. Detail of axon terminals. These contain several mitochondria, neurofilaments, neurotubules, and clear vesicles. (11,216X)

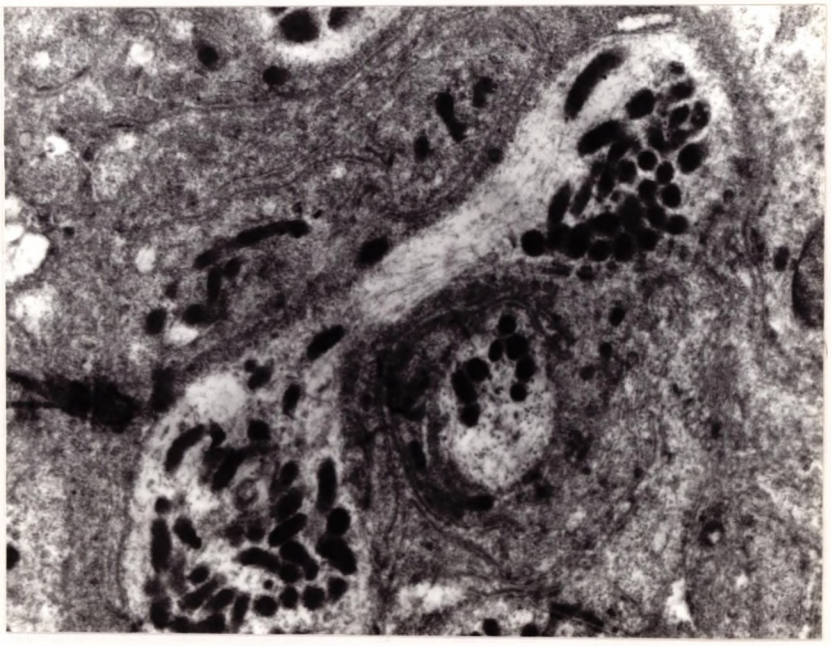


Figure 46. Two axon terminal varicosities linked by a narrow inter-varicose zone. (11,750X)

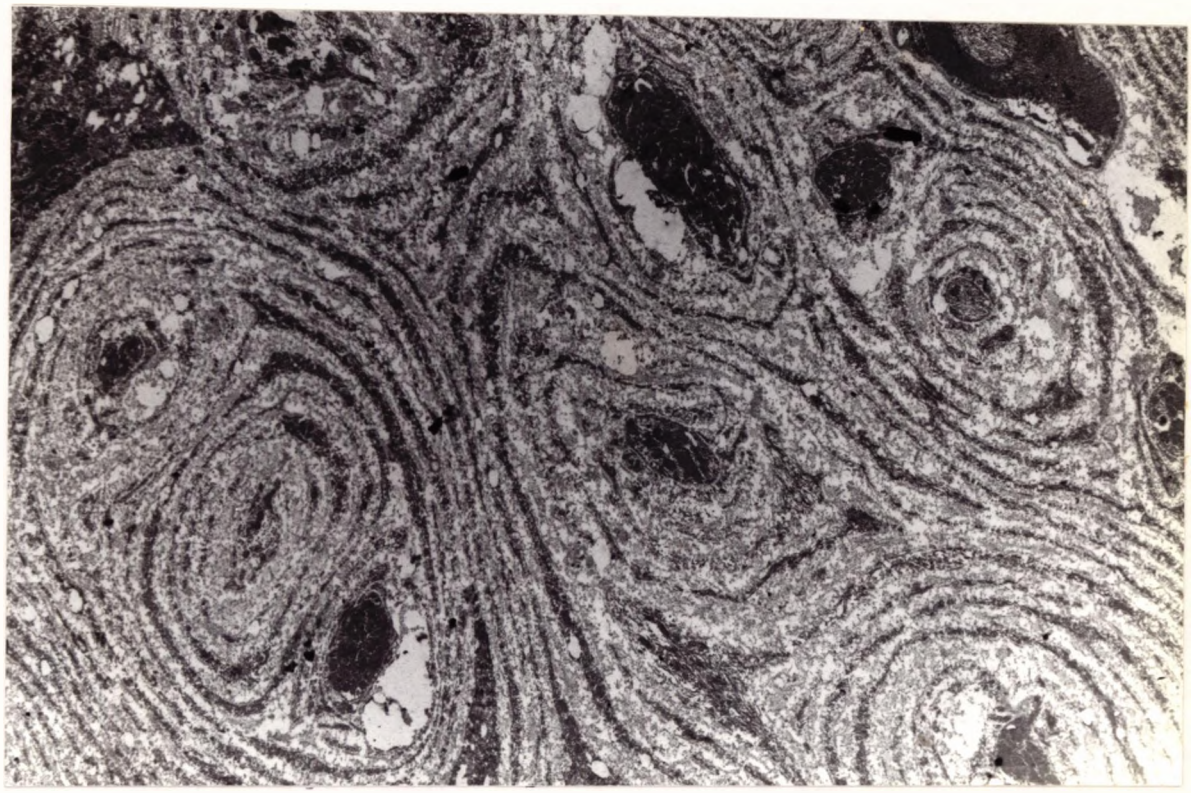


Figure 47. A largely concentric arrangement of Schwann cell lamellae surround axon terminals. (6,842X)

4.3. Eyelid Margin and Palpebral Conjunctiva.

4.3.1. Light microscopy (marginal conjunctiva).

A cross section through the eyelid close to the margin shows the transition from occlusal surface to marginal conjunctiva. A stratified squamous epithelium covers both surfaces. Over the occlusive zone the epithelium is thinly keratinised, and this is maintained until close to the tarsal gland openings. On passing into the conjunctiva, goblet cells are at first infrequently encountered and epithelial thickness gradually increases, being maximal over the marginal angle where the conjunctival surface first contacts the globe (Fig.48). A feature of both zones is the presence of stromal papillae projecting into the epithelium, although the surface remains flat until the tarsal conjunctiva is reached when the surface becomes less regular and papillae penetrate deeper into the epithelium.

The eyelid margin was found to be richly innervated with numerous small calibre mixed nerve fibre bundles distributed throughout the stroma. Corpuscular nerve endings were numerous within the marginal conjunctiva of the upper lid where they were particularly common close to the junction of the marginal conjunctiva and occlusal zone. On passing further into the conjunctiva away from the margin their incidence rapidly declined. Observation of a limited amount of marginal conjunctiva from the lower lid failed to reveal any corpuscular endings. Those corpuscles from the upper lid

were found at varying distances from the epithelium (Fig.49). Many were observed within stromal papillae (Fig.50) whereas others were located up to 175μm from the basal epithelial layer (Fig.51). Corpuscles varied in size (mean greater diameter 40μm, range 23-62μm) and in semi-thin section closely resembled those found at other conjunctival locations. The degree of encapsulation was variable. The capsule surrounding superficially located corpuscles tended to be thin and incomplete (Fig.50) whereas deeper lying structures often possessed a more substantial capsule which formed a complete investment (Fig.51).

4.3.2. Electron microscopy (marginal conjunctiva).

Electron microscopy confirmed a morphology similar to earlier descriptions of corpuscles in the limbal and bulbar conjunctiva (Fig.52). An irregular network of Schwann cell lamellae enveloped several axon terminal varicosities which contained an accumulation of mitochondria (Fig.53). Certain corpuscular endings at the lid margin, in contrast to those previously described in the limbal and bulbar conjunctiva, possessed a capsule which consisted of an outer layer of perineural cells (Fig.54) displaying a basal lamina, and an inner fibrocytic layer.

4.3.3. Incidence of marginal corpuscles.

The incidence of marginal corpuscles is shown in the Table.

REFERENCE	UPPER/LOWER EYELID	LINEAR DIST. ALONG MARGIN	NUMBER OF CORPUSCLES
HG9 BB1	UPPER	1.35mm	15
HG9C1	UPPER	0.92mm	13
HG16C	UPPER	0.81mm	21
HG16C1	UPPER	0.63mm	16
HG19C	LOWER	0.46mm	0
HG16A1	LOWER	0.10mm	0

Table 3. Incidence of marginal corpuscles.

Samples of eyelid tissue obtained from three eyes were sectioned perpendicular to the margin. Corpuscular nerve endings were found predominantly in an narrow strip of tissue close to the marginal angle of the upper lid. In total 65 corpuscles were observed in upper lid samples corresponding to 3.7mm of marginal tissue. This is equivalent to 17-18 corpuscles per mm. No corpuscular nerve endings were found however in two small pieces of lower eyelid.

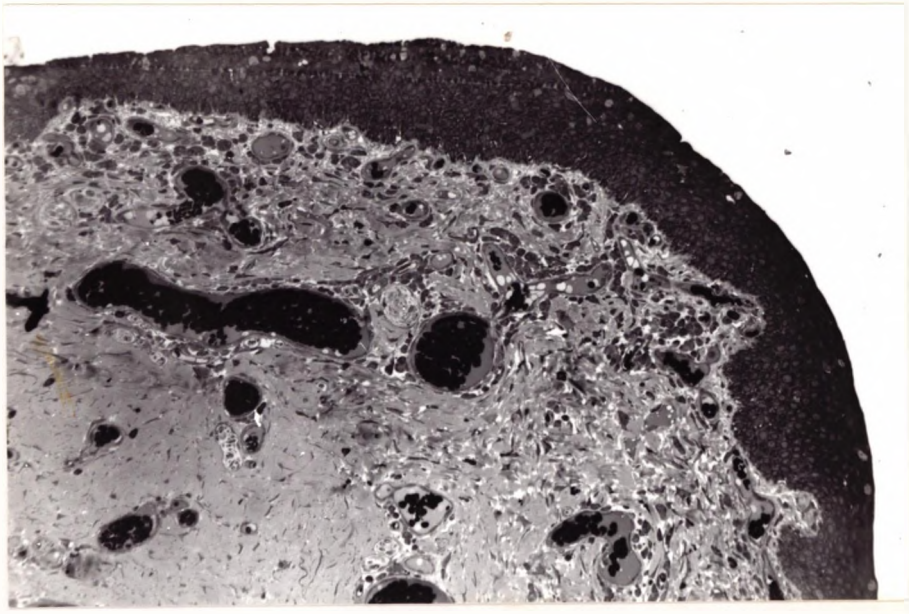


Figure 48. A section through the marginal conjunctiva of the upper lid. Stromal papillae penetrate the thick stratified squamous epithelium which is sparsely populated with goblet cells. (115X)

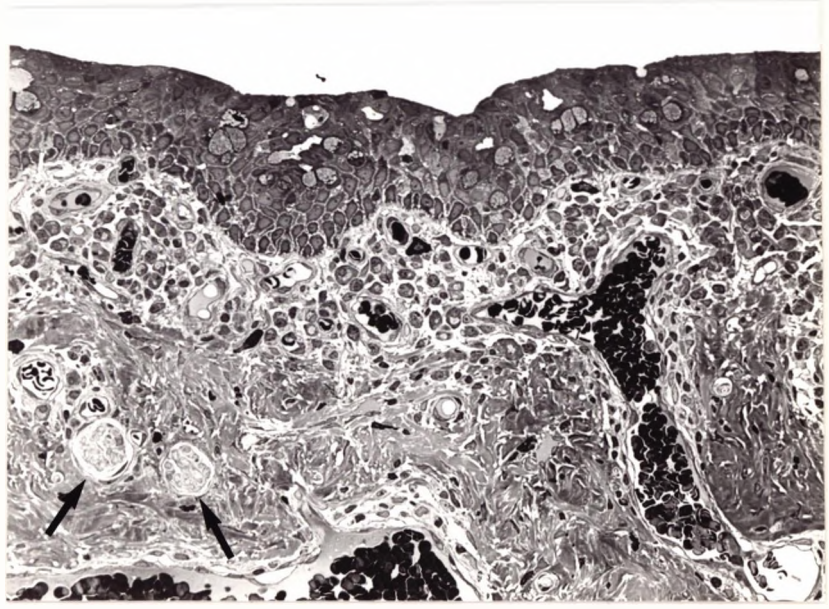


Figure 49. Two corpuscular nerve endings (arrowed) located in the marginal conjunctiva. (231X)

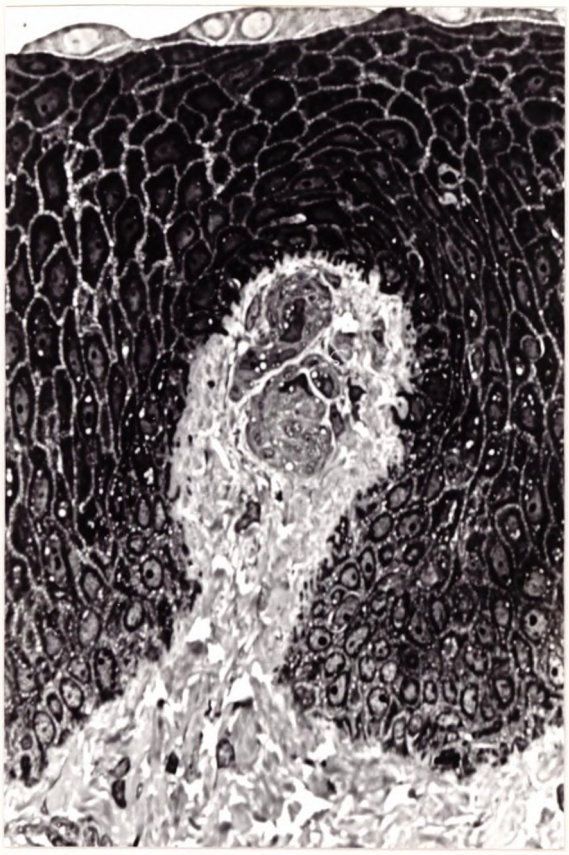


Figure 50. A multi-lobed complex nerve ending within a stromal papilla in marginal conjunctiva. The capsule is thin and indistinct. (413X)

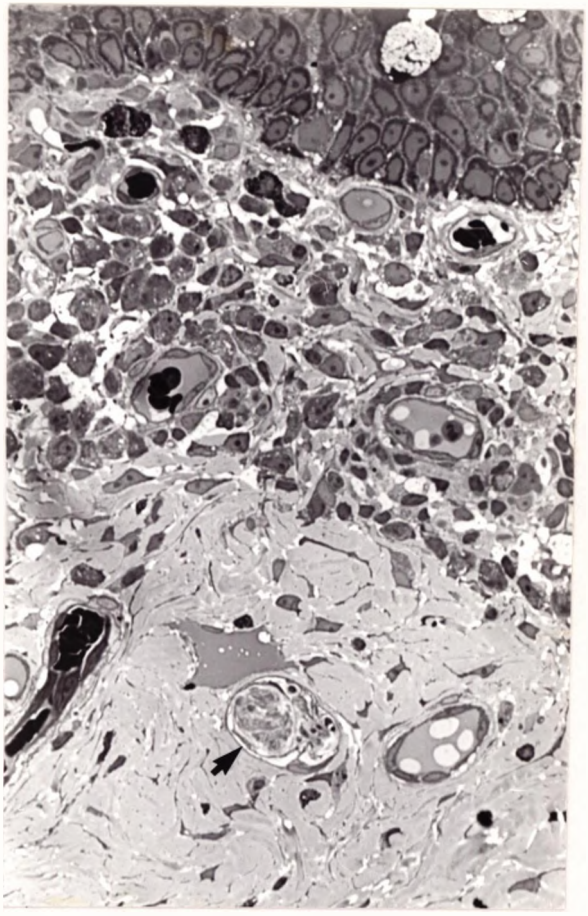


Figure 51. This example shows a thicker capsule completely investing the corpuscle (arrow). (397X)

