1. INTRODUCTION

Although some researchers have noted degeneration and edema of corneal stromal nerves in HSV-1 keratitis, such changes have not been described in detail or quantified.\(^1,2\) Work by Tullo et al. demonstrated a decrease in corneal sensitivity as well as levels of corneal substance P in a herpes simplex keratitis model in the mouse, using a radioimmuno assay for substance P.\(^3\) Metcalf et al. used a histochemical method for acetylcholine esterase, but failed to show loss of nerves in the stroma although a significant decrease in corneal sensitivity was found.\(^4\) These results suggest that changes in corneal sensitivity could be due to an altered function of corneal nerves, rather than to a decrease in corneal nerve density. However, recently Rozsa and Beuerman showed a parallel between corneal nerve density and psychophysical thresholds for corneal stimulation.\(^5\)

We compared the organization of the corneal innervation at both the intraepithelial and stromal levels in normal rabbits and following the development of herpetic dendritic keratitis.

2. MATERIALS AND METHODS

2.1 Animals: New Zealand albino rabbits (1.5 - 2.0 kg).

2.2 Virus: RE strain of HSV-1 (kindly supplied by Dr. Centifanto-Fitzgerald) was used. Virus stock was grown on Vero cells, and the titre of each viral stock was determined on monolayer cultures. Innoculum size per eye was approximately 0.100 ml of 10^6 PFU/ml.

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2.3 Innoculation of rabbit cornea: Innoculation was performed so as to minimize damage to the corneal epithelium and to the corneal nerves. Sterile paper strips (Schirmer tear test strips, Cooper Vision Pharmaceuticals, Inc, San Germain, PR) soaked in the viral suspension, was placed on the rabbit cornea after achieving anaesthesia with two drops of proparacaine 0.5% solution. The lids were then pulled closed and gently rubbed for 30 seconds, after which the Schirmer paper was removed. Control eyes were handled identically, except that the Schirmer paper was soaked in tissue culture media without virus.

2.4 Nerve staining. Rabbits were sacrificed by intravenous injection of sodium pentobarbitol. For orientation purposes, a small incision was made at the 12:00 o'clock position of the cornea at the limbus. Both corneas were incised and processed in parallel by a modified gold chloride technique. Briefly, the tissue was immersed in 1.0% gold chloride solution for 12 to 15 minutes, followed by incubation in acidulated water for 14 to 15 hours, at which time the solution was replaced with 70% alcohol to stop further staining. The tissue was dissected into 4-6 lamellae in the frontal plane before dehydration and mounted flat on slides for observation and photography. Some specimens were not dissected, but were imbedded in paraffin and then used to make 15 µ cross-sections.

2.5 Histology: Selected rabbit corneas were also evaluated for routine histology by fixation of the tissue in normal buffered formalin and then 5 µ cross-sections were stained by hemotoxin and eosin.

2.6 Procedure: After viral corneal innoculation, rabbits were followed by slit lamp examination. Photographs, with and without fluorescein, and viral cultures were made at periodic intervals. At selected times, animals were sacrificed for histological evaluation.

3.0 RESULTS

3.1 Normal innervation: The cornea is innervated by 12 to 16 large nerves which enter in the mid-stroma at the limbus. Some 2 to 3mm within the cornea, these nerves contain both