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Nerve Structures in Human Central Corneal Epithelium

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Abstract. Eight corneal buttons obtained after enucleations and keratoplasties were impregnated with gold chloride. In epithelial flat preparations dissected from the stroma, the topography of epithelial nerves was examined by light microscopy. Four main structures constituted the innervation of human central corneal epithelium: (1) Bowman's membrane penetrating stromal nerves; (2) basal epithelial nerve plexus; (3) dendritic cells that are interspersed among the basal plexus and possibly connected with nerve fibres; (4) nerve terminals, originating from the basal plexus and dividing dichotomously in the superficial cell layers.

Zusammenfassung. 8 Hornhauttrepanate, erhalten nach Enukleationen und Keratoplastiken wurden mit Goldchlorid imprägniert. Die Topographie der epithelialen Nerven konnte lichtmikroskopisch in Epithelflachpräparaten, die vom Stroma dissezierten waren, untersucht werden. Vier Hauptstrukturen konstituierten die Innervation des zentralen Epithels der Kornea: 1. Stromanerven, die die Bowman'sche Membran penetrieren. 2. Basaler Nervplexus. 3. Dendritische Zellen, die im basalen Plexus auftreten und möglicherweise mit Nervenfasern in Verbindung stehen. 4. Freie Nervenendigungen, die ihren Ursprung im basalen Plexus haben und sich in den oberflächlichen Zellschichten dichotom teilen.

Introduction

Sensation is a prominent feature of the human cornea. It serves the protective function of this anterior part of the eye, which is the best innervated surface tissue of the body. Mechanical forces of the order of 2.5×10^{-5} (Draeger et al. 1976) can elicit painful sensation. Moreover, it has been shown recently that trigeminal nerve fibres not only transmit sensation, but also have a trophic influence upon the corneal epithelium (Schimmelpfennig and Beuerman 1979). The perception and transmission of mechanical stimuli and trophic signals also inevitably depend on intact sensory corneal innervation. Its interruption, caused clinically (Davies 1970) or experimentally (Beuerman and Schimmelpfennig 1980), results in perceptive and trophic deficiencies. Our present knowledge about those physiological nerve functions in the human cornea is very limited. It can only be improved if future investigations are based on reliable anatomical facts. However, the literature of the past three decades is surprisingly sparse and inconclusive about anatomical details such as nerve access to the epithelium, existence of dendritic cells, structure of basal nerve plexus and ramification of epithelial nerve terminals.

The histological identification of nerve elements in the cornea depends mainly on a suitable staining procedure. Three appropriate techniques were developed in the past century. Methylene blue was introduced by Arnstein (1887) after Ehrlich (1886) had discovered its ability to stain nerves. Bielschowsky and Pollak (1904) first used silver to impregnate corneal nerves. The most convincing demonstrations of nerves in the skin and cornea of mammals and man were obtained by the gold-chloride-impregnation technique. Cohnheim (1867) and Ranvier (1881) who applied this procedure to animal corneas published detailed drawings of their observations. Since then, gold chloride has never been used systematically in human corneas and was abandoned in favour of the silver-impregnation method.

The purpose of this investigation is to reevaluate the gold chloride staining technique with regard to its suitability to identify human corneal nerves and to obtain additional information on the epithelial innervation.

Materials and Methods

1. Corneas

Central corneal buttons from eight eyes were impregnated with gold chloride (see below). The specimens, 5 and 7 mm in diameter, were obtained after enucleation for retinoblastoma (1) and choroidal melanoma (2), after keratoplasty for macular stromal dystrophy (2) and keratoconus (2), and after a fatal accident (1). With the exception of the last case, all corneas were examined by slit-lamp and their sensation tested with an anaesthesiometer (Beuerman and McCulley 1979).

2. Gold Chloride Impregnation

A modified method originally described by Ranvier (1881) was used. The corneal buttons were carefully trephined and excised, avoiding excessive distortion. They were then placed in fresh, filtered lemon juice for 15 min and washed rapidly in distilled water. This was followed by the impregnation in 1% or 2% gold chloride for 20 min. The specimens were then placed in distilled water to which had been added acetic acid (five drops of glacial acetic acid in 50 ml) and kept so for 8–12 h. Such preparations are very dark and cannot be examined under the light microscope. Therefore, thin tissue preparations were obtained by dissecting the epithelium from the underlying stroma by means of a Graefe's knife. These epithelial preparations with a thin layer of adherent stroma were then dehydrated in alcohol, cleared in xylene and mounted on a slide for examination.