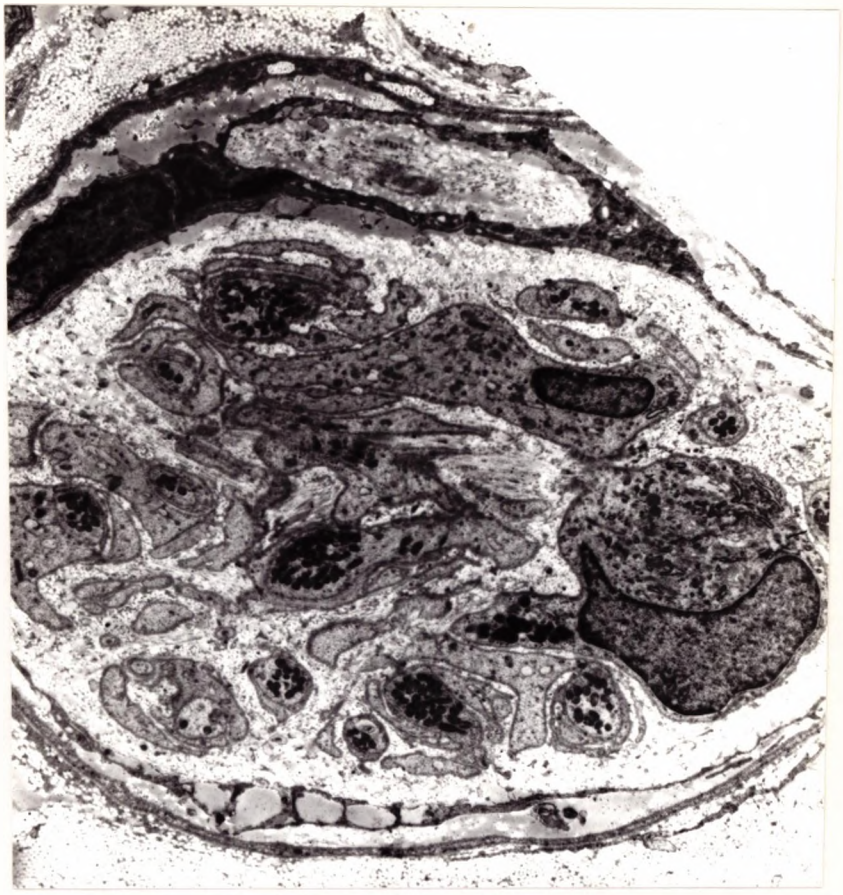


Figure 52. Electron micrograph of a corpuscular ending from the marginal conjunctiva showing a capsule of perineurium and fibrocytes enclosing several axon terminal varicosities and Schwann-like accessory cells. (4,836X)

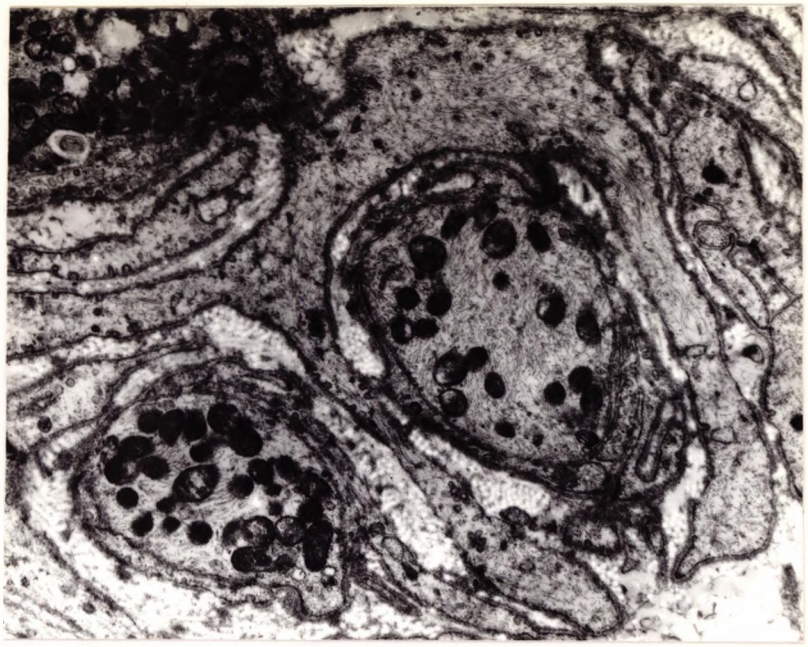


Figure 53. Detail of axon terminals. These contain an accumulation of mitochondria and are surrounded by cytoplasmic lamellae of Schwann cells. (13,934X)

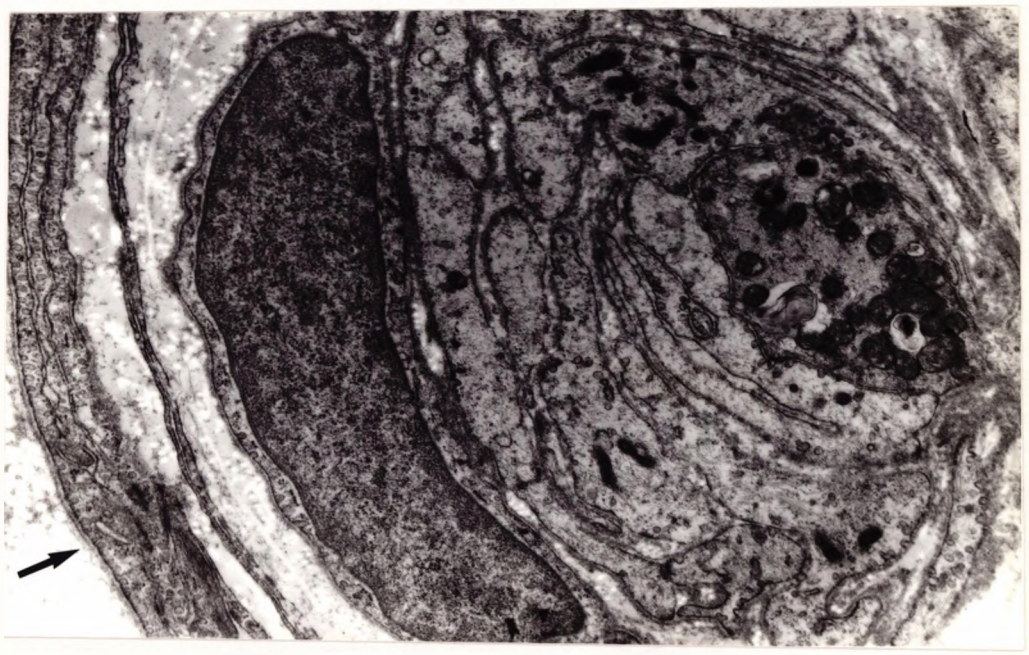


Figure 54. Detail of the capsule. A basal lamina (arrow) is visible at the external surface of the outer capsular layer .(13,459X)

CHAPTER 5

RESULTS

Intra-vital Staining of the Conjunctiva with Methylene Blue.

5.1. Qualitative Observations.

Following the instillation of the dye the first response was a marked vasodilation of the conjunctival and episcleral vasculature. This was followed by a patchy superficial punctate staining of the conjunctival epithelium which was particularly dense around the limbus. Conjunctival nerve fibres took up the stain at varying intervals from first instillation, ranging from a few minutes to over half an hour. These fibres were clearly visible as fine blue threads against the white background of the sclera, often following the course of conjunctival blood vessels.

Staining of corpuscular nerve endings soon followed. These consisted of round or oval forms which were variable in size and tended to have a superficial location within the stroma. They were supplied typically by a single nerve fibre (Figs. 55-59), although the axon sometimes bifurcated before reaching the corpuscle. At the magnifications afforded by convential biomicroscopy little detail was apparent within the body of the structure appearing uniformly stained (Figs. 56,59) or demonstrating focal areas of dense staining

against a paler blue background. Often this density was greatest at the point of entry of the axon. Using the higher magnifications of the non-contact endothelial camera in optimally stained terminals, a tangle of fine nerve fibres could made out.

Corpuscular nerve endings were observed in all subjects and there was a suggestion of individual variations in their incidence. They were found in groups separated by large areas from which they were absent. Although these observations were qualitative, the impression was gained that the greatest number of corpuscles were found in the upper conjunctiva and particularly the superior temporal quadrant. Fewest were found in the inter-palpebral zone, and suprisingly, in view of the histological findings, complex terminals were not observed in the limbal conjunctiva, and moreover the overall pattern of limbal innervation was largely indistinct, although stained corneal nerve fibres were visible beyond the limbus.

Staining was transient, although the dye was taken up and retained by nervous elements to different extents. This variability related to both subject and region of a particular eye: one sector achieving optimal staining as another began to fade. This often led to incomplete staining of nerve fibres and large calibre nerves often seemed to end abruptly. Corpuscular nerve endings remained visible for intervals ranging from a few minutes to over half an hour.

Once staining had faded it was not possible to restain corpuscles with further instillations of the dye.

In addition to nerve fibres, methylene blue was also taken up by lymphatics which were densely stained (Fig.60).

There were no persistent adverse effects associated with the procedure other than a slight residual hyperaemia usually present on the following day.

5.2. Quantitative observations.

In three subjects corpuscular nerve endings were counted, and their distribution was recorded by mapping the position of stained corpuscles on a photo-montage of the ocular surface which was made prior to the staining session. Conjunctival and episcleral blood vessels were used to aid localisation.

Corpuscular nerve endings took up the stain at varying intervals from first instillation, and this was retained for up to half an hour. The transient nature of the stain meant that the maximum useful period for mapping corpuscles was approximately 2hrs.

The results for these three subjects are given in Figs. 61-63.

These data confirm the qualitative observations with regard to the preponderance of corpuscles in the upper conjunctiva, and particularly the upper temporal quadrant. In each case more than half the stained endings were found in this zone. Although isolated corpuscles were observed, they tended to be clustered in groups of 3-7. As noted previously few were found in the inter-palpebral zone and none were observed at the limbus.

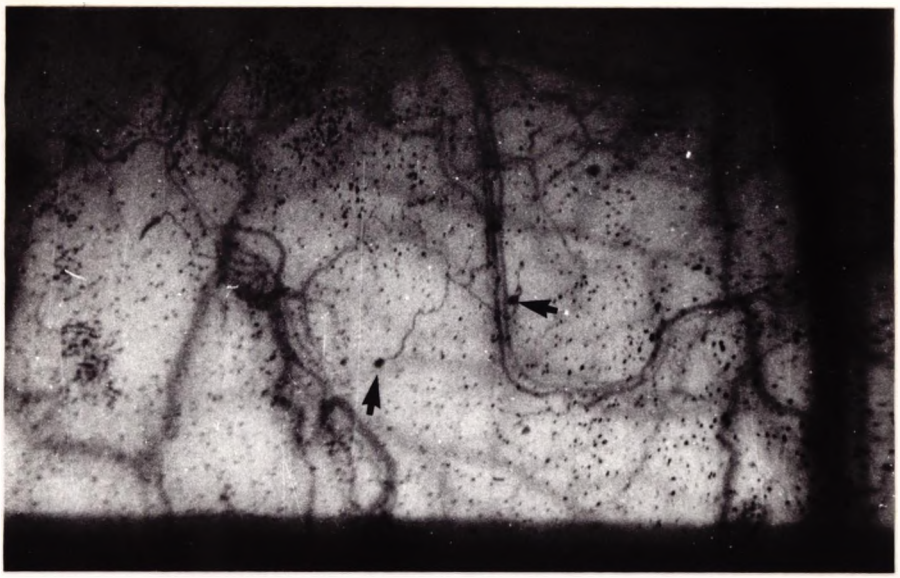


Figure 55. A slit lamp photograph of the bulbar conjunctiva stained in vivo with methylene blue showing several corpusclar nerve endings (arrowed). (35X)

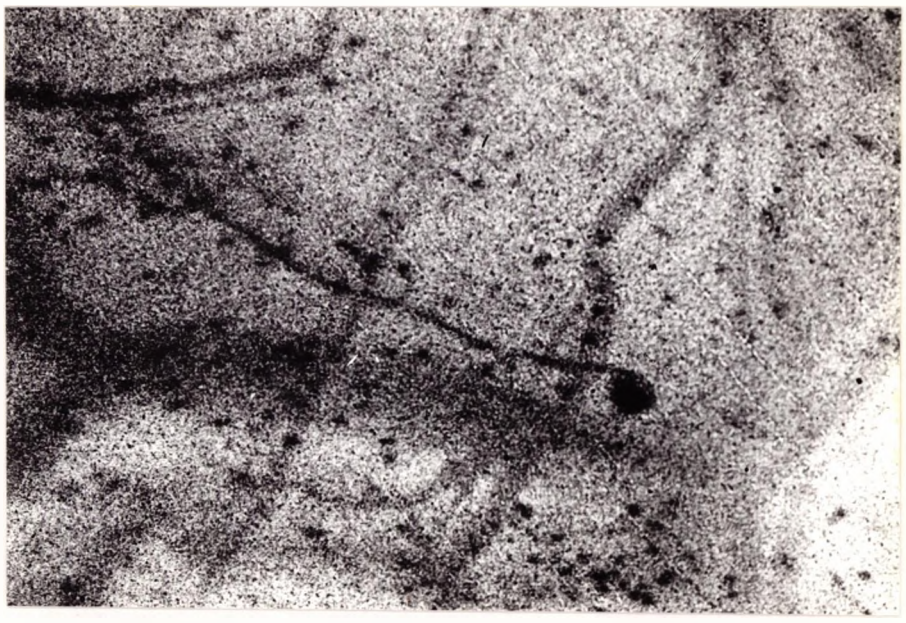


Figure 56. A higher powered view of a corpuscular nerve ending surrounded by scattered superficial punctate stain. (130X)

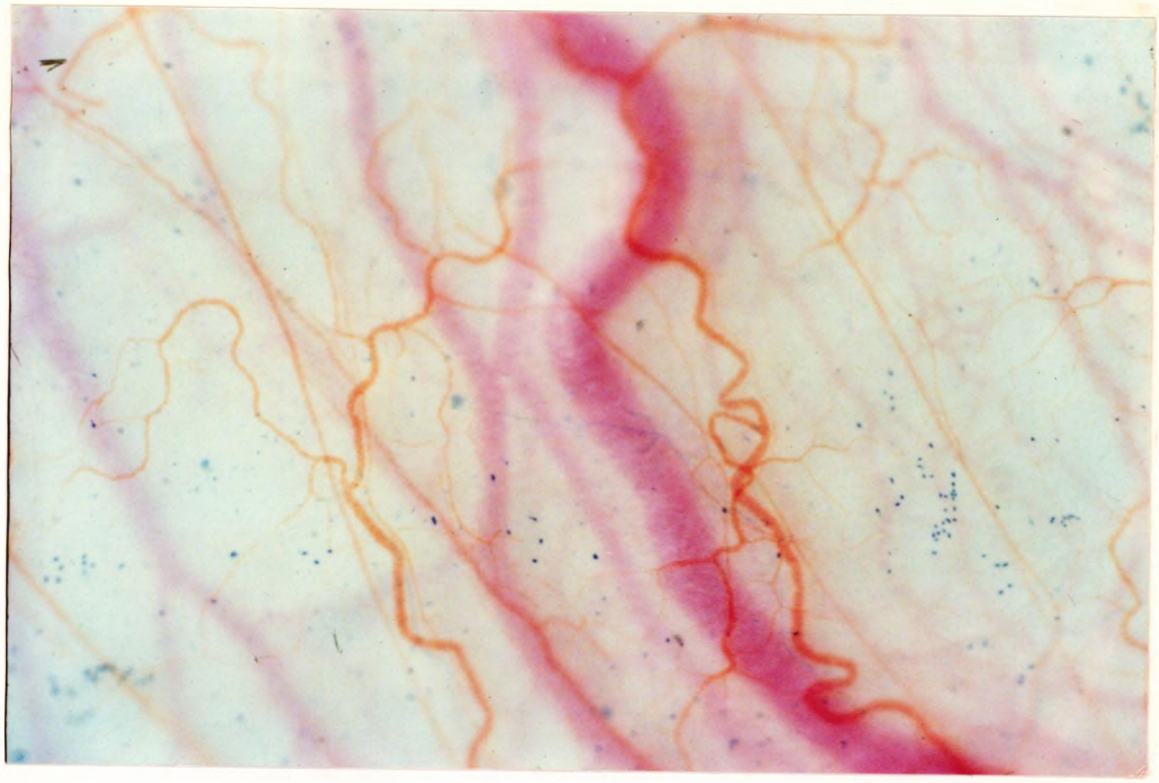


Figure 57. An axon is visible as a fine blue thread which courses around an episcleral vessel before terminating in a corpuscular ending. (40X)

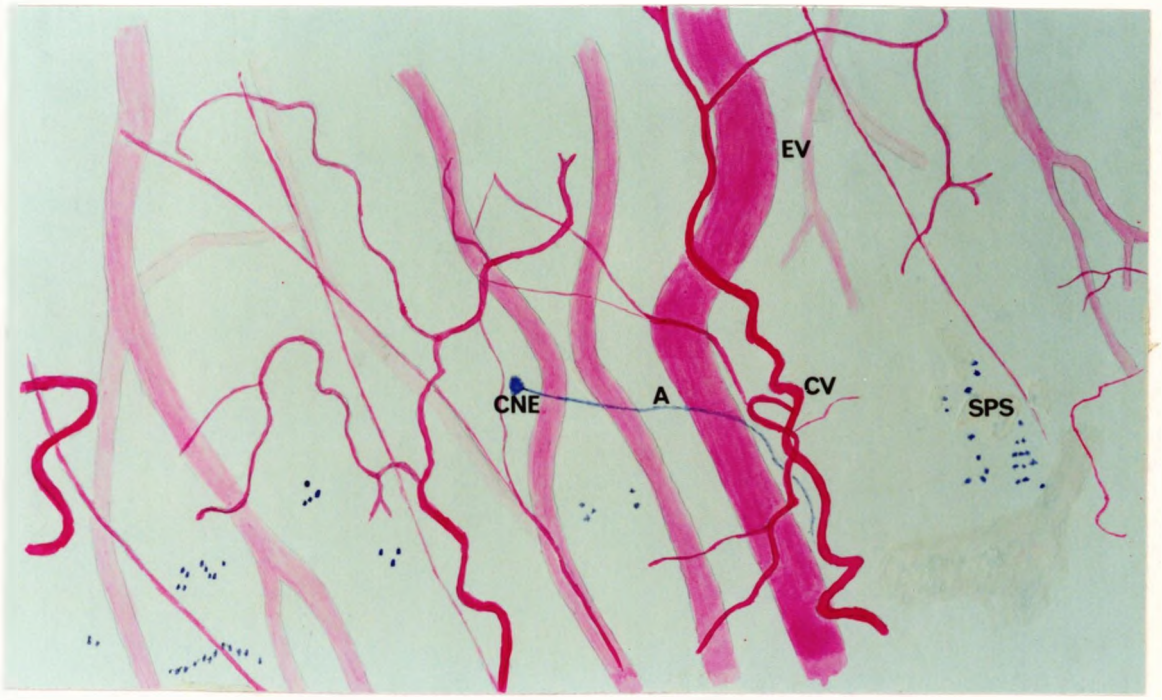


Figure 58. Key to figure 57. CNE= corpuscular nerve ending. SPS= superficial punctate stain EV= episcleral vessel. CV= conjunctival vessel. A= axon.

