THE CORNEAL EPITHELIUM IN EXPERIMENTAL HERPETIC KERATITIS IN RABBITS*

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INTRODUCTION

During the past half-century, the histopathology of Herpes simplex virus infection of the rabbit eye has been described in progressively greater detail as newer techniques of preparing tissues for examination have been developed. The earliest studies were hampered by the limitations inherent in the classical methods of staining and embedding tissue specimens (LOWENSTEIN 1919, 1920, DOERR 1920, GRUTER 1920, LUGER et al. 1921, FUCHS et al. 1923, BERGER 1926, FUCHS 1933). Fifteen years ago, silver staining methods were first used to study herpetic keratitis in the rabbit (WOLTER et al. 1956). Ten years ago, transmission electron microscopy and fluorescent antibody techniques were applied to the
study of herpetic keratitis (Hogan et al. 1962, 1964, Kimura et al. 1962, Dawson et al. 1966, 1968, Tanaka et al. 1967), and more recently, the use of the scanning electron microscope (Spencer et al. 1970).

In the present study, the replica method developed by Wolf (Wolf 1939), and reintroduced more recently (Vrabec 1970), was chosen to provide detailed examination of the events occurring on the epithelial surface, and to provide plastic images similar to those obtained by the scanning electron microscope. In addition, two different techniques of silver staining have been used, in order to better define the surfaces of cells and the corneal nerves. The sequence of events following infection of rabbit corneas with Herpes simplex virus was followed at intervals from three hours up to 25 days, by daily slit lamp observation of the corneas, until sacrifice of the rabbit and preparation of the cornea for histologic study.

MATERIALS AND METHODS

1. Virus

a) The Powers strain of Herpes simplex virus was isolated in 1966 at the Harkness Eye Institute from a conjunctival swab taken from a patient with chronic disciform and epithelial herpetic keratitis. Isolation and initial passages were carried out on HeLa cells, and the identity of the virus was confirmed by neutralization tests. Subsequent passages have been on both HeLa cells and FL amnion cells. The characteristic cytopathology is that of round cells in tissue culture. For the experiment, a 32 oz. bottle with a monolayer of FL amnion cells was infected with 1 cc of this material. At the time the virus was harvested, 1 cc of virus material was placed into multiple duplicate tubes and stored at -60°C until use in the experiment. Infectivity titration, done on RK 13 cells, showed a titer of \(1 \times 10^{-5} \text{TCID}_{50}\).

b) The Miyama strain of Herpes simplex virus was received in 1966 through the courtesy of Dr. Shiro Nii. The Miyama strain GC + was first isolated from the vesicular fluid of a patient who had herpes labialis (Nii et al. 1961). In tissue culture the characteristic cytopathology is that of numerous giant cells. The virus has been maintained in our laboratory by serial passage on HeLa cells, and after harvesting the material was frozen at -60°C in multiple tubes until use in the experiment. Titration on RK 13 revealed an infectivity titer of \(1 \times 10^{-4} \text{TCID}_{50}\).