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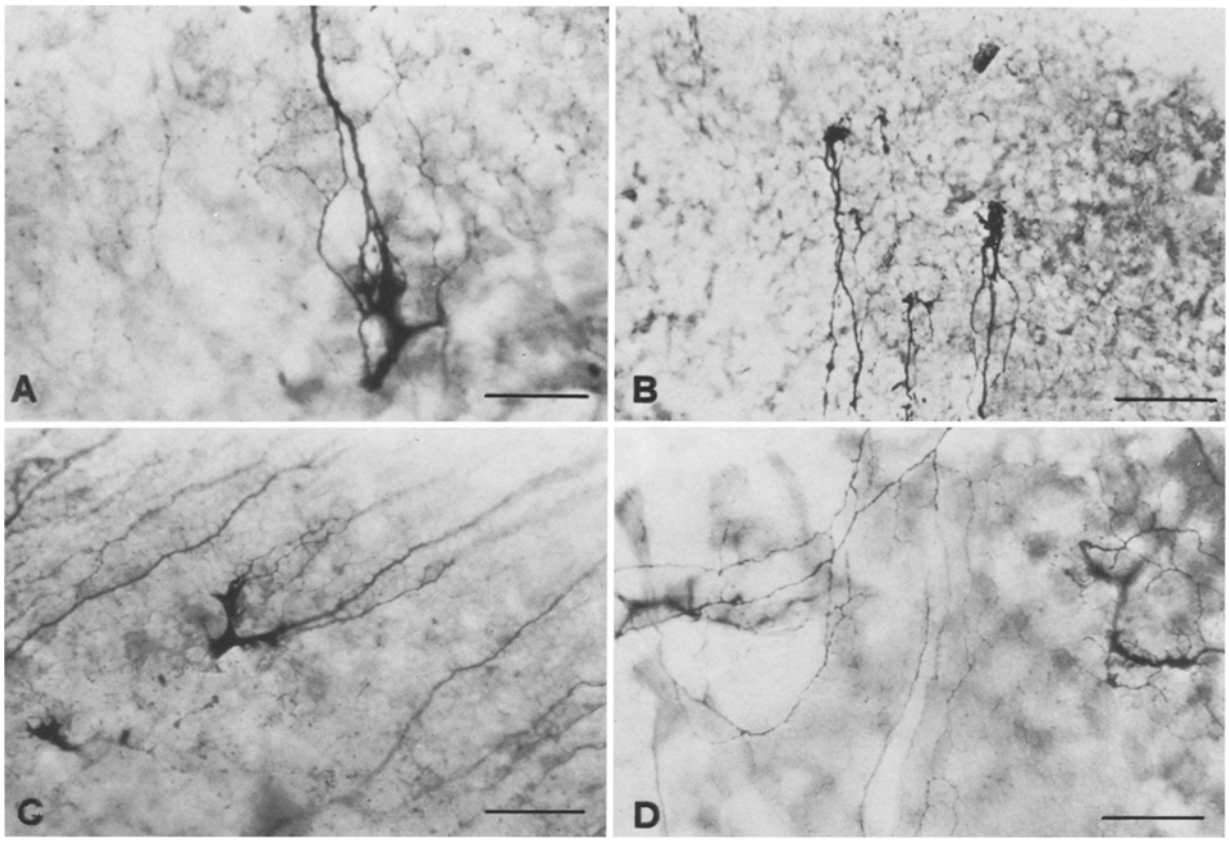

Fig. 1. Nerve access to central human corneal epithelium by penetrating stromal fibres. Corneas obtained immediately after enucleation for retinoblastoma (*A*), melanoma (*B*), fatal accident (*C*), and after keratoplasty for keratoconus (*D*). (Magnification bar = 100 μm)