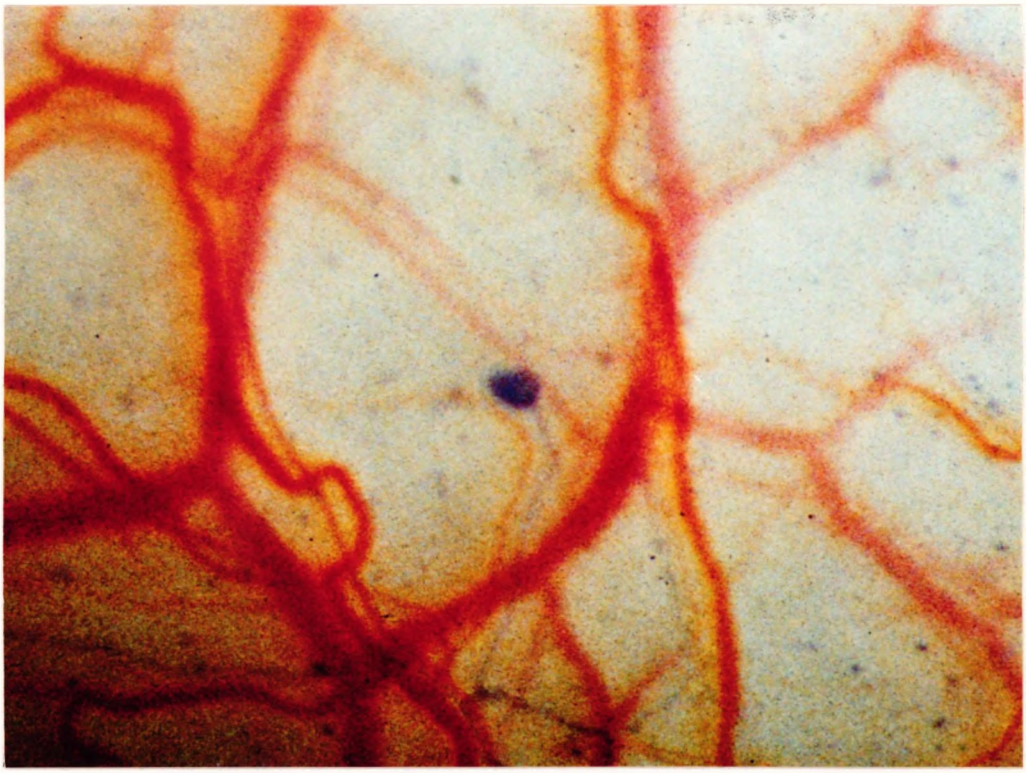


Figure 59. Another example of a stained corpuscle served by a single axon. (140X)

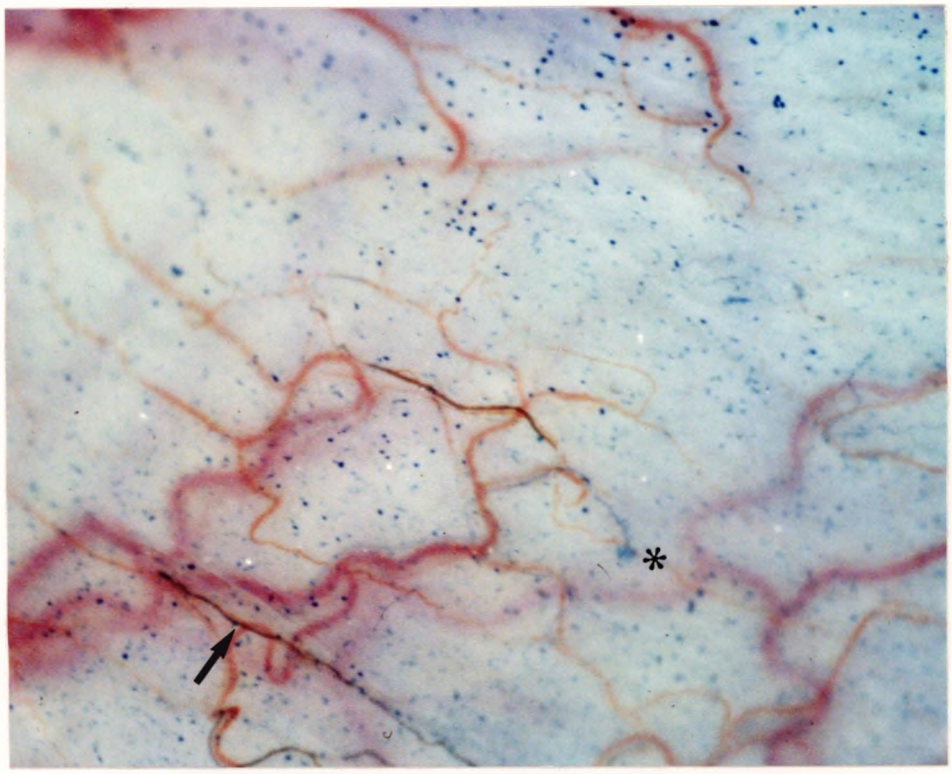


Figure 60. This example shows densely stained lymphatic vessels (arrowed). The asterisk marks a corpuscular nerve ending. (60X).

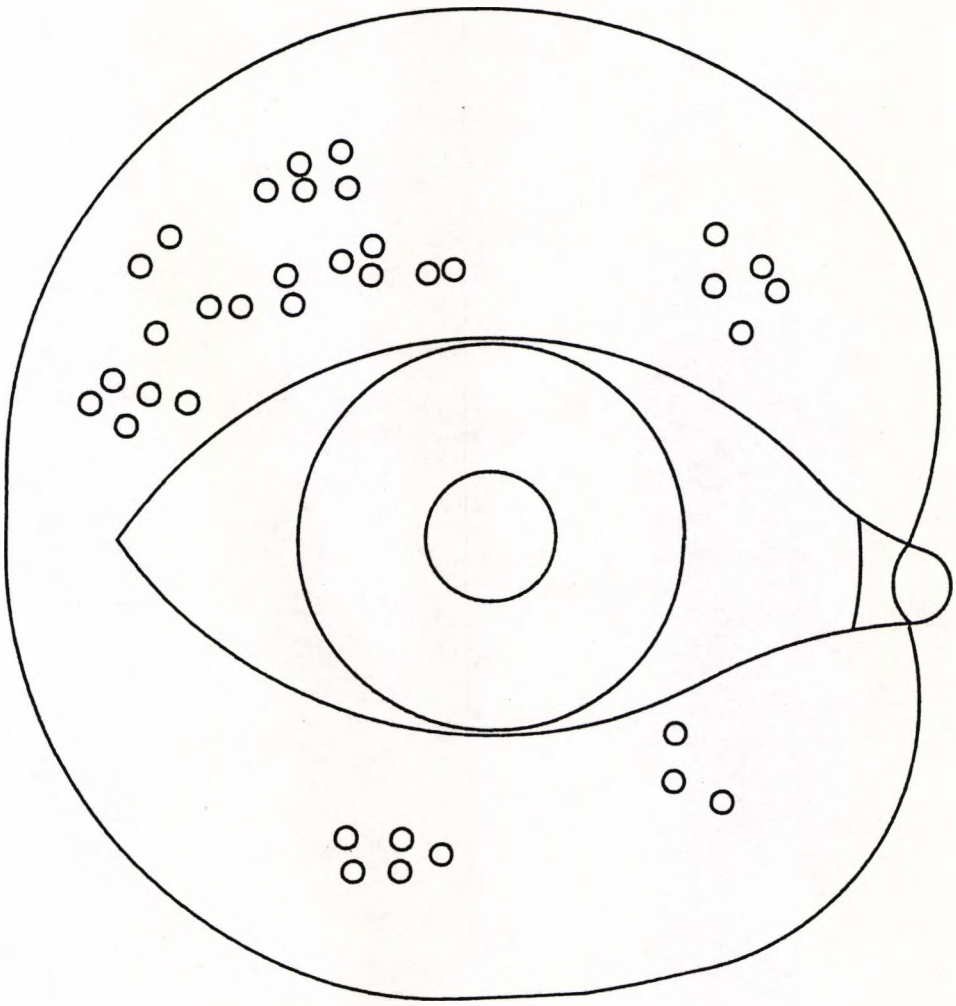


Figure 61. Distribution of methylene blue stained corpuscles (SD). (35 corpuscles stained).

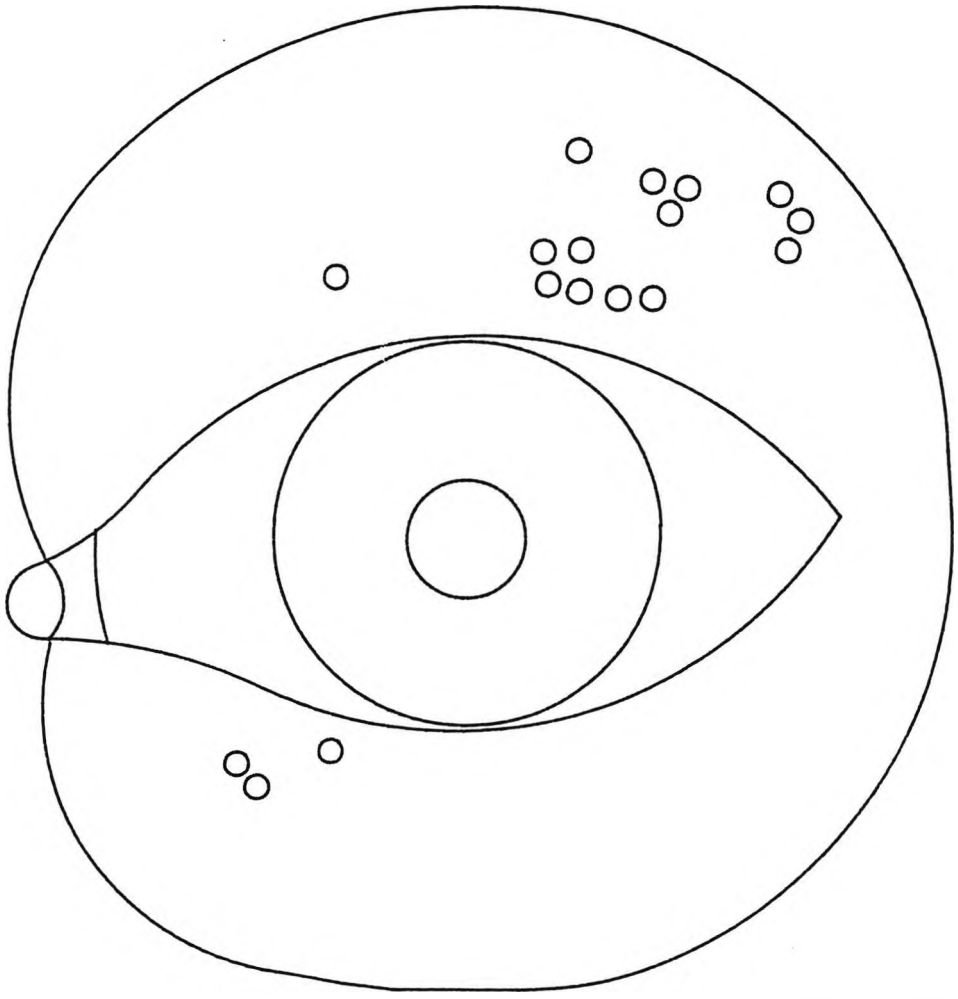


Figure 62.. Distribution of methylene blue stained corpuscles (AH). (17 corpuscles stained).

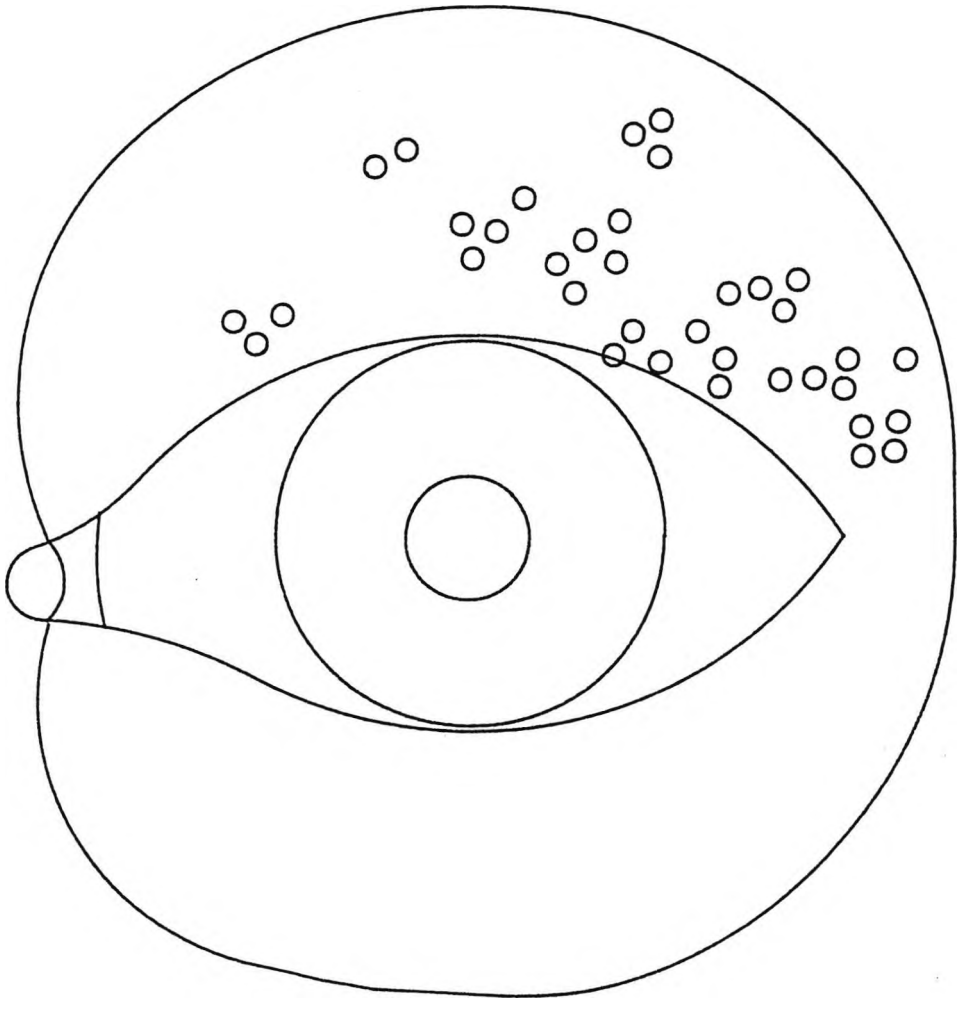


Figure 63. Distribution of methylene blue stained corpuscles (JL). (36 corpuscles stained).

CHAPTER 6.

RESULTS

LIMBAL CONJUNCTIVAL SENSITIVITY

6.1.Repeatability of Measurements.

Assessment of the repeatability of the method showed that although sensitivity values could not be accurately reproduced on a point to point basis, the repeatability of the median sensitivity for each zone was good. In 83% of cases the difference between the median sensitivities recorded on two separate occasions was either zero or 0.5 units. In the remainder the difference never exceeded 1.0 unit.

6.2. Statistical Analysis of Limbal Sensitivity

Because only three filament lengths were used, which were then converted onto an ordinal sensitivity scale, non-parametric statistical tests were utilised in the analysis (Seigel 1956), and the determination of medians rather than means was appropriate.

