Pseudomonas Aeruginosa Infections of the Eye

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• *Pseudomonas aeruginosa seldom invades the body except in persons or in organs lacking natural defenses, and usually the infection is chronic rather than acute, evoking little systemic response. When introduced into the cornea, however, as in penetration by a foreign body or in contaminated medicines, it acts with extreme virulence, in many cases causing blindness and even necessitating enucleation.*

Although many attempts at control of Ps. aeruginosa, even with powerful antibiotics, have been unsuccessful, polymyxin B appeared to have good effect and was tested in experimental infection of the cornea in rabbits.

It was demonstrated by preliminary studies in vitro that polymyxin B was effective against nine strains of Ps. aeruginosa which on inoculation caused rapidly progressive ulcers in the corneas of rabbits.

A strain of proved virulence was introduced into both eyes of each of 18 rabbits. The left eyes only were treated with subconjunctival injections at 48-hour intervals of a solution of polymyxin B, to which epinephrine was added as a vasoconstrictor to prevent rapid dispersion. The right eyes remained untreated as controls.

In five of the six rabbits treated immediately after inoculation, the treated eyes remained clear, while moderate infiltration developed in the sixth. In the six rabbits not treated for 24 hours after inoculation, ulcers developed but remained localized during therapy. In those not treated for 48 hours after inoculation, ulcers developed before treatment began but did not spread as rapidly as in the controls.

Hyaluronidase was added to the preparation for half the rabbits in each group but had no perceptible beneficial effect.

THE DEVASTATING EFFECTS of infection of the eye by Pseudomonas aeruginosa (Bacillus pyocyaneus) have received much comment in ophthalmological literature. The most common form of infection is corneal ulcer, which spreads with frightening rapidity to panophthalmitis. The infection often follows penetration of the cornea by a foreign body which becomes embedded there. The ulcer usually becomes necrotic and is resistant to ordinary therapy. A hypopyon forms and perforation occurs with resultant intraocular involvement. In the majority of cases loss of useful vision in the affected eye ensues or evisceration may be necessary.

Ps. aeruginosa has been reported as an uncommon cause of other infections of the eye and ocular adnexa. Jacobi[19] stated that the first published report (1885) of Ps. aeruginosa infection in the ophthalmologic literature was that of a case of dacryocystitis; he did not cite the reference. Terson[43] reported a case of dacryocystitis due to Ps. aeruginosa in 1895. Seven cases of conjunctivitis associated with Ps. aeruginosa have been recorded. Of these, five occurred in newborn infants,[46, 8, 17, 41] two in adults. The case reported by Derby[8] was associated with meibomitis. In 1943 McCulloch reported one case of meibomitis, one of endophthalmitis following iridectomy, and one of infection following trephining, all thought to be caused by Ps. aeruginosa.

The first report of a corneal ulcer due to Ps. aeruginosa was that in 1891 of Sattler,[38] who, after isolating the causative organism, stated: "If we now compare the depicted bacteria with some of the known bacterial species that have been identified we see that it offers by far the greatest agreement with the bacillus pyocyaneus. I have had the opportunity to make a number of corneal inoculations and hypodermic injections in rats and mice with several cultures that I obtained from the Institute of Hygiene and am now convinced that results agree to a very striking degree with the picture that I have described above." Since this first report, many cases of corneal infection have been published. Mauersberg (1910),[28] Jacobi (1912),[1] Morelli (1922),[30] Garretson and Cosgrove (1927),[14] and Joy (1942),[22] have presented excellent summaries of a total of 64 cases occurring prior to 1939. In the interval 1939-1952, an additional 40 cases have been noted.*

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*References 1, 4, 5, 7, 23, 24, 27, 33, 40, 44, 45.

The relative increase in the number of reported cases during this latter period may be due partly to improvement in the methods of bacteriologic examination and diagnosis coincident with the onset of the antibiotic era.

PATHOGENESIS

Although almost all infections of the eye due to Ps. aeruginosa are exogenous, Jacobi[19] cited a case reported by Pergens in which a metastatic orbital abscess was found to contain Ps. aeruginosa. This is the only recorded case of endogenous Ps. aeruginosa infection of the eye or its adnexa in a human being. Panas[32] demonstrated that Ps. aeruginosa may enter the eye of rabbits from the blood stream. He recovered Ps. aeruginosa from the aqueous humor of rabbits injected intravenously with cultures of this organism and believed that a predisposing factor for the immigration of the organism was irritation of the eye. However, Stock[42] produced nodular iritis and disseminated choroiditis in rabbits without irritation of the eye by injecting Ps. aeruginosa into a vein of the ear.

