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The role of mucin on experimental *Pseudomonas* keratitis in rabbits.

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The role of mucin in the manifestation of Pseudomonas *keratitis was studied.* Pseudomonas *was cultivated in solutions of mucin, in which it grew rapidly and then inoculated into rabbit cornea by needle pricks. When the organism was inoculated as a suspension in saline, infection infrequently occurred as small ring abscesses of short duration around a few sites of inoculation. When the organism was inoculated as a suspension in a solution of gastric mucin, infection was usually observed as severe hypopyon-keratitis with formation of a huge ring abscess. Corneal perforation and panophthalmitis resulted in some cases. It was thus concluded that the pathogenicity of* Pseudomonas *is definitely increased when it was inoculated into the cornea with mucin solution.*

Hypopyon-keratitis due to infection with *Pseudomonas* was relatively rare before antibiotics became widely used.[1] However, the increase of this type of keratitis has been increasingly reported during the past 15 years throughout the world.[2] Topical use of broad-spectrum antibiotics such as chloramphenicol for minor injuries of the cornea is often associated with this type of keratitis.[3] The topical use of chloramphenicol definitely accelerates this keratitis in rabbits[4] and, at the same time, it causes a heavy deposit of mucosubstances on the corneal surface.[5] Mucin has the property to increase pathogenicity of bacteria.[8] Thus, this study was undertaken to investigate the role of mucin in the experimental production of *Pseudomonas* keratitis.

Method.

Strain. A strain of *Pseudomonas aeruginosa* isolated from a clinical case of hypopyon-keratitis was used after 12 to 20 subcultures in our laboratory. This strain was identified as type F-27 by phage-typing.[6] It was grown in broth culture and then washed three times with saline. Then it was suspended in saline or in 15 per cent mucin solution, at a concentration of about 10^7 cells per milliliter, for inoculation. The suspension was inoculated either into in vitro culture media or into rabbit eyes immediately after preparation.

Mucin. A gastric mucin (Difco) was used. The mucin was sterilized by heating at 70° C. for 24 hours in 95 per cent ethanol, and sterile mucin powder was obtained by evaporating off the ethanol.

In vitro cultivation. Pseudomonas was cultivated in 0.3, 0.03 and 0.003 per cent solutions of mucin in saline, adjusted to pH 7.2 with sodium bicarbonate solution. The concentration of mucin in normal tear fluid is reported to be about 0.003 per cent.[7] The organism suspended in saline was inoculated into the media to give final dilution of about 10^3 organisms per milliliter, and growth was measured at 33° C. for 24 hours. Broth and saline were used as control culture media.

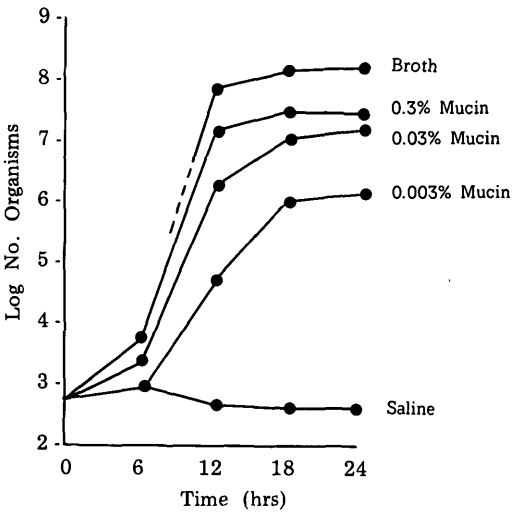

Fig. 1. Growth curves of *Pseudomonas* in solutions of mucin, broth and saline at 33° C.

Inoculation into rabbits. Ten albino rabbits weighing about 2.5 kilograms each were used. The animals were inoculated into the right eye with a suspension of the organism in 15 per cent mucin, and into the left eye with cell suspension in saline. For this retrobulbar anesthesia was used and one drop of the inoculum was instilled into a cul-de-sac. Then slight needle pricks were made in about 15 sites on the cornea using a 27G3/4-gauge needle. Attempts to reisolate the organism from the cul-de-sacs were made every day for one week after the inoculation and then usually every other day.