Results were analysed using the Microstat II statistical computer package (Ecosoft. USA. 1989).

For a single sample case paired measures were analysed using the Wilcoxon signed ranks test. Two independent samples were compared using the Mann-Whitney test. The Freidman two-way analysis of variance (ANOVA) was used when more than two related samples were tested, and the Kruskal-Wallis one way ANOVA for more than two independent samples.

6.3. Inter-zone Variation in Sensitivity in Non-lens Wearers.

Non-contact lens wearers (N=28) were used to determine the variation in limbal sensitivity with distance from the corneal margin. The median sensitivity was determined from the pooled sensitivity values from all subjects for zone 1 (ie.nearest to the cornea), and similarly for zones 2 and 3, and these were then plotted for each zone (Fig.64).

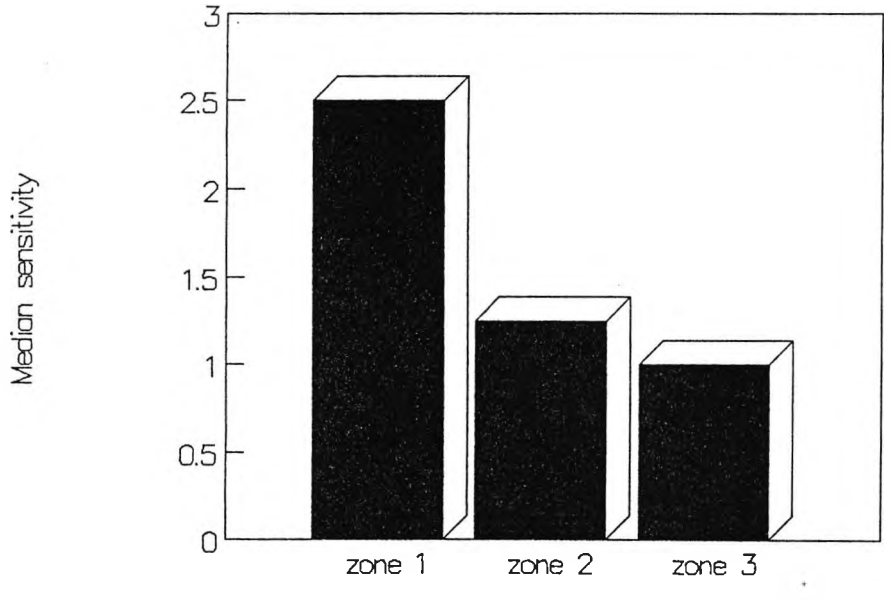


Figure 64. Graph showing the variation in median sensitivity between zones 1,2, and 3.(Non-lens wearers).

A comparison of the medians for all three zones shows a fall off in sensitivity with increasing distance from the cornea. A statistical analysis was performed using the Freidman test which indicated that the differences between zone 1 and zone 2, and also between zone 1 and zone 3 were significant ($p = <0.05$). However a comparison beteen zones 2 and 3 showed no significant difference.

6.4. Difference in median sensitivity between temporal and inferior limbal conjunctiva in non-lens wearers.

In order to test whether there was any significant difference between the sensitivity of the temporal and inferior limbus, the median of all the inferior limbal test points for each subject was subtracted from that of the equivalent temporal conjunctiva. These differences were then plotted for each subject (Fig.65).

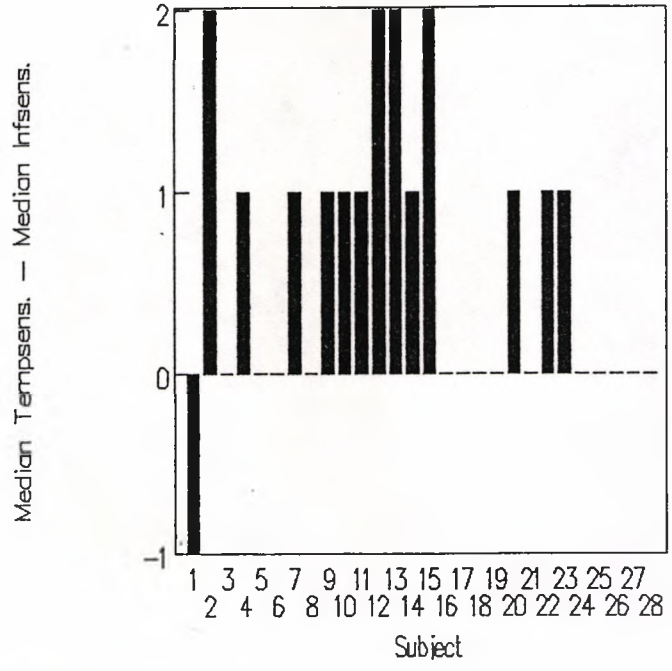


Figure 65. Graph showing the difference in median sensitivity between temporal and inferior conjunctiva plotted for each subject (non-lens wearers).

Only in one case did the sensitivity of the inferior limbus exceed the temporal. Applying the Wilcoxon signed ranks test demonstrated that the temporal limbus has a significantly greater sensitivity ($p = <0.001$).

6.5. Effects of Contact Lens Wear.

RGP lens wearers (N=12) and hydrogel wearers (N=10) were analysed independantly. The median sensitivity was calculated separately for zones 1, 2 and 3 for individual subjects, and an overall group median was determined for each zone. These were plotted against the equivalent values for a group of age matched non-contact lens wearers which acted as controls. The mean ages of both groups are given in the Table below:

	Mean age (years)	SD
RGP wearers.	34.3	10.6
Controls.	34.9	10.8
Hydrogel wearers.	33.0	11.3
Controls.	33.8	11.0

Table 4. Mean age and standard deviation of contact lens wearers compared with non-contact lens wearing controls.

A plot of the median sensitivity for zones 1, 2, and 3 for RGP wearers v's age matched controls (Fig. 66). appears to show a trend, such that the contact lens wearers have

a lower sensitivity, although when these individual differences when analysed using the Mann-Whitney test this was not statistically significant.

Fig. 67 indicates that there is no such trend for hydrogel wearers and the differences are similarly not significant.

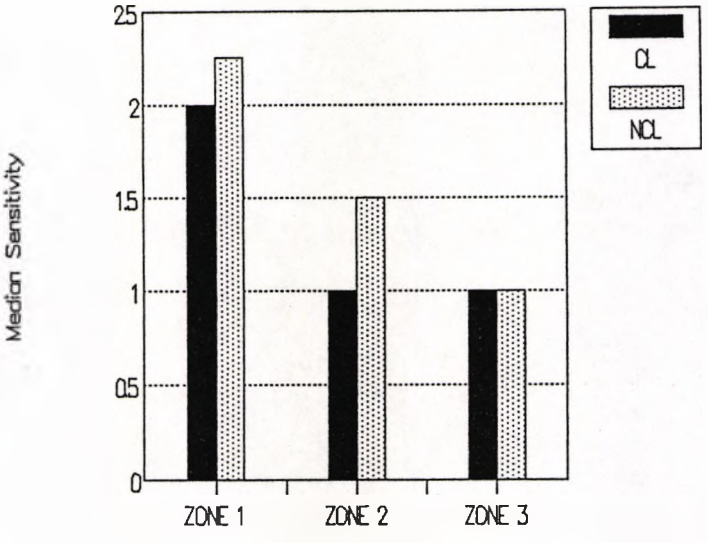


Figure 66. Graph of median sensitivity of RGP wearers for each zone plotted against age matched controls.

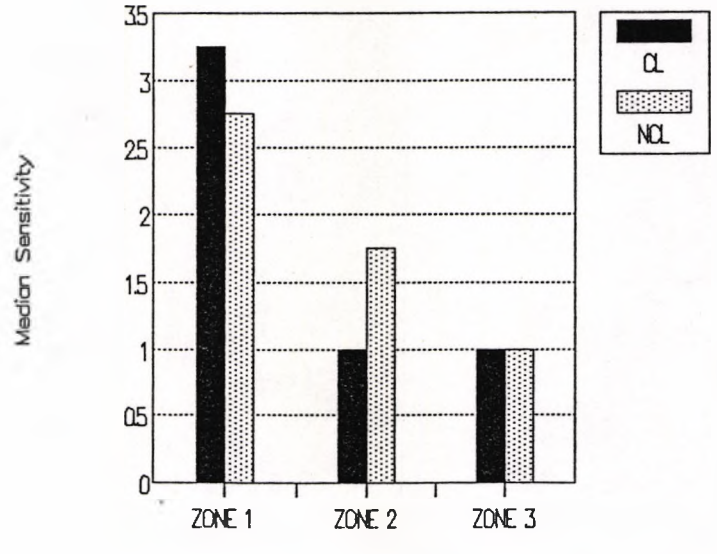


Figure 67. Graph of median sensitivity of hydrogel wearers for each zone plotted against age matched controls.

The influence of length of wear was investigated separately and found to be not significant (Kruskal Wallis 1-way ANOVA p= >0.05).

Because of the lack of a significant difference in sensitivity between contact lens wearers and non-lens wearers, it was felt valid to pool together all experimental groups for subsequent analyses.

6.6. Effects of Age.

The overall median sensitivity was plotted for all subjects (N=50) (including lens wearers and non-lens wearers) against subject age (Fig.68).

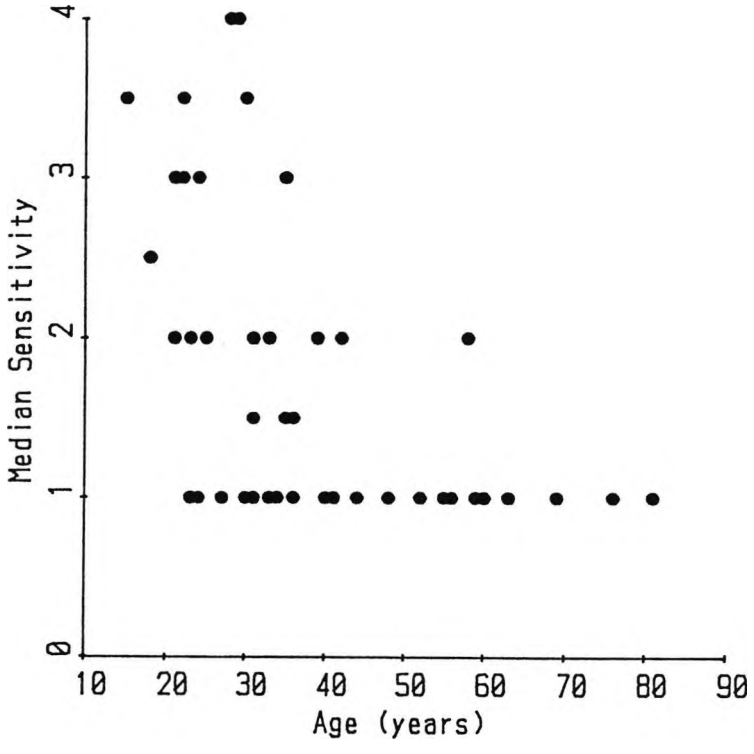


Figure 68. Graph showing overall sensitivity for all subjects plotted against age

The graph shows the large inter-subject variablity in limbal sensitivity, even for subjects falling within the same age decade. The overall trend however is a fall off in sensitivity with age.

The effect of age was analysed statistically by ranking subjects in order of increasing sensitivity. These assigned ranks were then grouped into 4 age profiles: 15-25, 25-35, 35-45, and >45yrs according to subject age, and the ranks were then averaged. Fig.69 shows a plot of this data.

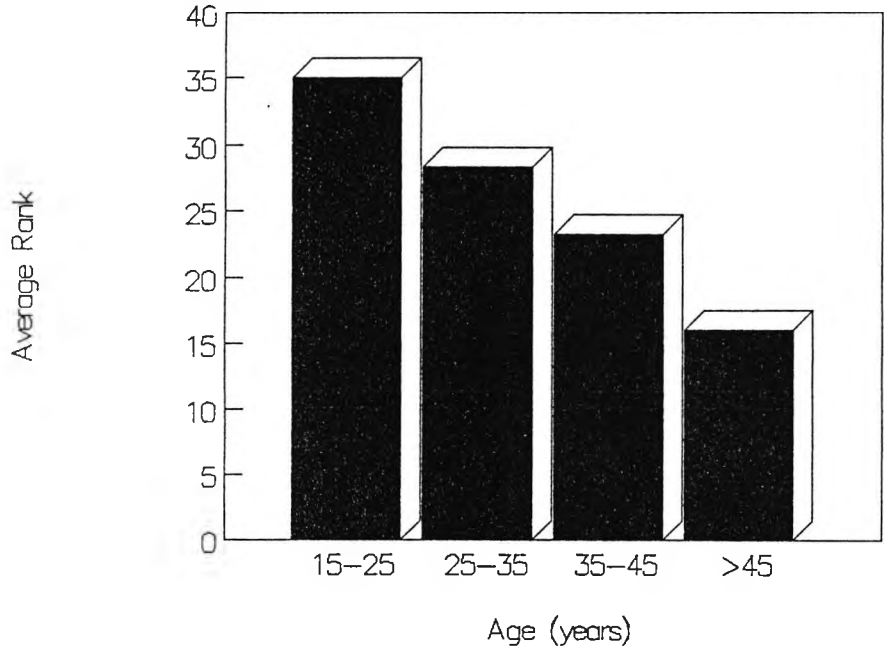


Figure 69. Effect of age on limbal sensitivity (ranked data).

Since a higher average rank reflects a subject group with a higher sensitivity the graph shows the decline in sensitivity with age. A Kruskal Wallis 1-way ANOVA indicated that all the individual group differences are significant ($p = <0.05$). The difference between the 15-25 age group and the >45 group has a higher level of significance ($p = <0.01$)

6.7. Effects of Iris Colour.

Iris colour was classified on a 1-5 arbitrary scale in order of increasing pigmentation (Seddon et al 1989).

In order to compensate for any bias in the grouped data due to age the subjects within each iris grouping were age matched as far as possible. This was difficult due to the limited numbers within certain groups. The mean ages for each group are given in the Table below:

	Number of Subjects	Mean Age (years)	SD
GRADE 1	13	31.4	6.4
GRADE 2	9	31.2	7.5
GRADE 3	5	24.6	7.1
GRADE 4	7	30.0	6.4
GRADE 5	2	26.5	8.5

Table 5. Mean age of subjects within each iris colour group.

Subjects (N= 36) were ranked in order of increasing median sensitivity and then grouped according to their particular iris grading. The ranks were then averaged and these were then plotted against iris grade (Fig.70). No overall trend is apparent with regard to iris colour and the Kruskal

Wallis test showed that these differences are not significant.

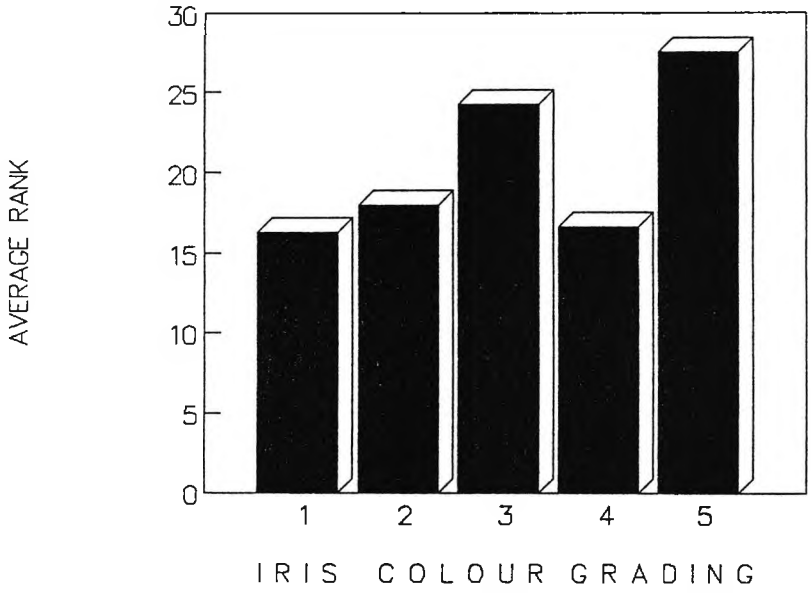


Figure 70 Graph showing variation in median sensitivity according to iris colour

CHAPTER 7.

DISCUSSION

The present study was designed to investigate aspects of nerve termination in the human conjunctiva. Particular emphasis was placed upon complex or corpuscular nerve endings, and was directed towards an understanding of their light and electron microscopic appearance, together with an investigation of their incidence and distribution throughout the conjunctiva. The limbal conjunctiva was noted to have a concentration of these terminals, and so this region was used in a complementary investigation which attempted to correlate an enhanced touch sensitivity to the position of these putative receptors.