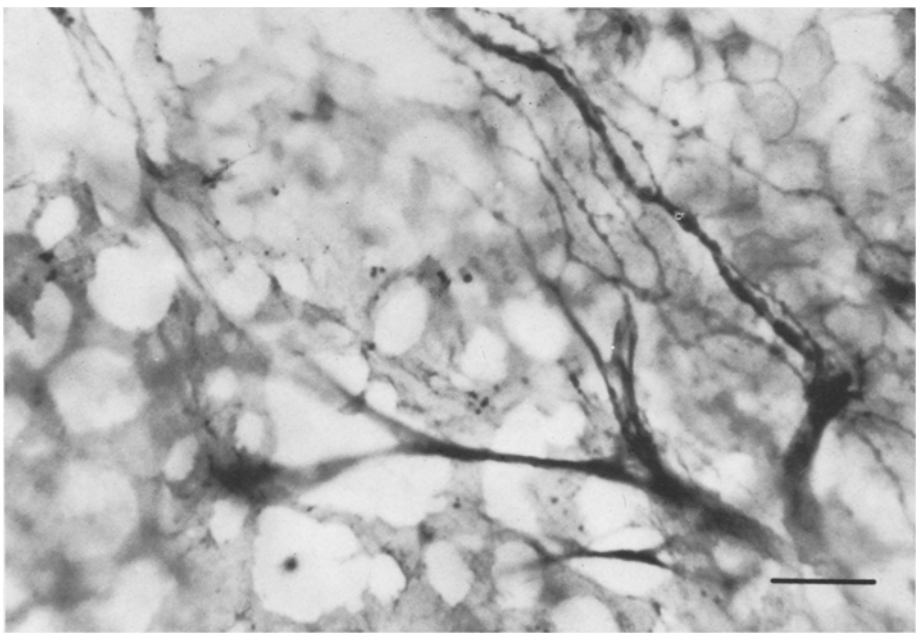

Fig. 2. Penetrating stromal nerve fibre dividing into two branches at the stromal-epithelial interface. Long fibres are given off from the branches to the basal plexus. Stromal lamellae on the left side. Basal epithelium on the upper right side. (Magnification bar = 20 μm)

Results

1. Nerve Access to the Epithelium

In all corneas, branches of stromal nerves ascended to the epithelium (Fig. 1). At their entrance to the basal cell layer they divided in most of the cases dichotomously and gave off numerous long fibres, which then proceeded in one direction within the basal cell layer (Fig. 2). Those fibres, together with the dividing penetrating stromal nerve, had the appearance of a 'leash'. In a corneal button 5–8 leashes could be seen.

2. Basal Epithelial Plexus

The long fibres originating from penetrating stromal nerves were the main constituents of the basal epithelial plexus (Fig. 3). They

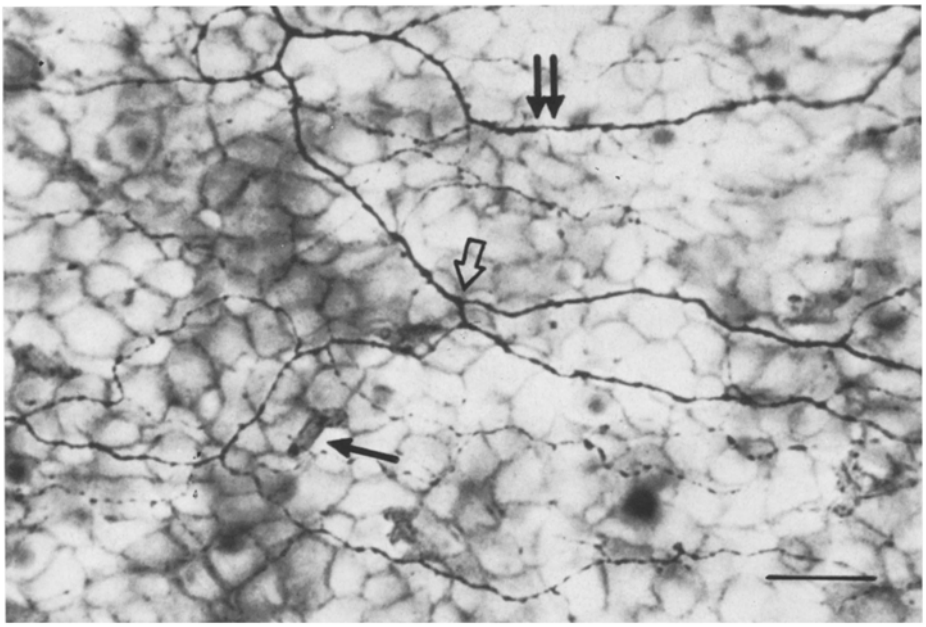

Fig. 3. Nerve fibres within basal epithelial cell layer (basal epithelial nerve plexus). Long fibres (*double arrow*) connected by thin, beaded fibres and occasionally bridged by short branches (*open arrow*). Dendritic cell (arrow). (Magnification bar = 20 μm)