Most infections of the eye with Ps. aeruginosa follow mechanical, chemical or thermal injury to the eye. It is believed that Ps. aeruginosa is an "opportunist," having no invasive power of its own, being ordinarily controlled by the defenses of the body. Regarding infection elsewhere in the body, Jawetz[20] wrote: "Should these defenses not be developed as in infants, or break down, as in debilitated persons, or should the bacillus be introduced passively into areas devoid of adequate natural defense mechanisms, it can set up an infection." As such infection elsewhere in the body is characterized by chronicity and by the fact that it evokes little systemic response and rarely disseminates, it is curious that in the cornea it should attain extreme virulence once the "opportunity" to gain a foothold has occurred.

The opportunity for invasion is often presented to the organism by the physician himself. Introduction of Ps. aeruginosa into an abrasion of the cornea is a major source of infection. Allen mentioned four cases of keratitis due to Ps. aeruginosa which were thought to be caused by contaminated sulfonamide stock solution in an industrial plant dispensary.[48] Garretson and Cosgrove reported seven cases in another industrial plant following removal of foreign bodies from the cornea; they assumed that the most likely source of infection was the 4 per cent boric acid solution used in the first aid room. Lepard[24] reported two cases of ulcerative keratitis due to Ps. aeruginosa which were thought to be caused by contaminated fluorescein solution. Thygeson[44] reported an ulcer of the cornea attributed to the same origin, and three similar cases have recently occurred in an industrial plant in the San Francisco Bay Area.[40] McCulloch[26] listed 18 cases in which Ps. aeruginosa had been identified on culture of material from the conjunctiva, in five of which contaminated eyedrops had been used, contaminated physostigmine solution in four, and contaminated fluorescein solution in one. In these cases the organism appeared to have caused two cases of corneal ulcer, one of endophthalmitis following iridectomy, one of meibomitis, and one of infection after trephining. Bignell[41] reported ten cases of infection of the cornea, noting that four cases occurred in one medical center and four in another. He urged regular sterilization of penicillin drop dispensers.

Many cases of corneal infection with Ps. aeruginosa at the site of injury are not directly traceable to any one source, and it is of course impossible to institute prophylactic measures for these. However, the incidence of "office infections" can be decreased by assuring the sterility of medicine containers. Besides the solutions already mentioned, McCulloch[26] noted contamination with Ps. aeruginosa in solutions of Pontocaine,® pilocarpine, ethylmorphine, scopolamine and atropine. In addition, cortisone solution is known to have been contaminated by the organism.

There have been cases of ulcerative keratitis of the eye due to Ps. aeruginosa in which apparently no corneal injury had been sustained. In 1899 Bietti reported a case caused by a blow upon the eye with a fist in which no corneal lesion was visible. Axenfeld[2] in 1917 was impressed by the toxic action of the organism, and discussed a case in which he made no mention of corneal injury. Ohm[31] noted one case in which the precipitating factor was thought to be drying of the cornea. Guyton[16] and Giannini[15] each reported a case in which no history of corneal injury was obtained. Braley noted five cases of rather severe burns, in which infection of the skin with Ps. aeruginosa extended to the eye.

INVASIVENESS OF THE ORGANISM

A high degree of variation exists in the invasiveness found in the many strains of Ps. aeruginosa. Some strains have proven relatively innocuous, while others are so highly invasive that Herrenheiser[18] considered them the most dangerous of the pyogenic organisms of the eye. Despite the wide variety of methods utilized in combating the infection, Joy[22] said: "It is probable that the degree of virulence of the organism is more responsible for the outcome than is any particular form of therapy." This statement would appear to be borne out by the many failures in treatment. Indeed, similar infections of the urinary tract, the meninges, the middle ear, and those of surgical wounds have stubbornly resisted the barrage of therapeutic agents employed against

them. It is thought that the diversified nutritional ability of Ps. aeruginosa enables the organism to circumvent the action of most antibacterial agents.

These findings, combined with the fact that Ps. aeruginosa itself produces bactericidal substances, have stimulated a great deal of experimental and clinical research with this organism. In 1860 Fordos[12] isolated the blue pigment pyocyanine from cultures of Ps. aeruginosa. This has proven to have antibacterial properties. It has since been shown by Wrede and Strack[49] to be only one of the antibacterial agents produced by this organism. The others as listed by Florey et al.[11] include the "pyo" products, of which there are four, as well as pyolipic acid, and hemipyocyanin.