The discussion will take place under three headings:

1. A reassessment of the structure, incidence, and distribution of conjunctival sensory nerve terminals in the context of the previous literature on the innervation of the conjunctiva.

2. A consideration of the ultrastructure of corpuscular nerve endings in the conjunctiva compared with complex sensory terminals throughout the body.

3. A discussion of the possible functional significance of sensory corpuscles in the human conjunctiva.

7.1. A Reassessment of the Structure and Distribution of Conjunctival Sensory Nerve Endings in the Context of Previous Literature on the Innervation of the Conjunctiva.

Much of our knowledge regarding the innervation of the conjunctiva derives largely from work carried out on monkeys (Macintosh 1974, Ruskell 1985, Oduntan 1989), and few authors refer specifically to man. The present study was able to confirm that in terms of the distribution and morphology of its nerves the human conjunctiva is similar to that described in monkeys. There are however some notable differences, and these relate to the mode of nerve termination.

Morphological studies detailing the sensory innervation of the monkey conjunctiva (Macintosh 1972, Ruskell 1985, Oduntan 1989) indicated that all sensory nerves terminated freely and without modification either in the stroma, where they frequently associated with blood vessels, or in the epithelium. No evidence was found for complex terminal forms. This is in sharp contrast to the findings reported here for the human eye which indicates that in addition to free nerve endings, specialised nerve endings of corpuscular form are widely distributed throughout the conjunctiva.

Free nerve endings were found within the walls of blood vessels, and throughout the stroma. They were especially concentrated close to the epithelium. Compared to monkeys

(Macintosh 1974, Oduntan 1989) the human conjunctiva contained fewer intra-epithelial terminals. These were infrequently observed, although when present were confined to the basal cell layers. Sampling for electron microsopy is not the technique of choice for quantifying free nerve endings because of the limited amount of tissue sampled. However, the scarcity of intra-epithelial free terminals was confirmed for the limbal conjunctiva using the gold chloride whole-mount technique.

Complex nerve endings were first described in the conjunctiva by Krause (1859). Krause believed that all nerves terminated in this way, but subsequent workers were able to demonstrate free nerve endings (Mauchle 1867, Ciaccio 1874). In order to study conjunctival innervation a variety of histological methods have been utilised, in particular whole-mount preparations with heavy metal stains eg. osmic acid, gold and silver chloride. In addition methylene blue has been commonly employed both in vitro and on the living eye. Using these methods several authors have described a variety of morphological forms of nerve ending which differ in size, shape, and degree of structural specialisation.

Most workers identified "complex" nerve endings in the conjunctiva as Krause corpuscles (endbulbs). These were typically round or oval encapsulated structures servedby 1-3 myelinated nerve fibres that lose their myelin upon entry. Despite several morphological studies a number of

equivocal points still persist with regard to their structure. These include, the presence and nature of a capsule, the organization of the interior of the corpuscle, and the existence of a secondary nerve supply. The present study addressed these points using the enhanced resolution gained by the electron microscope, which until now has not been applied to the study of these complex nerve endings in the conjunctiva.

Whether or not these structures are truly encapsulated has been debated over the years. Most workers were able to observe a distinct capsule, considered by some to be of perineural origin (Rouget 1868, Key and Retzius 1876), or alternatively consisting of connective tissue cells (Krause 1859, Ciaccio 1874, Dogiel 1891). In contrast however Oppenheimer and coworkers (1859) could not find any trace of a capsule in their preparations. The current study was able to confirm the presence of a distinct capsule. In sectioned material the capsule generally consisted of 1-2 layers of fibrocyte processes which appeared to form an incomplete investment. Certain corpuscles at the eyelid margin displayed a more prominent capsule, consisting of both fibrocytes and perineural cells. The significance of this distinction will be discussed later.

The observations of many of the early workers who employed a variety of neuron specific stains were often rather vague when it came to describing the internal organization of the corpuscle. This stemmed from the nature of the stain which

was either totally neuron specific and therefore failed to display non-neural components, or alternatively was less specific and thus stained both neural and non-neural cells resulting in a density of staining which precluded detailed observation. Although in most of these early accounts nuclei belonging to non-neural cells were often visible, they were considered to belong to capsular cells. The interior of the structure was thought to contain only the terminal axon embedded in a finely granular substance (Krause 1859, Ciaccio 1874, Longworth 1875). Other workers, however, were able to observe cells within the body of the structure which they considered to be distinct from those of the capsule (Rouget 1868, Poncet 1875, Key and Retzius 1876). With the electron microscope the ability to confirm the existence of these non-neural cells within the corpuscle has been possible. They were found to share many of the ultrastructural characteristics of Schwann cells, and could be differentiated from capsular cells by the presence of a basal lamina. The extensive network of cytoplasmic lamellae which derived from these Schwann-like cells showed a close relationship with the terminal axon in a similar manner to that seen in other corpuscular endings (vide infra).

With regard to the neural components, earlier studies generally agreed on the myelinated nature of the serving axon and its terminal course. Myelin is retained until soon after the point of entry of the axon which undergoes several divisions to form a dense entanglement. In some descriptions the myelinated part of the axon was shown to form a

coil-like arrangement on the surface of the corpuscle prior to entry (Krause 1859, Longworth 1875). This was a variant often encountered in the present study in sectioned bulbar conjunctiva. These corpuscles displayed multiple myelinated profiles around their circumference, although such profiles were occasionally observed deeper into the structure. Firm evidence of a secondary nerve supply as described originally by Lightbody (1867) and subsequently by Wolter (1964) was not obtained in the current investigation . Although fine calibre axons appeared occasionally to terminate close to the nerve ending in whole mount preparations, it was not possible with the electron microscope to decide whether or not these unmyelinated nerve fibres entered the corpuscle.

The presence of varicosities along the terminal ramifications of the axon, a consistent feature when viewed under the electron microscope, were not always apparent in many of the earlier morphological accounts, although in the drawings from gold and methylene blue preparations of Dogiel (1891) and Crevatin (1903) they can be clearly seen. Oppenheimer et al (1958) considered this beading to be a technical artifact and it was absent in their silver preparations.

Turning now to the question of the incidence of complex nerve endings in the human conjunctiva. This has been a controversial issue. The area of conjunctiva studied and the methods used often varied, and many of those who attempted to quantify corpuscular nerve endings obtained quite

different results. Longworth (1875) performed a complete analysis of a single eye by dividing the conjunctiva into 5 segments. In two segments no corpuscular endings were found and in the others between 30 and 60 were observed. Poncet (1875) recorded 5-6 per $40mm^2$ of bulbar conjunctiva. In contrast, Krause (1881) estimated a greater incidence for the whole conjunctiva of 2 per 5mm2 and found a considerable regional variation. The method of whole mount gold chloride impregnation, which was used in the present study, was found to be an unsatisfactory means of quantification. Non-specific staining, particularly of the epithelium, made observation difficult and careful attempts to remove the epithelium often resulted in trauma to the underlying tissues. Both these factors would tend to lead to an underestimate of the incidence of complex nerve endings. Potentially the in-vivo staining method with methylene blue represents a rapid non-invasive method of quantifying the total number of sensory corpuscles. This technique has been used by various workers (Knüsel and Vonwiller 1922, Oppenheimer and coworkers 1958, Riisager 1962). There was general agreement on the large inter-subject variation in the number of such nerve endings stained by this technique. Sometimes they were completely absent and in other subjects as many as 100 were observed. Furthermore, it was reported by Rissager (1962) that the number of complex nerve endings increased with age. The results of intra-vital staining obtained in the present study confirmed the inter-subject variation in the incidence of corpuscular nerve endings. Compared with histological methods it was found that this

technique appeared to considerably underestimate the number of corpuscles. It is worth considering possible reasons for this discrepancy. Staining with methylene blue was transient and often incomplete. This could relate to inadequate passage of the dye through to sub-epithelial tissues. It has been reported that penetration of the dye is enhanced by cocaine (Passmore and King 1955), which was used to obtain anaesthesia in earlier investigations. It is possible that the oxybuprocaine used in the current study did not have such a marked effect. Other factors include the existence of a non-specific background staining which meant that absolute terminal identity could only be made in the presence of the serving axon, and furthermore smaller corpuscles may have been missed at the levels of magnification available.

Although it is very time consuming, a histological method consisting of an interrupted serial sectioning procedure represents the most reliable method for quantification. The whole of the limbal conjunctiva from three eyes was treated in this way revealing 36, 53, and 122 corpuscles. In addition several samples of bulbar conjunctiva from 2 eyes, amounting to 61mm^2, contained 102 corpuscular endings. In view of the apparent inter-subject and regional variations in their incidence it is important to retain reservations regarding an extrapolation from a limited amount of tissue. However, the data from the histological study suggests that corpuscular nerve endings appear to be more numerous than previously documented.

The existence of a regional variation in the distribution of corpuscular nerve endings in the bulbar conjunctiva has been suggested by many authors . Based on histological (Ciaccio 1874, Poncet 1875) and intra-vital staining methods (Knüsel and Vonwiller 1922, Oppenheimer et al 1958, Rissager 1962) the superior temporal quadrant was found to contain a concentration of corpuscles. The results of methylene blue staining of the conjunctiva in the present study are in agreement with these observations. Generally few stained terminals were located within the inter-palpebral zone. Samples of bulbar conjunctiva for histology were taken from bulbar conjunctiva near the fornix and therefore these may not reflect the situation across the whole of the bulbar conjunctiva.

Many observers commented on the prevalence of corpuscular nerve endings at the corneo-conjunctival limbus (Key and Retzius 1876, Dogiel 1891, Crevatin 1903, Knüsel and Vonwiller 1922, Wolter 1962), and the current study was able to confirm these findings from histological observations which indicated that these nerve endings were concentrated in a 1mm wide annular zone of limbal conjunctiva located approximately 0.5mm from the termination of Bowman's membrane. Dogiel (1891) described complex nerve endings in the peripheral cornea. However, in the present study, in common with numerous other investigators, the cornea was found to contain only free nerve endings.

In view of the histological findings it was particularly surprising that the in-vivo methylene blue technique failed to stain corpuscular nerve endings in the limbal conjunctiva. This is in sharp contrast to the observations of Knüsel and Vonwiller (1922) who used the same technique and found numerous stained corpuscles at the limbus. It is difficult to explain this discrepancy. In the current study, although the limbus displayed a concentration of non-specific superficial staining, nerve fibres in this region had a poor affinity for the dye. Because of the consistent finding of corpuscular nerve endings at the limbus histologically, it seems unlikely that these particular corpuscles were absent in the subjects used for methylene blue staining. This is a further indication that methylene blue, when used intra-vitally fails to stain all corpuscles.

Few accounts exist of the innervation of the marginal palpebral conjunctiva (Dogiel 1894, Winkelman 1957, Munger and Halata 1984). The present study found that corpuscular nerve endings were common within a narrow marginal zone in the upper lid. An incidence equivalent to 17-18 corpuscles per mm of margin was counted. This represents the greatest concentration of all conjunctival locations studied so far. In view of these findings it was all the more unexpected to find no complex terminals in samples of marginal conjunctiva taken from the lower lid. However, this may relate to the limited amount of tissue available. Munger and

Halata (1984) commented that the small Meissner corpuscles located over the occlusal surface of the lids were relatively more common in the upper than the lower lid. More data are required to more fully quantify any differences.

7.2. A Consideration of the Structure and Distribution of Corpuscular Nerve Endings in the Conjunctiva Compared with Equivalent Complex Sensory Terminals throughout the Body.

Sensory nerves in the skin and mucosal surfaces of the body terminate in a variety of ways which range in structural complexity from the simplest, the "free nerve ending", to more complex forms including those belonging to the category "corpuscular nerve ending". The latter term,which has been adopted in this account for the complex nerve endings in the conjunctiva, refers to a family of sensory nerve endings which comprise one or more axon terminals together with non-neural accessory cells all enveloped by a capsule of varying thickness.

Sensory nerve endings have been classified in various ways. Classically the light microscopic observations of the early histologists gave rise to an eponymous classification which included a large number of complex sensory terminals. As early as 1905 Ruffini realised that such a classification, which recognised the slightest morphological variation, was an artificial one and that the number of supposedly distinct terminals far exeeded the number of known sensory modalities. Weddell and his colleagues in 1955 were of the opinion that this diversity in terminal morphology precluded any rigid classification. However modern histological techniques, particularly electron microscopy, when applied to sensory nerve terminals have indicated a greater degree of homogeneity.

The first electron microscopic studies of complex nerve endings were those of Pease and Quilliam (1957), and Cauna and Ross (1960) working on the Pacinian and Meissner corpuscle respectively. Following these initial observations many others have studied the fine structure of a variety of corpuscular receptors, and this literature is the subject of several reviews (Munger 1971, Andres and Von Düring 1973, Munger and Ide 1988).

On the basis of the ultrastructural appearance of sensory terminals several attempts have been made to to substitute a simpler classification scheme (Halata 1975, Chouchkov 1978, Malinovsky 1988).

Halata (1975) divided sensory nerve endings of mammalian skin into 3 subtypes.

Type 1 : includes terminals lacking a Schwann cell investment eg. epidermal free nerve endings, and the Merkel cell complex.

Type 2: are collectively referred to as the "bulboid endings of the dermis" and include " simple bulbous" nerve endings which consist of a single terminal expansion enveloped by Schwann cell processes , and the more complex " dendritic bulbous " nerve endings which contain multiple terminal expansions associated with 2 or more Schwann cell lamellae. The Meissner and lanceolate endings are included in the latter category.

Type 3: consist of encapsulated corpuscles where Schwann cell lamellae form a multi-layered concentric arrangement

arround terminal axons. The corpuscle is surrounded by a perineural capsule. The Pacinian corpuscle is considered a type 3 ending.

An alternative classification was proposed by Chouchkov (1978). In this scheme the main division is into encapsulated and non-encapsulated nerve endings. The latter include free nerve endings of the dermis and epidermis, the Merkel cell complex, and terminals associated with hairs. Encapsulated nerve endings are subdivided into three groups according to the presence and arrangement of Schwann cell lamellae within the interior of the terminal, which is referred to as the "inner core".

Group 1: lack a lamellated inner core eg. Ruffini corpuscle.

Group 2: possess an asymmetrical lamellated inner core eg Meissner corpuscle.

Group 3: include those which display a symmetrical lamellated pattern eg Pacinian corpuscle.

The most recent attempt at classifiying sensory formations was that of Malinovsky (1988). This scheme places less emphasis on encapsulation and concentrates on the arrangement of the Schwann cell lamellae in a similar manner to those previously outlined.

A summary of the classification of mucocutaneous sensory nerve endings is given in Figure 71.

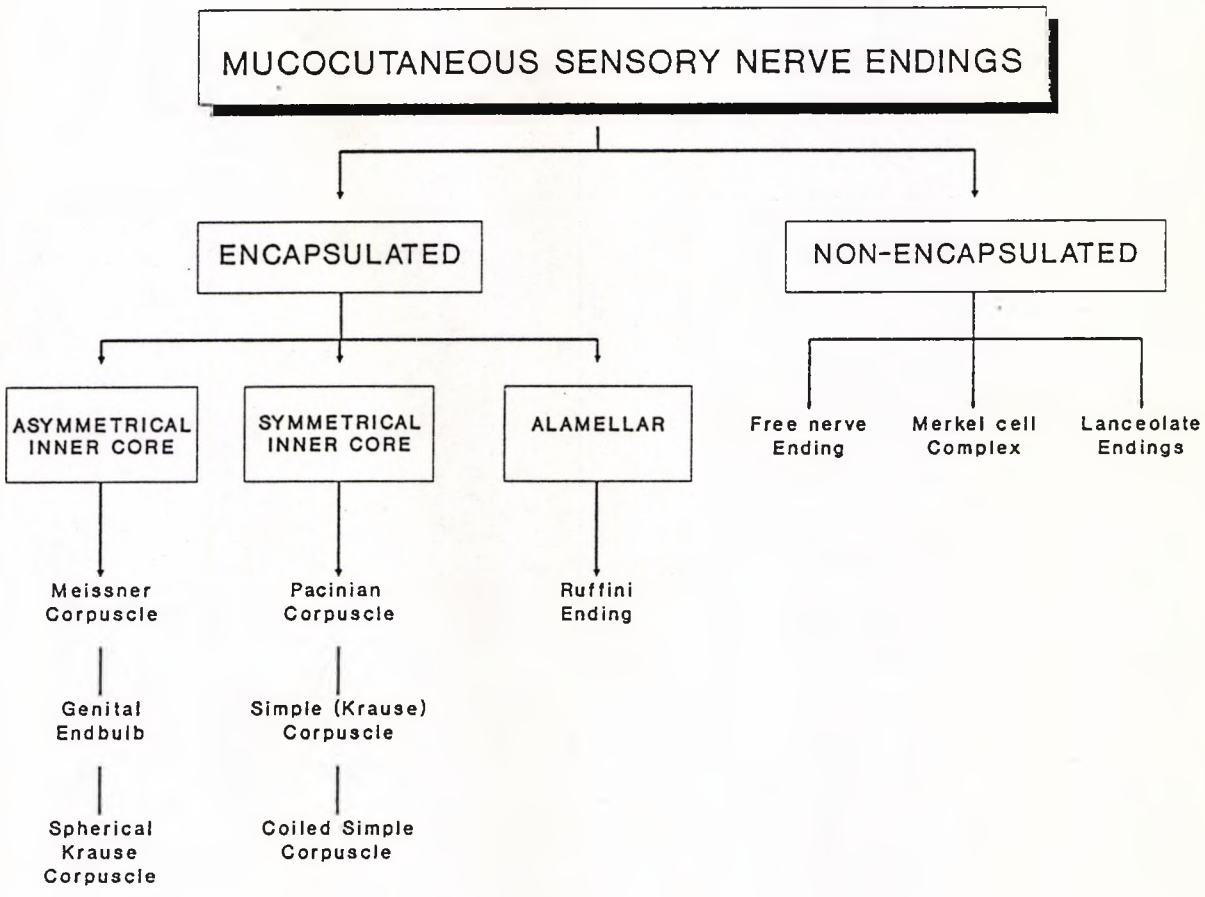


Figure. 71. Classification scheme for sensory nerve endings in the skin and mucosal sufaces of the body. Corpuscular nerve endings in the conjunctiva closely resemble the "spherical" Krause corpuscle.