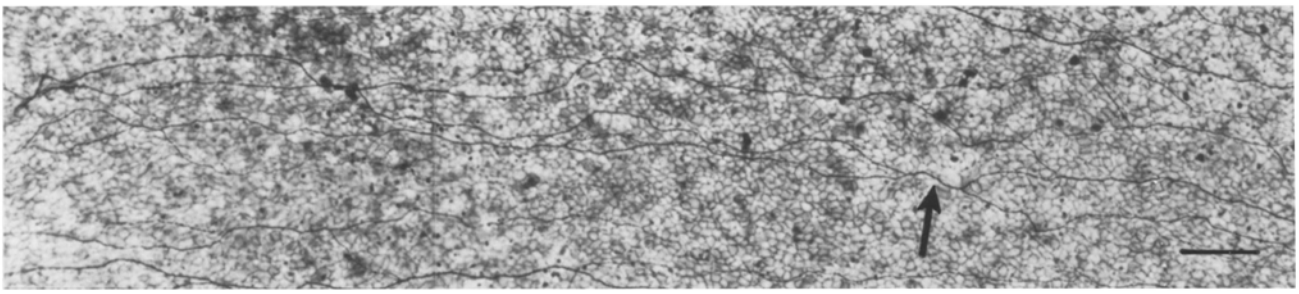

Fig. 4. Long nerve fibres in basal epithelial plexus. Penetrating stromal fibre on upper left side. Fusion of two fibres (*arrow*). Composite photograph. (Magnification bar = 100 μm)

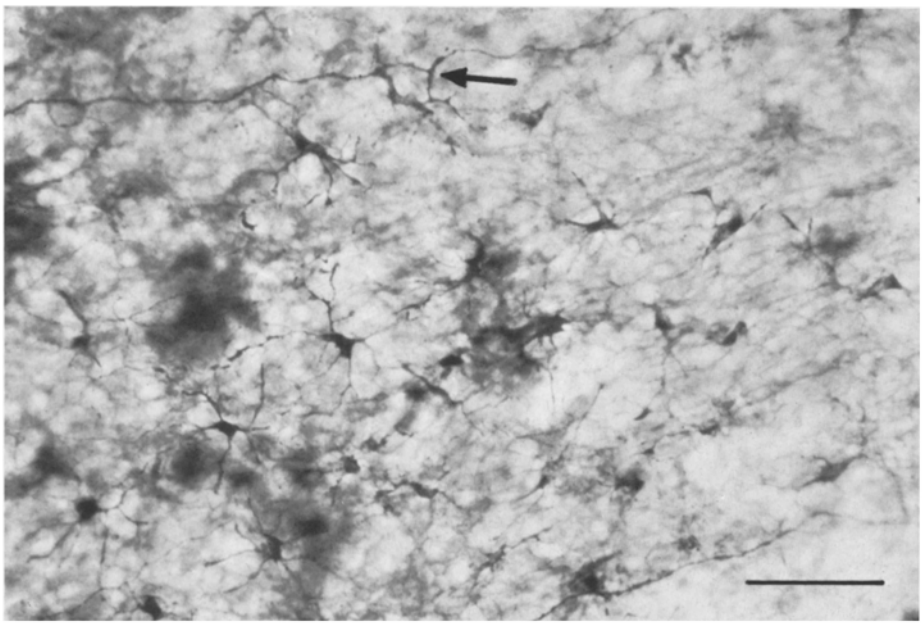

Fig. 5. Dendritic cells in basal epithelial cell layer. Contact of cell processes with nerve fibre (*arrow*). (Magnification bar = 50 μm)