In 1893, Rumpf reported beneficial results after injections of solutions of killed Ps. aeruginosa into patients with typhoid fever, according to Florey the first example of the treatment of human disease by the injection of products from an organism other than that which caused the disease. Emmerich and Löw[9] were the first to report on the antibacterial effectiveness of a crude extract of old cultures of Ps. aeruginosa. They called this preparation pyocyanase. Escherich[10] reported successful use of pyocyanase in treating diphtheria, and Lowenstein[25] reported the successful treatment of various infections of the eye and conjunctiva.

In discussing contamination of medicines with Ps. aeruginosa, McCulloch[26] made the interesting observation that if a solution contained Ps. aeruginosa it nearly always yielded a pure culture of the bacillus. Thygeson[44] observed in a case of meibomitis that culture of the lid margin disclosed "no staphylococci at all, not even non-pathogenic strains."

REVIEW AND PRESENT STATUS OF THERAPY

Because of the persistence of Ps. aeruginosa and its ability to acquire resistance,[13] a wide variety of therapeutic agents have been employed against it with little success: hot compresses, bichloride of mercury, boric acid, pilocarpine, and Mercurochrome,® among others. Joy[22] was the first to introduce sulfa therapy into the ever-growing list of agents. He showed that large doses of sodium sulfapyridine fed prophylactically to rabbits was of definite value in retarding infection, although the response progressively decreased with an increase in the interval between the inoculation and the institution of therapy. Good results were obtained when the agent was given orally before inoculation or within six hours after, but if it was not given within 18 hours the results were not significant. The work of Robson and Scott[35] also emphasizes the necessity for promptness in instituting therapy: Using instillations of 30 per cent solution of sodium sulfacetamide four or five times daily for experimentally induced lesions of the cornea, they found that if the instillations were begun within five hours after inoculation, favorable results could be attained; but if not until twelve hours had elapsed, the results were minimal.

Seeking a therapeutic procedure that would act rapidly and adequately against organisms that have penetrated into the stroma of the cornea, Von Sallmann[47] attempted a combination of oral sulfa therapy with local sulfa drugs introduced by iontophoresis. He used sulfapyridine, sulfadiazine and sulfacetamide on experimentally induced lesions in rabbits, and found that sulfadiazine entered the eye in greater amounts than the other drugs. The most favorable results were obtained by using a combination of sodium sulfadiazine introduced iontophoretically and sulfadiazine powder given locally and orally 24 to 30 hours after inoculation.

Reports conflict as to the efficacy of penicillin therapy. Juler and Young[23] treated a case with locally applied penicillin drops without beneficial effect. Sorsby[39] likewise reported no response to penicillin administered subconjunctivally (one million units at intervals of 24 to 48 hours). Alpert,[1] however, succeeded in curing ring abscess of the cornea caused by Ps. aeruginosa with injections of 75 units of penicillin into the anterior chamber of the affected eye combined with oral doses of sulfadiazine. Pendexter[33] treated a case with penicillin drops and intramuscular injection of penicillin; the ulcer healed and the vision was recorded as 20/200 three months after onset. Despite the use of streptomycin, penicillin, and Saemish section, however, Maschler[27] was unable to restore vision in an ulcerated eye, although removal of the eye was not necessary.

In a recent report Bignell[4] stressed the importance of clinical recognition of infection and institution of early and continued treatment. He obtained good results by subconjunctival administration of streptomycin plus streptomycin drops. He continued this therapy until "no dead white infiltration" remained.

Despite these successful results with sulfonamid, penicillin and streptomycin, most strains of Ps. aeruginosa have recently been shown to be resistant to these drugs in vitro.[13] It has also been shown that the most effective antimicrobial agent now available for the suppression of this organism is polymyxin.[21, 34] Wiggins[48] found polymyxin to be more effective than either aureomycin or streptomycin against 85 strains of Ps. aeruginosa in vitro. He also obtained beneficial effects upon severe experimental Ps. aeruginosa infection of the cornea in rabbits when polymyxin was started as late as 24 hours after inoculation. Ross,[36] in a similar trial, was unable to con-

clude that polymyxin was of practical value, but Braley pointed out that in Ross's experiment the infection was much more severe and therefore results would not be expected to be as good as those obtained by Wiggins.

Miller and Long[29] treated a case of Ps. aeruginosa infection of the eye with polymyxin given intramuscularly and in an eye bath in addition to streptokinase and streptodornase, the latter being used because of a thick mucopurulent exudate. Some vision was retained in the affected eye and they expressed the opinion that if therapy had been instituted earlier, a better result could have been obtained.

Jawetz[20] stated: "Polymyxin B does not permit readily the development of resistance in populations of microorganisms exposed to its action." He showed that in only one of a series of 35 cases of Ps. aeruginosa infection of various portions of the body did the bacteria reappear during a short course of treatment with polymyxin.