Where do the corpuscular nerve endings observed within the human conjunctiva fit into these morphological classification schemes? Firstly, they are encapsulated, and although they often possess axon terminals surrounded by a concentric network of Schwann cell lamellae they lack an overall symmetry and therefore would be classified on Chouchkov's scheme as an "encapsulated receptor with an asymmetrical inner-core". Other receptors included within this category include: Meissner corpuscles, "spherical" Krause corpuscles, and the genital endbulb.

The term "Krause corpuscle (or endbulb)" has been widely used since its first description in the conjunctiva (Krause 1859,1881) to describe terminals in the skin, oral cavity, rectum (Chouchkov 1973), vocal cord (Nagai 1982), and pharynx (Schumaker et al 1970). This nomenclature can however be misleading and illustrates the problems associated with an eponymous classification. Krause originally described two distinct sub-types of corpuscular ending: "cylindrical" (simple) corpuscles, which were considered typical of lower mammals, and the more complex "spherical" corpuscles found in primates. In subsequent accounts however the prefix is often ommitted. The ultrastructure of the simple corpuscle is well documented for example in the lip, tongue, and nasal mucosa of the cat (Spassova 1971, 1973, 1974). These consist of a single straight terminal axon surrounded by concentrically arranged Schwann cell lamellae and a capsule of perineurium, resembling small Pacinian corpuscles. Corpuscles of this

type, sometimes with a variable degree of coiling of the terminal axon, have been more recently described in primates (Halata and Munger 1983, Halata and Munger 1986) where they have been termed "simple" and "coiled simple" corpuscles respectively.

The electron microscopic observations of the spherical Krause corpuscle in various human mucosa (Chouchkov 1973, Nagai 1982), closely resemble the description of corpuscular nerve endings in the conjunctiva. Axon terminal varicosities are surrounded by an asymmetrical arrangement of accessory cell lamellae, and the capsule consisting of 1-2 layers of fibrocytes. Junctional complexes, described as desmosome -like (Chouchkov 1973, Nagai 1982) are observed between axon terminals and enveloping lamellae. These are similar to the attachment plaques seen in conjunctival corpuscles, but junctions betweeen lamellae which were illustrated by these workers could not be found in the conjuctiva. Morphological variations observed in conjunctival nerve endings eg. densely stained axon terminals and multiple myelinated axon profiles were not recorded in these corpuscles.

Differentiation between the spherical Krause corpuscle, the Meissner corpuscle, and the genital endbulb is often difficult. It has been questioned whether a distinction can be made between the Krause and the genital endbulb (Munger and Ide 1988). The course of the terminal axon is similar, both forming a branching glomerular network, and there is a similar pattern of Schwann cell lamellae associated with the

axon (Polacek and Malinovsky 1971, Halata and Munger 1985). The Meissner corpuscle is usually distinguished in whole mount preparations by the terminal axon which alternately loops back and forth in the plane of the epithelium (Cauna 1960). In section the axon terminals typically present flattened profiles separated by parallel stacks of lamellae (Cauna and Ross 1960, Hashimoto 1973).

Although it is common for studies to emphasise differences between sensory corpuscles it should be remembered that in terms of their basic structural components: namely capsule, terminal axon , and accessory cells they share many common features.

The significance of a distinct capsule has been classically used to differentiate corpuscular nerve endings, and these are still often collectively referred to as encapsulated nerve endings. However more recent evidence has shown that encapsulation amoung otherwise morphologically similar endings is variable. Halata and Munger (1983) demonstrated that the capsule of both Meissner and "simple" corpuscles within the lip and mucosa of primates varied with position within the dermis such that deeper lying corpuscles possessed a more substantial capsule. Similarly, genital endbulbs in the human glans penis which immediately underly the epithelium had a scant fibrocyte capsule, whereas corpuscles located deeper in the stroma typically possessed a complete perineural capsule. Observation of the capsule of corpuscular endings in the conjunctiva indicated that in the

vast majority of cases a thin fibrocyte capsule was present. This may relate more to their superficial location. Certain corpuscles at the eyelid margin, in contrast, possessed a more substantial capsule comprising both fibrocytes and perineural cells, and it was generally found that these particular nerve endings lay deeper in the stroma. Variabiltity in the degree of encapsulation would tend to underplay its role in the receptive capacity of the terminal. The Meissner corpuscle, for which there is evidence of a mechanoreceptive function, is enveloped largely by fibrocytes and collagen fibres.

The morphology of the terminal axon generally shows little variation between corpuscles. These are characterised by an accumulation of mitochondria, with neurofilaments, neurotubules, and scattered vesicles. The occasional finding in this study of axon terminals which contain an electron dense axoplasm was therefore unexpected. It must be emphasised that the available eye-bank material was subject to a variable amount of delay prior to fixation and this may have influenced terminal morphology, however the observation of intact cell organelles would suggest that necrosis was not an important factor. The presence of varicosities along the terminal axon which are a consistent feature in the conjunctiva, have not attracted much attention in equivalent structures. However, it is possible to see evidence of varicosities in many illustrations.

Non-neural accessory cells form a homogeneous population between corpusclar nerve endings throughout the body. Their similarity to Schwann cells was first noted at the ultrastructural level by Cauna and Ross (1960), and a common lineage has been subsequently demonstrated by both developmental (Ide 1977) and histochemical studies. The latter include the identification of non-specific cholinesterase (Ide 1982) S-100 protein (Iwagaya et al 1982) and dipeptidylpeptidase IV (Dubovy 1988).

In summary therfore, the ultrastructural appearance of corpuscular nerve endings in the human conjunctiva places them into the category of complex endings referred to collectively as "encapsulated receptors with an asymmetrical inner core" (Chouchkov 1978). Within this group they most closely resemble the "spherical" Krause corpuscle as described by Chouchkov (1973), and Nagai (1982) in other tissues.

A Discussion of the Possible Functional Significance of Sensory Corpuscles within the Human Conjunctiva.

Before attempting to discuss the function of corpuscular nerve endings in the conjunctiva it is worthwhile examining how our current thinking on cutaneous sensibility has evolved.

Müller (1838) applied the concepts of "specific energy" and "specific irritability" to nerves. He postulated that there was an intrinsic difference between nerves subserving each of the 5 senses. This distinction was expressed in the term specific energy. Moreover, he maintained that the terminations of these nerves were in some way specialised to respond to a particular stimulus, that is, they possessed a specific irritability.

Blix (1884) determined the sensitivity of the skin to a variety of stimuli. He found an arrangement of "spots" which maximally responded to each stimulus. There were spots for warm which could be differentiated from those for cold, and in the same way sensory spots existed for touch and pain.

During this period there was an intense series of morphological investigations into the ways in which nerves terminate in skin and mucosa, and a variety of terminal forms were recognised which included free nerve endings, Meissner, Pacinian, Krause, and Ruffini corpuscles. Von Frey

(1884) combined physiological and anatomical data and suggested that under each sensory spot in the skin lay a distinct terminal form which was solely responsible for a particular sensation. Thus Meissner corpuscles were thought to subserve the sensation of touch, Krause endings cold, Ruffini endings warm, and free nerve endings pain. Although strong evidence to support these assertions was lacking Von Frey's theory was almost universally accepted and its influence within neurology persisted for many years.

An alternative theory was proposed following evidence that structurally specialised nerve endings were not necessary for cutaneous sensibility (Sinclair, Weddell and Zander 1952, Lele and Weddell 1956). This so called "pattern" theory, which denied the existence of specificity, maintained that sensations were differentiated by central processing of nerve fibre discharge which varied both temporally and spatially.

The modern concept combines something of both theories. There has been a return to the classical notion of specificity although this is rarely absolute: mechanoreceptors are selectively tuned to respond to a mechanical stimulus, thermoreceptors to temperature, and nociceptors to damaging stimuli. Less is known about central processing of the complex information arising from these receptors, however it seems likely that the final perception of a stimulus is the result of substantial central influence. Studies of conjunctival neurology in the past

have interpreted their data in the light of the prevalent theory of cutaneous sensibility. For example Strughold and Karbe (1925a, 1925b, 1925c), who were very much part of the Von Frey school, attempted to correlate the Krause corpuscle with the sensation of cold. This involved determining the position of cold spots within the conjunctiva and relating these to the position of Krause corpuscles as defined by the in-vivo methylene blue technique. The limitation of these experiments was that the stimulus was not purely thermal and incorporated a significant mechanical component. The investigations of Strughold and Karbe represent isolated studies which have not been reproduced since, either for the conjunctiva or for other areas known to contain Krause corpuscles, and therefore these results need to be interpreted with caution.

Oppenheimer and coworkers (1958) used combined histological and intra-vital staining methods to study conjunctival innervation. Their observations of complex nerve endings led them to conclude that rather than representing specific sensory receptors these structures were formed as part of a cycle of the growth and decay of the terminals of peripheral nerves. Evidence in support of this conclusion came from the reported heterogeneity in form of complex nerve endings together with their variable incidence and irregular distribution. Data on the number and distribution of these nerve endings were derived largely from the results of in-vivo staining which is claimed here to give substantial underestimates of the total number.

Furthermore, ultrastructural observations reported here have revealed that corpuscular endings in the conjunctiva show a high degree of specialisation, and although a variety in form is suggested at light microscopic level, a common ultrastructural organisation is maintained. Furthermore, if these complex nerve endings were formed as a result of some degenerative process then this would have been apparent with the electron microscope. Although axon terminals within corpuscles occasionally showed a dense background staining, there was no evidence of degeneration particularly affecting the nerve ending. These observations are contrary to such an arbitrary dismissal of their functional significance. If the assumption is made that they are acting as sensory receptors, then what particular stimulus are they detecting? Obvious possibilities include thermoreception or mechanoreception.

The thermal sensitivity of the anterior segment has been investigated using a variety of techniques (Kenshalo 1960, Beuerman et al 1977, Beuerman and Tanelian 1979). There is general agreement that the conjunctiva, in contrast to the cornea, possesses the ability to discriminate a warm or a cool stimulus. Stimulation of the cornea only produce a sensation which is perceived as irritation.

Attempts have been made to determine the neural structure involved in thermoreception. Hensel et al (1974) identified a complex receptor in the nose of the cat under a spot which displayed a marked cold sensitivity. However these have not

been found in any other species and current opinion maintains that free nerve endings are responsible for the transduction of thermal stimuli. Although the question of corpuscular thermoreceptors remains a possibility, it is generally acknowledge that their most likely function is in mechanoreception.

Studies of conjunctival touch sensitivity (Boberg-Ans 1955, Norn 1973, Draeger 1984, Winter et al 1986) have suggested large regional variations. Draeger (1984) measured touch thresholds in a small conjunctival area and found up to an 850 fold variation. He postulated that specific touch receptors underlay points of high sensitivity.

In order to prove an exclusive association between a particular sensation and a receptor, the requirement must be met that the receptor be always present when the sensation is present, and be always absent when the receptor is absent. For corpuscular nerve endings this has never been shown to be the case. Furthermore, hairy skin, which does not possess complex nerve endings, is still capable of registering a full range of sensory modalities. It is therefore important to think of complex nerve endings as an adjunct to other receptors rather than being exclusively responsible for a particular sensory modality. This could include the enhancement of a particular sensation or adding a particular quality to the sensation. At this point it is worth summarising the evidence for the role of these receptors in cutaneous sensibility?

The introduction of the microneurographic technique by Vallbo and Hagbarth (1968) provided an important tool which has furthered our understanding of the somatosensory system. This method enables the recording of single nerve fibre activity in awake human subjects. Microelectrodes are inserted percutaneously into a peripheral nerve and the response of the nerve to a variety of stimuli is recorded together with the subjective experience of the resulting sensation. Using this technique 4 types of mechanoreceptive afferent nerve have been identified which serve the glabrous skin of the human hand. These can be distinguished by their receptive field properties and their adaptation to sustained indentation (Vallbo et al 1979, Johansson and Vallbo 1982) and have been designated RA (rapidly adapting), PC (Pacinian corpuscle), SAI (slowly adapting I) and SAII (slowly adapting II) units. RA and PC units are rapidly adapting, that is they respond to the onset and removal of the stimulus, whereas SAI and SAII adapt slowly and respond with a sustained discharge. In terms of their receptive fields RA and SAI units have small fields with sharp borders whereas PC and SAII display large fields with obscure borders. Based on these properties RA units have been indirectly linked with Meissner corpuscles, SAI with the Merkel cell complex, PC with Pacinian corpuscles, and Ruffini endings with SAII

With regard to the other corpuscular endings very little data exists. Iggo and Ogawa (1977) identified receptive

fields of RA units in the cat. The most sensitive parts of the field were determined and marked with tungsten wires. When the tissue was subsequently examined histologically (cylindrical) Krause corpuscles were identified as the receptor underlying many of these points.

Returning now to the conjunctiva. The decision to investigate touch sensitivity, as opposed to any other modality, was primarily based on the ultrastuctural resemblance of corpuscular nerve endings in the conjunctiva to putatively identified RA mechanoreceptors in other tissues eg. the Meissner corpuscle. Touch sensitivity was measured with a slit-lamp mounted Cochet-Bonnet aesthesiometer which allows precise control of the stimulus application. The limbal conjunctiva was chosen as the preferred site for this investigation because corpuscular nerve endings were consistently found in large numbers within a narrow annular zone of limbal conjunctiva corresponding approximately to the extent of the palisades of Vogt. A systematic investigation of touch thresholds within a 1.5mm pericorneal zone indicated a threshold for the palisade zone which was significantly lower than the adjacent conjunctiva. Furthermore, these findings are contrary to the generally accepted view that there is a rapid fall off in sensitivity immediately on leaving the cornea. As with other sensory functions an overall reduction in limbal sensitivity with age was observed, although there were marked individual differences for subjects of similar age. It is tempting to correlate this variation with

the apparent variation in the incidence of corpuscular endings obtained from the histological study. It is possible to also speculate that the large regional variations of bulbar conjunctival touch sensitivity reported by Draeger (1984) and Winter et al (1986) may be related to the incidence of sensory corpuscles.

A statistically sigificant difference was found between the inferior and temporal limbus. This could not be substantiated in terms of the distribution of complex endings from the limited number of eyes studied. However, it is interesting that both Draeger (1984) and Winter et al (1986) found the temporal bulbar conjunctiva adjacent to the limbus to be the most sensitive conjunctival region.

The effect of contact lens wear on limbal touch sensitivity was tested for subjects wearing both rigid gas permeable and hydrogel lenses. No statistically significant differences were found between lens wearers and age matched controls for either lens type. This is not a surprising finding in view of the large inter-subject differences in sensitivity which would mask any contact lens effect. This is worthy of further study since differences in limbal sensitivity may be important in determining successful contact lens adaptation.

The results for the distribution of these putative receptors at the lid margin yielded some surprising results. They were found in large numbers within several samples of marginal conjunctiva taken from two upper lids, however two small

segments of lower lid displayed no complex nerve endings. Insufficient data is available to draw any definite conclusions. Studies which have investigated lid margin touch sensitivity indicate that the upper lid has the greater sensitivity although differences found to be small (Norn 1973, Bleshoy 1990). This area warrants further study both in terms of quantifying differences between the two lids in terms of their innervation, together with a systematic investigation of the sensitivity of both lid margins.

