Deposits of mucosubstances on the cornea by topical chloramphenicol: an electron microscopic study. YUKIHIKO MITSUI, REIKO TAKASHIMA, MICHIMASA FUJIMOTO, AND TATSUO KASHIYAMA.

Chloramphenicol was instilled into rabbit eyes and five days later the corneal surface was examined by scanning electron microscopy. The corneal surface was roughened and showed a proliferative appearance. Examination of thin sections of the cornea by transmission electron microscopy showed that the corneal surface stained strongly with alcian blue. It was thus supposed to be a deposit of mucosubstances. Mucosubstances increase pathogenicity of bacteria and, therefore, this deposit may accelerate Pseudomonas keratitis after chloramphenicol application.

The topical use of broad-spectrum antibiotics for minor injuries of the cornea is often followed by hypopyon-keratitis due to infection with Pseudomonas. It has been demonstrated in rabbits that the topical use of chloramphenicol increased the sensitivity of corneal infections with Pseudomonas, and that this increase in sensitivity may be due to some alterations of the cornea by this antibiotic. We have examined the cornea of rabbits by electron microscopy after instillation of chloramphenicol to learn what kinds of changes were brought about on the corneal surface by this antibiotic.

Method. Ten adult albino rabbits, weighing about 2.5 kilograms were used. A 1 per cent solution in saline of chloramphenicol succinate (pH 5.4) was instilled into the right eye of the rabbits and saline solution (pH 5.4) into the left eye, five times a day for five days. Then the eyes were removed under retrobulbar anesthesia. After removal, the precorneal tear film was removed as far as possible by flushing with warm saline from a pipette. Then the cornea was excised from the eyeball at the limbus by a routine procedure. The corneal specimen was divided into two pieces. One of the other pieces was fixed for two hours in cold 2.5 per cent glutaraldehyde in cacodylate buffer (pH 6.5) containing 1 per cent alcian blue SGN, and then for three hours in 1 per cent osmic acid in the same buffer containing 1 per cent alcian blue SGN. It was then dehydrated in alcohol and embedded in Epon. Thin sections were prepared with an LKB-microtome and stained with uranyl acetate and lead citrate. Specimens were examined in a Hitachi HU-12 electron microscope.

Results. The normal structure of the corneal surface seen by scanning electron microscopy was essentially almost the same as described by others. Fig. 1, A shows the appearance of normal cornea at high magnification. Three surface cells are seen, and borders between cells are sharply defined. The cell surface consists of a carpet of microvilli. The corneal surface after instillation of saline (pH 5.4) showed the same structure as that of the normal control. The corneal surface after application of chloramphenicol showed two significant changes when examined at low magnification. One was roughened appearance of the corneal surface and the other was desquamation of superficial cells. These changes were present in all portions of the cornea examined. Fig. 1, B shows the appearance at high magnification of an area of cornea where the superficial cells were preserved. Borders between cells cannot be detected. The microvilli of the cells are extremely deformed and proliferative appearance is obvious.

Fig. 2 shows sections of the surface epithelium stained with alcian blue. In the normal cornea (Fig. 2, A) a thin layer, 10 to 20 nm. thick, staining strongly with alcian blue, covered the corneal surface. The cornea treated with saline (pH 5.4) showed the same appearance with the normal cornea. After treatment with chloramphenicol (Fig. 2, B) the cell coat stained with alcian blue was increased to 50 to 120 nm. in thickness. In addition, material staining with alcian blue was diffusely scattered above the dense layer.

The cell coat as stained with alcian blue on the corneal surface could not be seen by electron microscopy in sections prepared by the standard procedure.

Discussion. Corneal infection due to antibiotic-resistant bacteria often occurs after topical application of antibiotics. From experiments on animals these bacterial infections are suspected to be due to the influence of the antibiotics on the host rather than that on the micro-organisms.
Fig. 1. Appearance of the surface of rabbit cornea by scanning electron microscopy. ×7,000. 
A, normal cornea. The cell surface consists of a carpet of microvilli. B, cornea after instillation 
of chloramphenicol. The microvilli are extremely deformed and proliferative appearance is 
obvious.
Fig. 2. Sections of the surface of rabbit cornea epithelium, stained with alcian blue, and observed by transmission electron microscopy. *22,000. A, normal cornea. The layer of probable mucosubstances on the cell surface measures 10 to 20 nm. in thickness. B, cornea after instillation of chloramphenicol. The layer of probable mucosubstances on the surface increased to 50 to 120 nm. in thickness.

but the pathogenesis of this kind of infection is obscure.

A thin layer of mucosubstances has been demonstrated in normal cornea as the deepest layer of the precorneal tear film.1* Physiologically, this layer of mucosubstances on the cornea may have a protective function increasing the resistance of corneal surface against external influences. After application of chloramphenicol a heavy cell coat, stained strongly with alcian blue, appeared on the corneal surface. This coat is supposed in all probability to be deposits of mucosubstances or the like. The developmental mechanism of this cell coat is obscure, but mucosubstances have the property of increasing pathogenicity of bacteria.5, 10 The formation of the heavy cell coat of mucosubstances may, therefore, accelerate corneal infections with resistant organisms after application of chloramphenicol. The possible relationship between the deposition of mucosubstances on the corneal surface after the instillation of chloramphenicol and development of corneal infections due to antibiotic-resistant bacteria after topical application of this antibiotic warrants further investigation.

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Key words: corneal surface, mucosubstance, electron microscopy, chloramphenicol, alcian blue.

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