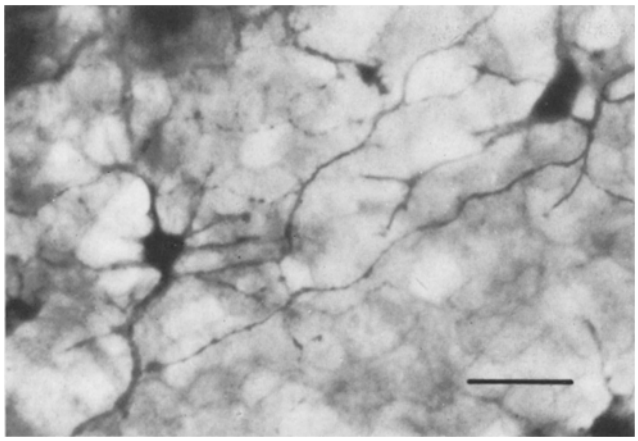

Fig. 6. Two dendritic cells in contact by long processes. (Magnification bar = 20 μm)

were mostly arranged in a parallel fashion and extended for a considerable distance. In Fig. 4, a segment of these fibres can be seen up to a length of almost 2 mm. During their course they were either connected by multiple, thin, beaded fibres or just bridged by a short branch. Occasionally, fibres seemed to fuse for a short distance.

3. Dendritic Cells

Within the basal cell layer, a population of cells was noticeable by its dark staining, which was comparable with that of the aureophilic nerve fibres (Figs. 5 and 7). The individual cells were dendritic in shape. They seemed to be interconnected by long processes (Fig. 6). Some of these were approached by thin, beaded nerve fibres (Fig. 8). Dendritic cells occurred in all corneas; however, their staining density and number varied.

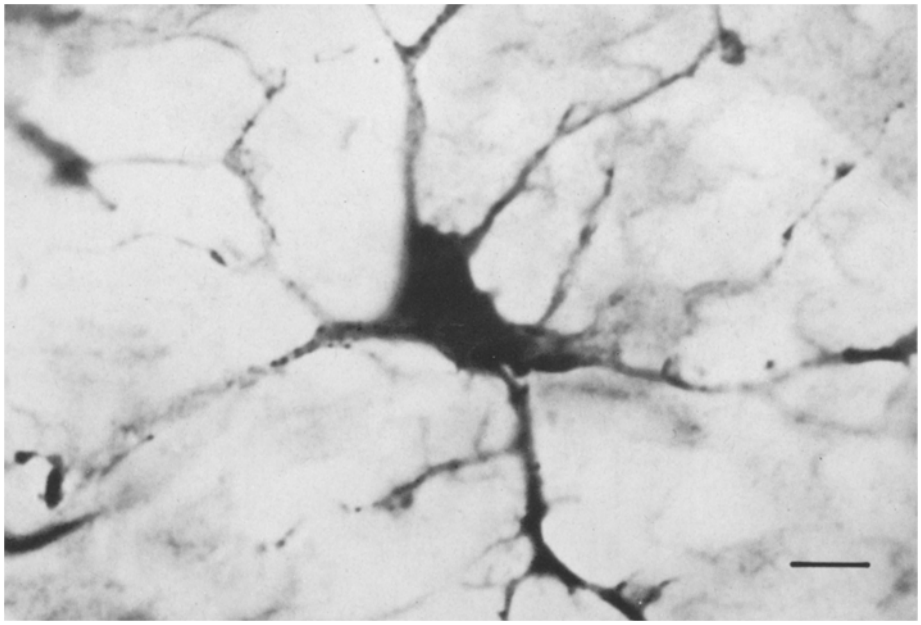

Fig. 7. Dendritic cell (Magnification bar = 5 μm)