The method of aesthesiometry employing the Cochet-Bonnet instrument is limited in the sense that it only represents one particular form of mechanical stimulus, which causes a transient indentation of the surface of the eye. It is possible that the putative receptors in the conjunctiva respond maximally to sustained indentation, or to a stimulus moving across the conjunctival surface. Future work, could therefore investigate the response to these different forms of mechanical stimulation.

CHAPTER 8.

SUMMARY AND CONCLUSIONS.

The objectives of this study were to investigate the morphology, distribution and possible functional significance of corpuscular nerve endings in the human conjunctiva. The existence of these structures has been known since the middle of the last century although investigators often disagreed as to their precise structural organization, and furthermore opinion differed as to their possible role in the conjunctiva. Although the ultrastructure of similar nerve endings in the skin and mucosa has been well documented, the present study is the first to investigate the electron microscopic appearance of complex nerve endings in the conjunctiva.

Corpuscular nerve endings in the conjuctiva are encapsulated and are typically served by a single myelinated nerve fibre which sheds its myelin sheath upon passing into the corpuscle. It then branches several times to form a series of axon terminal varicosities which are characterised by an accumulation of mitochondria. These neural elements are surrounded by several layers of cytoplasmic lamellae which derived from non-neural Schwann-like cells. At the ultrastructural level these complex nerve endings most closely resemble the spherical Krause corpuscle which has been described in the human skin and various mucosae.
Various methods were used to examine the incidence and

distribution of these nerve endings including: semi-serial sectioning for light microscopy, whole-mount gold chloride impregnation, and in-vivo staining of the human conjunctiva with methylene blue. It was felt that the semi-serial technique, although labour intensive, gave the most reliable estimate of their incidence. The whole of the limbal conjunctiva from three eyes was examined although it was impractical to study further areas other than by sampling. The reliance of earlier workers on the in-vivo methylene blue technique may have led to a considerable underestimate of their number.

Corpuscular nerve endings were found in large numbers at the corneo-conjunctival limbus, concentrated within a narrow annular zone of conjunctiva approximately 1mm wide and located 0.5mm from the termination of Bowman's membrane. Many were observed within the connective tissue elevations which form the palisades of Vogt. From a limited amount of bulbar conjunctiva corpuscular endings were found to be locally abundant with an incidence of approximately 2 per mm^2. An examination of the eyelid margin showed that in the upper lid complex nerve endings were present in large numbers, although surprisingly in the small amount of tissue examined were absent from the lower lid margin.

Previous investigators have proposed various functions for complex nerve endings in the conjunctiva, including mechanoreception, or thermoreception. Other workers have suggested that they correspond to stages in the degeneration

and regeneration of peripheral nerve terminals. The present study found no evidence to substantiate this latter claim and the high degree of structural specialisation which was apparent at the electron microscopic level is suggestive of functional potential. It was felt that their most likely role is in mechanoreception, as structurally similar nerve endings in other tissues have been putatively identified as mechanoreceptors.

A study of the touch sensitivity of the limbal conjunctiva indicated that sensitivity was greatest in the palisade zone which was interesting in view of the histological data. Large intersubject variations existed in limbal sensitivity, and there was a significant fall off in sensitivity with age. It was found that the temporal limbus had a greater sensitivity than the inferior limbus, but from the few eyes studied this could not be correlated with the distribution of sensory corpuscles. The effect of contact lens wear on limbal touch sensitivity indicated no significant difference between groups of wearers when compared to age matched non-wearers. However, in view of the large inter-subject variation in sensitivity any effect could easily have been lost, and future work would aim to examine individual variations in sensitivity pre- and post wear.

In conclusion, if corpuscular nerve endings are acting as exteroreceptors then a concentration of specific receptors at the limbus so close to the highly sensitive cornea would seem surprising. However, if their particular responsiveness

demanded aggregation together with a compact form then there would be no place for them in the cornea in the interests of vision. Since the maintenance of corneal integrity is of the utmost importance, a mechanism which could signal a potentially damaging stimulus would be conveniently sited within the pericorneal zone. However, the demonstration of a wider distribution of corpuscular nerve endings throughout the bulbar and marginal conjunctiva may indicate a role in monitoring eyelid/conjunctival relationships and in the maintenance of a close apposition between the eyelid margin and ocular surface which is essential for the spreading of the tear film.

Corpuscular nerve endings are not uniformly distributed around the limbus and similarly bulbar corpuscles display local aggregation. Conjunctival sensitivity data appears not to substantiate such a point related function for these putative receptors, and therefore their particular role may be independent of specific location.

APPENDIX A

Details of histological specimens.

Limbal conjunctiva.

SPECIMEN REF.	AGE	SEX	RACE	FIXATIVE
HW22	12	F	C	FORMALIN
HL2	31	M	N	GLUT/PARA
HL4	69	F	C	GLUT/PARA
HL5	67	?	?	UNFIXED
HL6	64	?	?	UNFIXED
HL8	61	?	?	UNFIXED

Table 6. Anterior eye segments used to study limbal conjunctival innervation.

Bulbar and marginal palpebral conjunctiva.

SPECIMEN REF.	AGE	SEX	RACE	FIXATIVE
HW19	35	F	C	FORMALIN
HG9	72	F	C	GLUT/PARA
HG16	71	M	C	FORMALIN

Table 7. Details of bulbar and marginal palpebral specimens.

APPENDIX B.

Subject details for intra-vital staining with methylene blue.

SUBJECT	AGE	SEX	RACE
CC	21	M	C
SD	22	M	A
AH	31	F	C
JGL	31	M	C
TS	36	F	C
GLR	60	M	C

Table 8. Subjects used for methylene blue staining.

APPENDIX C.

Subject details for limbal conjunctival touch sensitivity.

SUBJECT	AGE	SEX	IRIS	MEDIAN SENSITIVITY					
				Z1	Z2	Z3	T	I	O
CW	15	F	3	4	3.5	2.5	3	4	3.5
CD	21	F	2	3.5	2.5	2	4	2	2
BS	21	F	3	4	2.5	1	3	3	3
SD	23	M	4	4	2.5	1	3	2	2
AR	24	F	3	3	1	1	1	1	1
NG	24	F	1	4	3	2	3	3	3
EA	28	F	1	4	3.5	3	4	3	4
RB	29	M	3	4	4	2.5	4	4	4
DE	30	M	2	4	3.5	2	4	3	3.5
AL	30	F	3	2.5	1	1	1	1	1
JL	31	M	1	3	1.5	1	2	1	1
CW	31	F	4	4	2	1	3	1	1.5
MT	34	F	1	2.5	1	1	3	1	1
CA	35	F	3	2.5	1.5	1.5	2	1	1.5

Table 9. Non-contact lens wearers.

Z1= zone 1, Z2= zone 2, Z3= zone 3, T= temporal, I= inferior, O= overall.

SUBJECT	AGE	SEX	IRIS	MEDIAN SENSITIVITY					
				Z1	Z2	Z3	T	I	O
EC	39	F	1	4	1.5	1.5	4	2	2
MT	40	M	2	1	1	1	1	1	1
PB	42	M	1	3.5	2	2	2	2	2
RH	48	M	2	1	1	1	1	1	1
AP	52	F	1	1	1	1	1	1	1
EH	55	F	1	2	1	1	2	1	1
AG	56	M	1	1.5	1	1	1	1	1
AE	58	M	1	1.5	1.5	1.5	2	1	2
GR	60	M	2	2	1	1	2	1	1
GD	60	F	2	1	1	1	1	1	1
PC	63	F	1	1.5	1	1	1	1	1
MP	69	M	1	1	1	1	1	1	1
RW	76	F	1	1	1	1	1	1	1
WN	81	M	2	1	1	1	1	1	1

Table 9 (Cont). Non-contact lens wearers.

SUBJECT	AGE	SEX	YEARS OF WEAR	IRIS	MEDIAN SENSITIVITY					
					Z1	Z2	Z3	T	I	O
ML	21	F	10	3	3.5	3.5	2	4	3	3
AR	22	F	7	3	4	3.5	2	3	4	3.5
AT	23	F	0.5	1	1.5	1	1	1	1	1
AK	25	F	7	4	3	2	1.5	2	2	2
JJ	31	F	18	1	3	2	1.5	2	2	2
AZ	33	M	6	4	2	1.5	1	2	1	1
TS	36	F	20	1	2	2	1.5	2	2	2
DW	36	M	6	4	2.5	1	1	1	1	1
MS	40	M	10	4	1	1	1	1	1	1
PM	41	M	9	2	1	1	1	1	1	1
MN	44	F	8	1	1.5	1	1	1	1	1
JM	59	F	16	1	1	1	1	1	1	1

Table 10. Contact lens wearers (Rigid Gas Permeable).

SUBJECT	AGE	SEX	YEARS OF WEAR	IRIS	MEDIAN SENSITIVITY					
					Z1	Z2	Z3	T	I	O
DN	18	F	11	5	4	2	2	3	2	2.5
PG	22	F	0.5	4	3.5	3.5	2	4	1	3
AT	23	F	4	1	1.5	1	1	2	1	1
JA	27	F	10	2	2	1	1	1	1	1
WS	31	F	13	2	4	1	1	1	1	1
CW	33	F	4	1	4	2	1	2	1	2
HO	35	F	3	5	4	2.5	2	3	2	3
CR	41	F	11	1	2.5	1	1	1	1	1
JP	41	F	11	2	3	1	1	1	1	1
MS	60	F	6	1	1	1	1	1	1	1

Table 11. Contact lens wearers (Hydrogel).

APPENDIX D.

Calibration of the Cochet-Bonnet aesthesiometer.

Diameter of filament = 0.137mm

FILAMENT LENGTH (cm)	MEAN BALANCE READING (mg)
6	23.6 (+/- 2.4)
5	31.4 (+/- 3.1)
4	40.8 (+/- 5.5)
3	67.5 (+/- 11.9)
2	168.4 (+/- 30.8)
1	845.6 (+/- 79.4)

Table 12 Milligrams of force measured on a laboratory balance for each filament length.

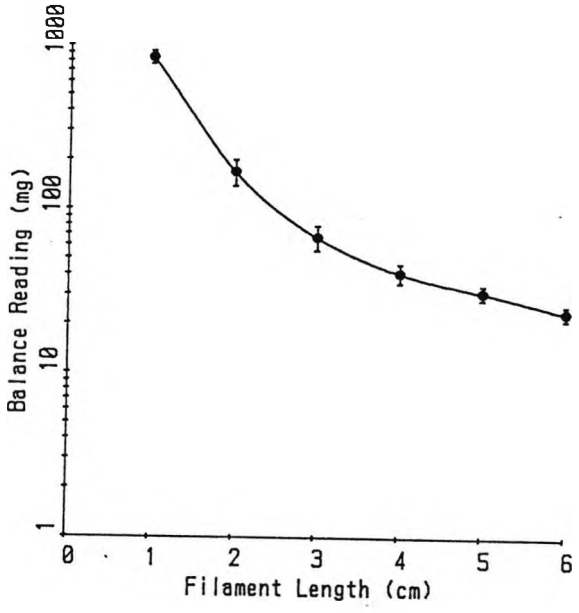


Figure 72. Calibration curve for the Cochet-Bonnet aesthesiometer

REFERENCES

ANDRES, K.H. DÜRING, M.von. (1973)
Morphology of cutaneous receptors.In:Handbook of Sensory Physiology. Vol.11. Somatosensory System. Ed. A.Iggo. Springer, New York. 3-28

ARNOLD, J. (1862)
Über die Endigung der Nerven in der Bindehaut des Augapfels und die Krause'schen Endkolben.
Arch.path.Anat. 24. 250-286

BELMONTE, C. GIRALDEZ, F. (1981)
Responses of cat corneal sensory receptors to mechanical and thermal stimulation.
J.Physiol. 198. 355-368

BEUERMAN, R.W.,MAURICE,D.M., TANELIAN,D.L. (1977)
Thermal stimulation of the cornea. In:Pain in the Trigeminal Region. Eds. D. Anderson, and B. Matthews. Elsevier, Amsterdam. 413-427

BEUERMAN,R.W., TANELIAN, D.L. (1979)
Corneal pain evoked by thermal stimulation.
Pain. 7. 1-14

BLESHOY, H. (1986)
Corneal sensitivity in keratoconus.
Trans.BCLA. 9-12

BLESHOY,H. (1990)
Neuropathology and Sensitivity in the Keratoconic Cornea.
PhD Thesis. City University. London.

BLIX, M. (1884)
Experimentelle Beiträge zur Lösung der Frage über die specifische Energie der Hautnerven.
Z.Biol. 20. 141-156

BOBERG-ANS, J. (1955)
Experience in clinical examination of corneal sensitivity.
Br.J.Ophthal. 39. 705-726

BONNET, R., MILLODOT, M. (1966)
Corneal aesthesiometry. Its measurement in the dark.
Am. J. Optom.Arch.Am.Acad.Optom. 43. 238-243

BURNSTOCK, G. (1986)
The changing face of autonomic neurotransmission.
Acta.Physiol.Scand. 126. 67-91

BUTLER,J.M.,RUSKELL,G.L., COLE,D.F., UNGER,W.G.,ZHANG,S.Q., BLANK,M.A., McGREGOR,G.P.,BLOOM,S.R. (1984)
Effects of VII (facial) nerve degeneration on vasoactive intestinal polypeptide and substance P levels in ocular and orbital tissues of the rabbit.
Exp.Eye Res. 39. 523-532

CAUNA, N. (1960)
Nerve supply and nerve endings in Meissner's corpuscles.
Am.J.Anat. 99. 315-350

CAUNA,N.,ROSS,L.L. (1960)
The fine structure of Meissner's touch corpuscles of human fingers.
J.Biophys.Biochem.Cytol. 8. 467-482

CHOUCHKOV,C. (1973)
On the fine structure of Krause's end bulbs in human skin, oral cavity, and rectum.
Arch.Histol.Jap. 35. 364-374

CHOUCHKOV, C. (1978)
Cutaneous receptors.
Adv.Anat.Embryol.Cell Biol. 54. 1-62

CIACCIO, G.V. (1874)
Osservazioni intorno alla struttura della congiuntiva umana.
Mem.Accad.Sci.Bologna.3rd Ser. 4. 469-524

COCHET, P., BONNET, R. (1960)
L'esthesie corneenne.
Clin.Ophthal. 4. 2-27

COLLINS, M.,SEETO, R.,CAMPBELL,L.,ROSS, M. (1989)
Blinking and corneal sensitivity.
Acta Ophthalmol. 67. 525-531

CREVATIN, F. (1903)
Le terminazioni nervose nel corio della congiuntiva.
Mem.Accad.Sci.Bologna 5th Ser. 10. 409-436

DAVANGER, M., EVENSEN, A. (1971)
Role of the pericorneal papillary structure in renewal of corneal epithelium.
Nature. 229. 560-561

DIXON, J. (1964)
Ocular changes due to contact lenses.
Am.J.Ophthalmol. 58. 424-379

DOGIEL, A.S. (1891)
Die Nervendkörperchen (Endkolben W.Krause) in der Cornea und Conjunctiva Bulbi des Menschen.
Arch.mikr.Anat. 37. 602-619

DOGIEL, A.S. (1894)
Die Nervenendigungen im Lidrande und in der Conjunctiva palpebr. des Menschen.
Arch.mikr.Anat. 44. 15-25

DRAEGER, J. (1984)
Corneal Sensitivity:Measurement and Clinical Importance.
Springer Verlag. Vienna.

DUBOVY, P. (1988)
Histochemical evidence of dipeptidylpeptidase activity in the Schwann cells surrounding unmyelinated portions of axons. In: Mechanoreceptors. Development,Structure and Function. Eds.P.Hnik, T.Soukup, R.Vejsada, and J.Zelena. Plenum Press. New York. 307-308

DUKE-ELDER,S.,WYBAR,K.C. (1961)
System of Ophthalmology. Vol II. The Anatomy of the Visual System.
Henry Kimpton. London.