Two other rabbits were inoculated similarly by simple instillation of the inoculums, the mucin-suspension into the right eye, and the saline-suspension into the left eye. Reisolation of the organism was tried every two hours after the inoculation.

Results.

In vitro cultivation. Results are shown in Fig. 1. *Pseudomonas* did not grow in saline at 33° C. During 24 hours, *Pseudomonas* grew from a cell density of about 10^3 to about 10^8 in broth, and to $10^{7.5}$, $10^{7.2}$, and 10^6 in 0.3 per cent, 0.03 per cent, and 0.003 per cent mucin, respectively.

Inoculation into rabbits by needle pricks. Inoculation of the saline-suspended organism into the left eye had no effect in 2 of 10 rabbits and the organism could not be reisolated 24 hours later

(Fig. 2, *A*). In two other rabbits very small punctate infiltrations surrounding the sites of some pricks were seen after 24 hours, but they disappeared by two days, and the organism could not be reisolated from the cul-de-sacs after 48 hours. In the other six rabbits small abscess-like infiltrations of the cornea developed in two days, but they generally disappeared within seven or ten days leaving only corneal maculae. In no case did a huge confluent abscess of the cornea develop. A pannus appeared in four of the ten eyes, with only one being a severe epaulette pannus.

After inoculation of the organism suspended in mucin solution into the right eye, abscess-like infiltrations with ulceration appeared in all 10 rabbits within 24 to 48 hours (Fig. 2, *B*). The infiltrations enlarged rapidly in most cases, and adjacent infiltrations became confluent during the following 24 to 48 hours, involving a large area of the cornea. Hypopyon appeared on the second or third day of keratitis. Subsequently, the anterior chamber could no longer be seen owing to the great opacity of the cornea. The ulceration of the cornea, as shown by fluorescein staining, lasted for two to three weeks in most cases. In one case (Rabbit No. 6), the cornea perforated on the fifteenth day. In another case (Rabbit No. 5) typical panophthalmitis developed in two weeks and the organism was isolated from the aqueous humor on the sixteenth day. Vascularization of the cornea was seen in all ten eyes within one to two weeks, and in eight of the ten eyes it developed a severe epaulette form. Inflammation did not subside for three to four weeks in most cases, and the organism could be reisolated from the cul-de-sacs for three to twenty days. The results are summarized in Table I.

Inoculation into rabbits by simple instillation. Neither saline-suspension nor mucin-suspension of the organism caused keratitis in two rabbits. From two eyes inoculated with the mucin-suspension, the organism could be reisolated for 10 and 12 hours, respectively. From two other eyes inoculated with the saline-suspension, reisolation of the organism was positive for eight and twelve hours, respectively.

Discussion. Hypopyon-keratitis due to *Pseudomonas* infection often occurs after topical application of broad-spectrum antibiotics in cases of minor injury of the cornea, such as foreign body removal.[3] It has been clearly demonstrated in rabbits that the corneal infection with *Pseudomonas* was definitely accelerated when the eyes were treated with chloramphenicol for four days prior to the inoculation.[4] By our previous study,[5] using electron microscopy, a heavy deposit of mucosubstances was demonstrated on the corneal surface after topical application of chloramphenicol. Mucin has the biological property of increasing the pathogenicity of bacteria. Thus it has been demonstrated in animals that a given dose of bacteria may be sublethal when injected in saline but lethal when injected in mucin solution.[6]