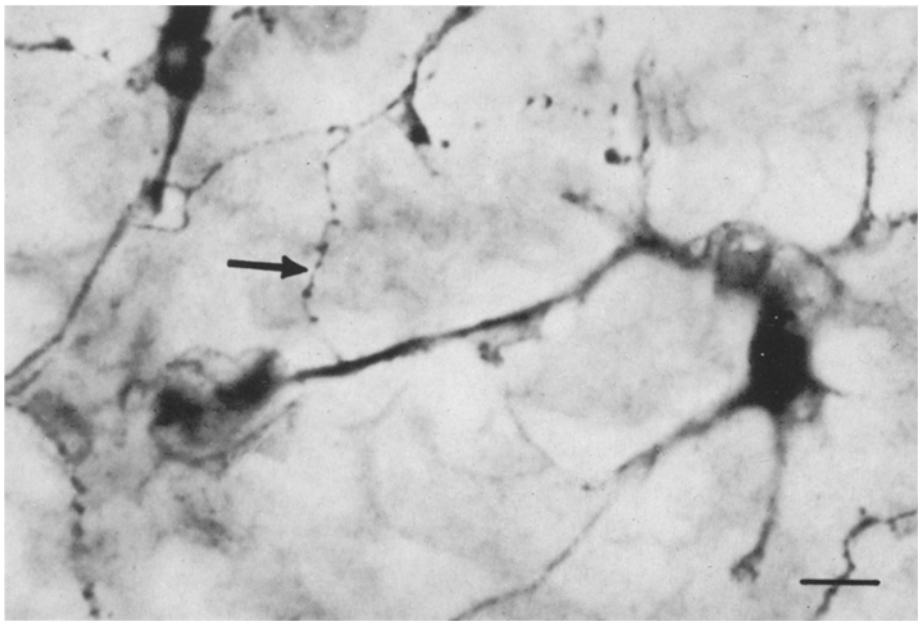

Fig. 8. Processes of dendritic cells connected by thin, beaded nerve fibre (*arrow*) (Magnification bar = 5 μm)

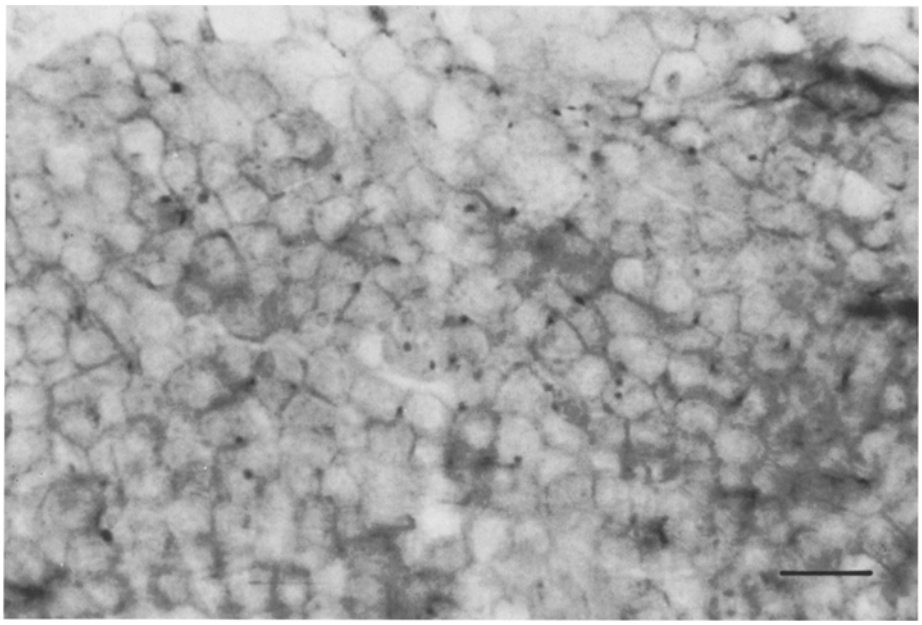

Fig. 9. Cross sections (black dots) of ascending nerve terminals above basal epithelial cell layer. (Magnification bar = 20 μm)

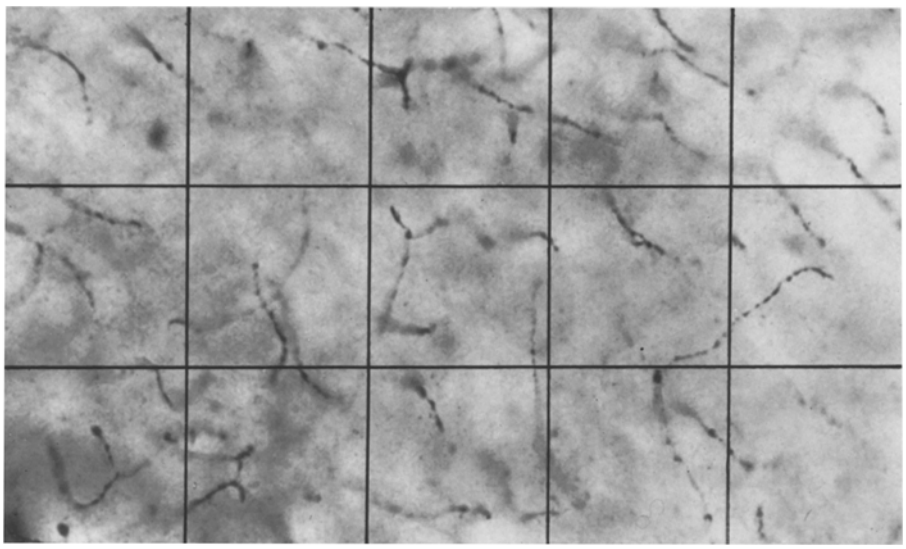

Fig. 10. Nerve terminals in superficial epithelium. (Square = 20 μm^2)