EHINGER, B. (1971)
A comparative study of the adrenergic nerves to the anterior eye segment of some primates.
Z.Zellforsch.mikroscop.Anat. 116. 157-197

FREY, M. von. (1894)
Beiträge zur Physiologie des Schmerzsinns.
Ber.sächs.Ges.Wiss.math-phys.Cl. 46. 185-196

FREY, M. von. (1895)
Beiträge zur Sinnesphysiologie der Haut.
Ber.sachs.Ges.Wiss.math-phys.Cl. 47. 166-184

FREY, M. von., STRUGHOLD, H. (1926)
Weitere Untersuchungen über das Verhalten von Hornhaut und Bindehaut des menschlichen Auges gegen Berührungsreize.
Z.Biol. 84. 321-334

GIRALDEZ, F., GEIJO, E., BELMONTE, C. (1979)
Response characteristics of corneal sensory fibres to mechanical and thermal stimulation.
Brain Res. 177. 571-576

HALATA, Z. (1975)
The mechanoreceptors of the mammalian skin: ultrastructure and morphological classification.
Adv.Anat.Embryol.Cell Biol. 50. 1-77

HALATA, Z., MUNGER, B.L. (1983)
The sensory innervation of primate facial skin.II.Vermilion border and mucosa of the lip.
Brain Res.Rev. 5. 81-107

HALATA, Z., MUNGER, B.L. (1986)
The neuroanatomical basis for the protopathic sensibility of the human glans penis.
Brain Res. 371. 205-230

HASHIMOTO, K. (1973)
Fine structure of the Meissner corpuscle of human palmar skin.
J.Invest.Dermatol. 60. 20-28

HENSEL, H., ANDRES, K.H., DÜRING M. von. (1974)
Structure and function of cold receptors
Pflugers Arch. 352. 1-10

IDE,C. (1977)
Development of the Meissner corpuscle of mouse toe pad.
Anat.Rec. 188. 49-67

IDE, C. (1982)
Histochemical study of lamellar cell development of the Meissner corpuscle.
Arch.histol.jap. 45. 83-97

IGGO, A.,OGAWA,H. (1977)
Correlative physiological and morphological studies of rapidly adapting mechanoreceptors in cat's glabrous skin.
J.Phvsiol. 266. 275-296

IWANAGA, T., FUJITA, T., TAKAHASHI, Y., NAKAJIMA, T. (1982)
Meissner's and Pacinian corpuscles as studied by immunohistochemistry for S-100 protein, neuron-specific enolase, and neurofilament protein.
Neurosci.Lett. 31. 117-121

JOHANSSON, R.S., VALLBO, Å.B. (1982)
Tactile sensory coding in the glabrous skin of the human hand.
TINS. 6. 27-32

KENSHALO, D.R. (1960)
Comparison of the thermal sensitivity of the forehead, lip, conjunctiva and cornea.
J.appl.Physiol. 15. 987-991

KESSING, S.V. (1968)
Mucous gland system of the conjunctiva.
Acta Ophalmol (Suppl). 95. 1-126

KEY, A. RETZIUS, G, (1876)
Studien in der Anatomie des Nerven systems und des Bindegewebes..
P.A. Norstedt & Soner,Stockholm. P211-220

KNÜSEL, O.,VONWILLER, P. (1922)
Vitale Färbungen am menschlichen.
Z.Augenheilk. 49. 157-188

KRAUSE, W. (1859)
Über Nervenendigungen.
Z.ration.Med.3rd Ser. 5. 28-43

KRAUSE, W. (1881)
Die Nervenendigung innerhalb der terminalen Körperchen.
Arch.mikr.Anat. 19. 53-136

LANDIS, S.C., JACKSON, P.C., FREDIEU, J.R.,THIBAULT, J. (1987)
Catecholaminergic properties of cholinergic neurones and synapses in adult rat ciliary ganglion.
J.Neurosci. 7. 3574-3587

LELE, P.P.,WEDDELL, G. (1956)
The relationship between neurohistology and corneal sensitivity.
Brain. 79. 119-154

LIGHTBODY, W.H. (1867)
Observations on the comparative microscopic anatomy of the cornea of vertebrates.
J.Anat. 1. 15-43

LONGWORTH, L.R. (1875)
Über die Endkolben der Conjunctiva.
Arch.mikr.Anat. 11. 653-660

LOWTHER, G.E. HILL, R.M. (1968)
Sensitivity of the lower lid margin in the course of adaption to contact lenses.
Am.J.Optom. 45. 587-594

LUHTALA, J., PALKAMA A., UUSITALO, H. (1991)
Distribution and origin of substance P, CGRP, and neuropeptide Y, immunoreactive nerves in the rat conjunctiva.
Invest.Ophthalmol.Vis.Sci. 32. 1285

MACINTOSH, S.R. (1974)
The innervation of the conjunctiva in monkeys.
Graefes Arch.Ophthalmol. 192. 105-116

MALINOVSKY, L. (1988)
What is a sensory corpuscle? In: Mechanoreceptors. Development, Structure, and Function. Eds.R. Hnik, T.Soukup, R. Vejsada, J.Zelena. Plenum Press,New York. 283-286

MAUCHLE,T. (1867)
Die Nervenendigungen in der Conjunctiva bulbi.
Arch.path.Anat. 41. 48-55

MILLER,A.S., COSTER, D.J.,COSTA, M.,FURNESS, J.B. (1983)
Vasoactive intestinal polypeptide immunoreactive nerve fibres in the human eye.
Aust.J.Ophthalmol. 11. 185-193

MILLODOT, M. (1969)
Studies on the sensitivity of the cornea.
The Optician. 157. 267-271

MILLODOT, M. (1972a)
Diurnal variation of corneal sensitivity.
Br.J.Ophthal. 56. 844-847

MILLODOT, M. (1972b)
The influence of pregnancy on the sensitivity of the cornea.
Br.J.Ophthal. 61. 46-49

MILLODOT, M. (1974)
Effect of soft lenses on corneal sensitivity.
Acta Ophthalmol. 52. 603-608

MILLODOT, M. (1975a)
Do blue eyed people have more sensitive corneas than brown eyed people?
Nature. 255.151

MILLODOT, M. (1975b)
Effect of hard contact lenses on corneal sensitivity and thickness.
Acta Ophthalmol. 53. 576-574

MILLODOT, M. (1976)
Effect of length of wear of contact lenses on corneal sensitivity.
Acta Ophthalmol. 54. 721-730

MILLODOT ,M. (1977)
The influence of age on the sensitivity of the cornea.
Invest.Ophthalmol. 16. 240-242

MILLODOT, M. (1978)
Effect of long term wear of hard contact lenses on corneal sensitivity.
Arch. Ophthal. 96. 1225-1227

MILLODOT, M.,LAMONT, A. (1974)
Influence of menstruation on corneal sensitivity.
Br.J.Ophthal. 58. 752-756

MILLODOT, M.,O'LEARY, D. (1979)
Loss of corneal sensitivity with lid closure in humans.
Exp.Eye.Res. 29. 117-121

MILLODOT, M.,O'LEARY, D.J. (1980)
Loss of corneal sensitivity with lid closure in humans.
Acta Ophthalmol. 58. 434-439

MÜLLER, J. (1838)
Handbuch der Physiologie des Menschen.
Coblenz. (Cited by Sinclair 1955).

MUNGER, B.L. (1971)
Patterns of organization of peripheral sensory receptors.
In:Handbook of Sensory Physiology. Vol. 1.Principles of Receptor Physiology. Ed. W.R.Lowenstein. Springer. Berlin. 523-556

MUNGER, B.L.,HALATA, Z. (1984)
The sensorineural apparatus of the human eyelid.
Am.J.Anat. 170. 181-204

MUNGER,B.L.,IDE,C. (1988)
The structure and function of cutaneous sensory receptors.
Arch.Histol.Cytol. 51. 1-34

NAGAI, T. (1982)
Encapsulated sensory corpuscle in the mucosa of human vocal cord:an electron microscopic study.
Arch.histol.jap. 45. 145-153

NILSSON,S.F.E., BILL, A. (1984)
Vasoactive intestinal polypeptide (VIP) effects in the eye and on regional blood flows.
Acta Physiol.Scand. 121. 385-392

NORN, M.S. (1973)
Conjunctival sensitivity in normal eyes.
Acta Ophthalmol. 51. 58-66

ODUNTAN, A.O. (1989)
Aspects of the Structure and Innervation of the Primate Conjunctiva and its Response to Denervation.
PhD thesis. City University. London.

OPPENHEIMER,D.R., PALMER, E., WEDDELL,G. (1958)
Nerve endings in the conjunctiva.
J.Anat. 92. 321-352

PACINI, F. (1840)
Nuovi organi scoperti nel corpo humano.
Pistoja. Tipgratia Cino. (Cited by Sinclair 1955).

PASSMORE, J.W., KING, J.H. (1955)
Vital staining of the conjunctiva and cornea.
Arch.Ophthal. 53.568-574

PEASE, D.C., QUILLIAM, T.A. (1957)
Electron microscopy of the Pacinian corpuscle.
J.Biophys.Biochem.Cytol. 3. 331-342

POLACEK,P., MALINOVSKY L. (1971)
Die Ultrastruktur der Genitalkörperchen in der Clitoris.
Z.mikr.anat.Forsch. 84. 293-310

PONCET, F. (1875)
Recherches critiques et histologiques sur la terminaison des nerfs dans la conjonctive.
Arch.physiol.norm.path. 2. 545-561

POZO, M.A., GALLAR,J.,BELMONTE,C. (1990)
Thermosensitive afferents encoding temperature changes in the anterior segment of the cats' eye.
Invest.Ophthalmol.Vis.Sci. 31.169.

RIISAGER, P.M. (1962)
A clinical study of the numerical variation with age of compact nerve endings in the human conjunctiva.
J.Neurol.Neurosurg.Psychiat. 25.134-136

RISS, B.,RISS, P. (1981)
Corneal sensitivity in pregnancy.
Ophthalmologica. 183.57-62

RISS, N., BINDER, S.,RISS,P.,KEMETER,P. (1982)
Corneal sensitivity during the menstrual cycle.
Br.J.Ophthalmol. 66.123-126

RIVAS,L., OROZA, M.A., PEREZ-ESTEBAN, A., MURUBE-DEL-CASTILLO, J. (1991)
Topographical distribution of ocular surface cells by the use of impression cytology.
Acta Ophthalmol. 69.371-376

ROUGET,C.H. (1868)
Mémoire sur les corpuscles nerveux qui se rencontrent a l'origine des nerfs sensitifs dans les papilles de la peau et des muqueuses.
Arch.physiol.norm.path. 1.591-609.

RUFFINI, A. (1905)
Les dispositifs anatomiques de la sensibilité cutanée sur les expansions nerveuses de la peau chez l'homme et quelques autres mammifères.
Rev.gen.Hist. 1. 421-538

RUSKELL,G.L. (1965)
The orbital distribution of the sphenopalatine ganglion in the rabbit. In:The Structure of the Eye. Vol. II. Ed. J.W.Rohen. F.K. Schattauer-Verlag.Stuttgart. 355-368

RUSKELL, G.L. (1970)
An ocular parasympathetic pathway of facial nerve origin and its influence on intraocular pressure.
Exp.Eye Res. 10. 319-330

RUSKELL, G.L. (1985)
Innervation of the conjunctiva.
Trans.Ophthalmol.Soc.U.K. 104. 390-395

RUSKELL, G.L. (1991)
The Conjunctiva. In: Clinical Contact Lens Practice.Eds E.S.Bennett and B.A. Weissman. J.B. Lippincott Company. Philadelphia. 1-18

RUSKELL,G.L.,SIMONS,T. (1990)
Distribution of scleral sensory nerves to the cornea and adjacent structures.
Ophthal.Physiol.Opt. 10. 409

SCHIMMELPFENNIG, B. (1982)
Nerve structures in the human central corneal epithelium.
Graefes Arch.klin.exp.Ophthalmol. 218. 14-20

SCHIRMER,K.E. (1963)
Corneal sensitivity and contact lenses.
Br.J.Ophthalmol. 47. 493-495

SCHUMACHER,G.H.,GRUBER,D.,STACH,W.,KLUGER,S. (1970)
Zur Innervation der Mundhöle und des Sclundes.
I.Mitteilung:Korpuskuläre Nervendigungen beim Menschen.
Dtsch.Zahn.Mund.Kieferheilkd. 54. 324-334

SEDDEN,J.M.,SAHAGIAN,C.R.,GLYNN,R.J.,SPERDUTO,R.D.,GRAGOUDAS,E.S (1990)
Evaluation of an iris color classification system.
Invest.Ophthalmol.Vis.Sci. 31. 1592-1598

SEIGEL, S. (1956)
Non-parametric Statistics for the Behavioral Sciences.
Mc Graw-Hill Book Co. New York.

SINCLAIR,D.C. (1955)
Cutaneous sensation and the doctrine of specific energy.
Brain. 78. 584-614

SINCLAIR,D.C., WEDDELL,G.,ZANDER,E. (1952)
The relationship of cutaneous sensibility to neurohistology in the human pinna.
J.Anat. 86. 402-411

SPASSOVA,I. (1971)
Ultrastructure of the encapsulated nerve endings in the lips of the cat.
J.Submicr.Cytol. 3. 339-352

SPASSOVA,I. (1973)
Ultrastructure of Krause endbulbs in the nasal skin of the cat.
Acta Anat. 84. 224-236

SPASSOVA, I. (1974)
Ultrastructure of the simple encapsulated nerve endings (simple endbulbs of Krause) in the tongue of the cat.
J.Anat. 118. 1-9

STONE,R.A. (1986)
Neuropeptide Y and the innervation of the human eye.
Exp.Eye Res. 42. 349-355

STONE,R.A.,KUWAYAMA,Y. (1985)
Substance P-like immunoreactive nerves in the human eye.
Arch.Ophthalmol. 103. 1207-1211

STONE,R.A.,KUWAYAMA,Y.,LATIES,A.M. (1987)
Regulatory peptides in the eye.
Experimentia. 43. 791-800

STONE,R.A.,McGLINN,A.M. (1988)
Calcitonin gene-related peptide immunoreactive nerves in the human and rhesus monkey eyes.
Invest.Ophthalmol.Vis.Sci. 29. 305-310

STRUGHOLD,H. (1925)
Die Schwellen des Kaltesinnes am Auge
Z.Biol. 83. 201-206

STRUGHOLD,H., KARBE, M. (1925a)
Die Topographie des Kaltesinnes auf Cornea und Conjunctiva.
Z.Biol. 83. 189-200

STRUGHOLD,H.,KARBE,M. (1925b)
Die Dichte der Kaltpunkte im Lidspaltenbereiche des Auges.
Z.Biol. 83. 207-212

STRUGHOLD,H., KARBE,M. (1925c)
Vitale Färbung des Auges und experimentelle Untersuchung der gefärbten Nervenelemente.
Z.Biol. 83. 297-308

SUCHARD,E. (1884)
Recherches sur la structure des corpuscles nerveux terminaux de la conjonctive et des organes génitaux.
Arch.Physiol. 4. 337-347

TEN TUSSCHER,M.P.M.,KLOUSTER,J.,VRENSON,G.F.J.M. (1988)
The innervation of the rabbit's anterior eye segment.
Exp.Eye.Res. 46. 717-730

TSENG,S.C.G. (1989)
Concept and application of limbal stem cells.
Eye. 3. 141-157

UDDMAN,R.,ALUMENTS,J.,EHINGER,B.,HANKANSON,R.,LOREN,I.,SUNDLER,F (1980)
Vasoactive intestinal polypeptide nerves in ocular and orbital structures of the cat
Invest.Ophthalmol.Vis.Sci. 19. 878-885

UNGER,W.G.,BUTLER,J.M. (1988)
Neuropeptides in the uveal tract.
Eye. 2. 202-212

VALLBO,Å.B.,HAGBARTH,K.E. (1968)
Activity from skin mechanoreceptors recorded percutaneously in awake human subjects.
Exp.Neurol. 21. 270-289

VALLBO,Å.B.,HAGBARTH,K.E.,TOREBJORK,H.E.,WALLIN,B.G. (1979)
Somatosensory,proprioceptive, and sympathetic activity in human peripheral nerves.
Physiol.Rev. 59. 919-957

VOGT,A. (1921)
Atlas of Slit-Lamp Microscopy of the Living Eye.
Springer.Berlin.

WAGNER,R.,MEISSNER,G. (1852)
Über das Vorhandensein bisher unbekannter eigenthümlicher Tastkörperchen (corpuscula tactus) in den Gefühlswärzchen der menschlichen Haut und über die Endausbreitung sensitiver Nerven.
Nachrichten Georg-August-Uni. konigl.Ges.Wiss. Gottingen. 2. 17-30

WARWICK,R. (1976)
Anatomy of the Eye and Orbit.
H.K. Lewis and Co. Ltd. London.

WEDDELL,G.,TAYLOR,D.A.,WILLIAMS,C.M. (1955)
Studies on the sensory innervation of the skin III. The patterned arrangement of the spinal sensory nerves to the rabbit ear.
J.Anat. 89. 317-342

WINKELMANN,R.K. (1957)
The mucocutaneous end-organ.
A.M.A. Arch.Dermatol. 76. 225-235

WINTER,R.,DRAEGER,J.,RUMBERGER,E.,LUCAS,L. (1986)
Zur Sensibilitätsbestimmung an der Bindehaut-Methodik und erste klinische Befunde.
Fortschr.Ophthalmol. 83. 176-178

WOLTER,J.R. (1964)
Double nerve supply of Krause corpuscles at the human corneal limbus.
Arch.Ophthalmol. 71. 409-411