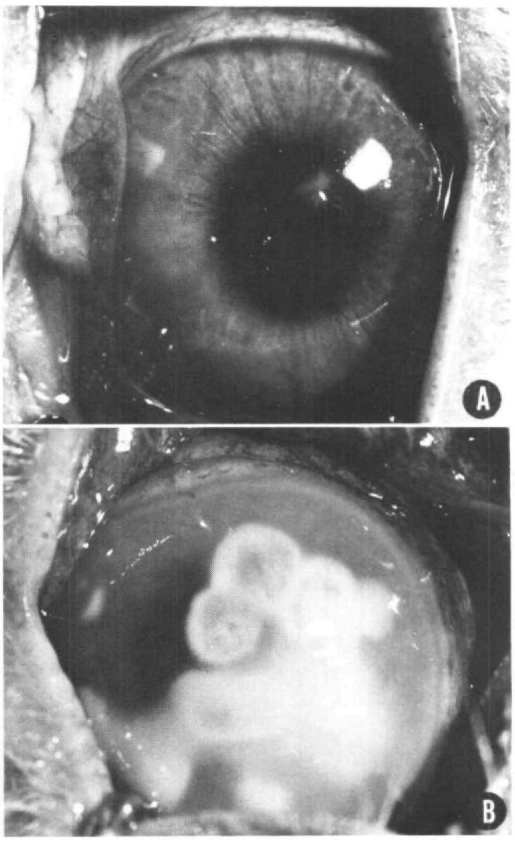

Fig. 2. *A*, rabbit cornea 48 hours after inoculation of *Pseudomonas* suspended in saline. Left eye, rabbit No. 2. Almost no change is seen. *B*, rabbit cornea 48 hours after inoculation of *Pseudomonas* suspended in mucin solution. Right eye, rabbit No. 2. Severe infiltrations with ulceration are seen at the sites of needlepricks.

Our present experiments in rabbits indicated that inoculation of *Pseudomonas* suspended in saline into the cornea by superficial needlepricks seldom produced severe infection. Moreover, when infection did occur, only small infiltrations of short duration developed round a few needle pricks. Whereas inoculation of the organism suspended in solution of gastric mucin by the same technique usually resulted in severe hypopyon-keratitis, characterized by ring-shaped abscesses developing at the sites of pricks within 24 to 48 hours. The keratitis progressed rapidly in most cases resulting in large abscesses of the cornea, sometimes with corneal perforation or panophthalmitis.

We have shown that the pathogenicity of

Table I. Summary of results on inoculation of *Pseudomonas* into rabbit cornea

Rabbit No.	*Inoculum in mucin (right eye)*			*Inoculum in saline (left eye)*		
	*Degree of keratitis**	*Duration of ulceration† (Days)*	*Period when re-isolation achieved (Days)*	*Degree of keratitis**	*Duration of ulceration† (Days)*	*Period when re-isolation achieved (Days)*
1	+++	18	20	±	2	1
2	+++	20	18	−	0	0
3	++	11	3	+	2	1
4	+++	>18§	6	++	6	4
5	++++	>28§	5(>16‡)	++	16	6
6	++++	25	5	±	2	1
7	++	10	4	+	4	3
8	++	10	8	++	10	7
9	+++	21	4	−	0	0
10	+++	20	4	++	5	4

*Keratitis − : No infiltration.
± : Negligible infiltration.
+ : Punctate infiltrations.
++ : Infiltrations and ulcerations as ring-shaped abscesses.
+++ : Confluent infiltration and ulceration as a huge abscess.
++++ : Perforation of the cornea or panophthalmitis.
†Period when fluorescein staining is grossly visible.
‡Re-isolation from the aqueous humor.
§Died on the nineteenth day (No. 4) and on the twenty-ninth day (No. 5).

Pseudomonas was increased when inoculated into the cornea as a suspension in mucin solution. A heavy deposit of mucosubstances on the corneal surface produced by chloramphenicol may, therefore, result in an acceleration of the manifestation of *Pseudomonas* keratitis, particularly when the cornea is slightly damaged.

The possible mechanism of the mucin-bacteria interaction is obscure, despite the efforts of a series of investigators.[8-10] *Pseudomonas* persisted slightly longer in the cul-de-sac of one rabbit when instilled in mucin suspension than instilled in saline suspension, probably due to the viscosity of mucin solution. The viscosity of mucin alone, however, is not a determining factor, because other more viscous substances are inactive.[9] The growth of *Pseudomonas* in mucin solution is slower than that in broth as shown by the present experiment. The effect of mucin may be due chiefly to its action on the defense mechanisms of the hosts, such as the inhibition of the bactericidal properties of phagocytic cells[8] or the interference with the sensitization of organisms by antibody.[10] However, nothing is known with certainty.

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