4. Nerve Terminals

Free nerve endings in the superficial epithelial layers were a constant observation in all preparations. Their density was considerable and is reflected in Fig. 10. At least one terminal can be identified per 20 μm^2. By focussing, the terminals could be followed from their origin in the basal plexus up to the superficial epithelium, where they divided dichotomously (Figs. 9 and 11). Bead-like enlargements of the axon terminals were a characteristic anatomical quality and made their detection easy.

Discussion

The pattern of innervation of the human cornea has been the subject of research since Schlemm (1831) initially discovered nerves at the limbus, which proceeded into the stroma. Later, stromal nerves and their ramifications were identified in human and animal corneas with the use of methylene blue (Dogiel 1890), silver (Cajal 1904) and gold chloride (Hoyer 1873). Despite many investigations, the literature does not reveal a satisfactory description of the innervation of the human corneal epithelium. Related observations had been discussed in earlier publications; however, the illustrations were limited to a few drawings even in the first third of this century (Reiser 1936). In 1950, Zander and Weddell's comprehensive study of corneal innervation in a number of vertebrates including man became a standard reference, but it does not show any photographs of human corneal epithelial nerves. Also, Duke-Elder's System of Ophthalmology (1961) presents the epithelial innervation of the human cornea rather briefly. Thus, in a review Mensher (1974), citing Wolter (1971), comes to the conclusion: "It remains as a fact, however, that the normal source and pathway of the corneal nerves are not as yet entirely understood."

The access of nerves to the corneal epithelium was first described by Attias (1912). In fresh gold-chloride-impregnated human corneas he observed stromal nerve fibres ascending to the epithelium and dividing there into numerous branches. Vrabec (1954), using silver-impregnation methods, made similar observations in the peripheral human cornea. Recently, Lim and Ruskell (1978) and Ruskell and Lim (1980) questioned the existence of these structures in monkeys and man on the basis of histologi-

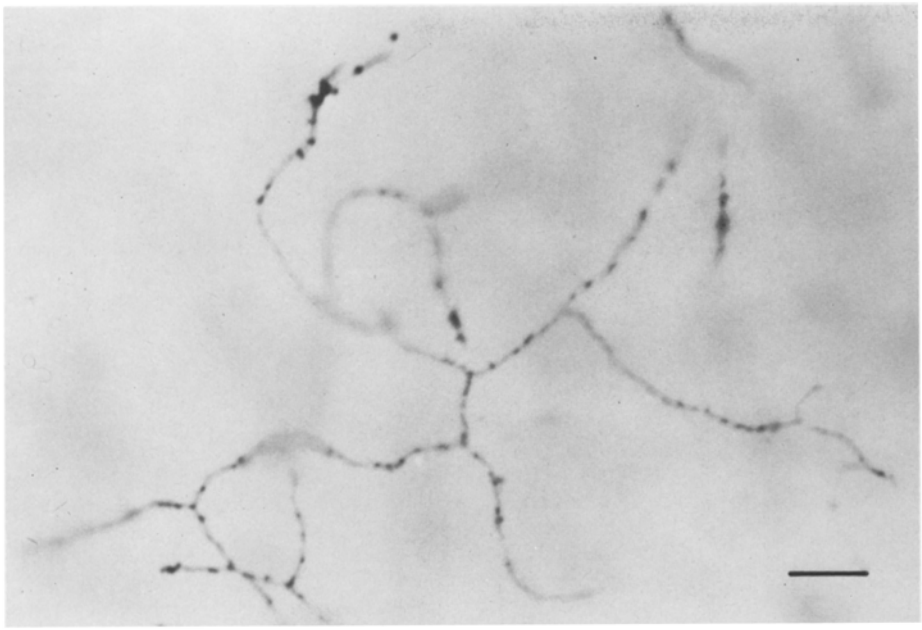

Fig. 11. Dichotomous arborization of nerve terminals. (Magnification bar = 5 μm)

cal and electron-microscopical investigations and favoured exclusively nerve access from the conjunctiva. The present findings clearly demonstrate the occurrence of penetrating stromal nerve fibres and their importance for the constitution of the basal epithelial plexus. Other peripheral structures, which cannot, of course, be excluded, may play an additional role in the epithelial nerve supply.

The existence of a nerve plexus in the subepithelial layer of the cornea has been known since Cohnheim's (1867) original investigation. However, the exact location of this plexus has since then been in dispute. Attias (1912) localized it above Bowman's membrane and reported that its fibres gradually ascended to the upper epithelium. In 1956, Wolter demonstrated nerve fibres within the basal cell layer in silver-impregnated cross sections of the peripheral human cornea. In cross sections, however, the general topography cannot be illustrated. Only in flat preparations can the relationships be seen between perforating stromal nerves, their branches and the constituting fibres of the plexus. It is evident that the plexus consists of two types of fibres: (1) branches of penetrating stromal nerves, which seem to be the 'skeleton' of the plexus, proceed several millimetres at the level of the basal cells (Fig. 4) and (2) thin, beaded fibres that connect the long fibres. The entire plexus is located within the basal epithelial cell layer.

Dendritic cells have been described histologically by silver impregnation (Pau 1957) and electronmicroscopically (Segawa 1964) in the human cornea, since Langerhans (1868) first observed them in gold-chloride-impregnated human skin. As yet, their occurrence in the central human corneal epithelium has not been confirmed by gold chloride, which is suggested to be specific for Langerhans cells (Zelickson and Mottaz 1968). Their possible function there is unknown. Recent experimental data consider an immunological role for Langerhans cells in the skin (Rowden 1980). The present study shows the existence of these aureophilic cells in the central corneal epithelium. They appear to be arranged in a syncytial pattern, and some dendrites seem to be in contact with thin, beaded nerve fibres (Fig. 8). The number of dendritic cells and their staining density varied in the preparations. This might have been more the expression of postsurgical cellular changes than differences in diagnosis.

The terminal nerve supply in the superficial human corneal epithelium has been more a matter of speculation than of convincing demonstrations. The minute structure of axon terminals makes the histological and ultrastructural identification very difficult. Only a few publications communicate observations on nerve terminals in the cornea (Klein 1980; Boeke 1925; Engelbrecht 1953; Scharenberg 1955). Wolter (1956) demonstrated their origin in the basal plexus of the peripheral human cornea. In flat preparations they can be identified as black dots between cells, when they ascend to the upper epithelium (Fig. 9). There, the terminals divide dichotomously; this has not yet been established histologically. Matsuda (1968) was unable to observe axonal arborization electronmicroscopically in human corneas. The bead-like enlargements of axon terminals could be seen in all preparations. According to Matsuda (1968), they represent local accumulations of mitochondriae.

In conclusion, gold-chloride impregnation of fresh human corneas provides additional information on the innervation of its central epithelium. Four main structures (Fig. 12) can be distinguished. The first is Bowman's membrane penetrating stro-

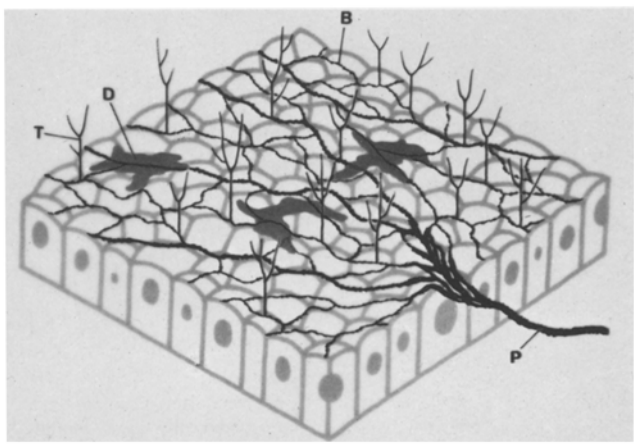

Fig. 12. Pattern of innervation in central human corneal epithelium (artist's view). Penetrating stromal nerve (*P*), basal epithelial plexus (*B*), ascending nerve terminals (*T*), dendritic cells (*D*)

mal nerves, which give off several fibres of considerable length. They are interconnected by thin, beaded fibres and form together the second structure, the basal nerve plexus within the basal epithelial cell layer. Dendritic cells are the third and are interspersed among the basal plexus and possibly in connection with nerves. Fourth, the nerve terminals originate from the basal plexus and ascend to the superficial epithelium, where they divide dichotomously.

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