It would seem, therefore, that some form of treatment with polymyxin might be effective against Ps. aeruginosa in ulceration of the cornea. With this in mind, the effect of polymyxin was tested in vitro and on experimental Ps. aeruginosa infections of the cornea in rabbits.

EXPERIMENTAL STUDIES

A. Sensitivity Tests in Vitro

1. Disc sensitivity tests:

Nine strains of Ps. aeruginosa isolated from patients at the University of California Hospital were used. The 24-hour growth of the organism on proteose No. 3 slants was suspended in 0.9 per cent saline solution. A 10^{-4} dilution of this suspension was evenly spread over proteose No. 3 agar plates and discs of filter paper containing 5 mcg., 10 mcg., and 30 mcg. respectively of polymyxin were placed upon each plate. The plates were incubated at 37 degrees C. for 18 hours. The presence of a zone of inhibition surrounding the discs was taken as qualitative evidence of the sensitivity of the strain of Ps. aeruginosa to polymyxin.

With all nine strains zones of inhibition surrounded the three discs on each plate. The largest zone of inhibition was noted about the 30 mcg. disc.

2. Tube dilution sensitivity tests:

Nine strains of the organism were tested against serial twofold dilutions of polymyxin, 0.2 ml. of the 10^{-4} dilution of each strain being placed in ten tubes containing 2 ml. of sterile proteose No. 3 broth and the serial dilutions of polymyxin. Turbidity indicated resistance of the strain to polymyxin as diluted. The results of this test are given in Table 1.

TABLE 1.—*Tube tests of sensitivity of nine strains of Ps. aeruginosa to serial dilutions of polymyxin B sulfate*

Tube	Units of Polymyxin in 2 Ml. of Broth	Survival of Strains
1	500 (0.5 ml.)	None
2	250 (Dilution of 1)	1 and 9
3	125 (Dilution of 2)	1 and 9
4	63 (Dilution of 3)	All
5	31 (Dilution of 4)	All
6	16 (Dilution of 5)	All
7	8 (Dilution of 6)	All
8	4 (Dilution of 7)	All
9	2 (Dilution of 8)	All
10	1 (Dilution of 9)	All

Two loopsful from the tubes showing no turbidity were subcultured in sterile broth containing no antibiotic. None of the subcultured tubes showed turbidity after 72 hours of incubation at 37 degrees C. This indicated that the polymyxin had a bactericidal, rather than a bacteriostatic effect upon all the strains of Ps. aeruginosa tested.

B. Virulence Tests in Vivo

1. Plate counts:

In order to determine the approximate number of organisms to be inoculated into the corneas of test rabbits, plate counts were made of three dilutions of each of the nine strains of the organism. One ml. of a 10^{-4} dilution of each was placed in nine separate petri dishes and mixed with melted agar (45 degrees C.). After cooling, the plates were incubated at 37 degrees C. for 24 hours and the colonies counted through a 3-power magnifying lens. Similar counts were made upon the 10^{-5} and the 10^{-6} dilutions.

Plates having between 30 and 300 colonies each were used in determining the number of organisms per milliliter of inoculum.

It is estimated that on the average 0.02 ml. of the 10^{-4} dilution of each of the nine strains contained approximately 200 organisms.

2. Inoculation of rabbit corneas:

Intracorneal inoculations containing 0.02 ml. of the 10^{-4} dilution of each of the nine strains of Ps. aeruginosa were made. Within 24 hours a very definite corneal ulcer was visible in all eyes. The infections were observed for four days and were seen to progress rapidly to involve the entire cornea in all cases.

C. Local Treatment of Experimentally Produced Ps. Aeruginosa Infections of Rabbit Corneas

1. Methods:

One virulent strain of Ps. aeruginosa was selected from the nine strains previously tested. Intracorneal inoculations of both eyes of 18 rabbits were made, each inoculation containing 0.02 ml. of a 10^{-5} dilu-

tion, in 0.9 per cent saline solution, of a 24-hour culture of the strain (approximately 50 organisms in each cornea).

Six rabbits received treatment immediately after inoculation with the organisms. Six were not treated until 24 hours after inoculation, and the remaining six received no treatment for 48 hours. Treatments in all cases were continued for six days.

Half the rabbits in each group received subconjunctival injections containing 200,000 units (20 mg.) of polymyxin, in 0.5 ml. of 0.9 per cent saline solution. To retard dispersion of the drug by the circulation, 0.1 ml. of a 1:1000 solution of epinephrine was added as a vasoconstrictor. Only the left eyes were treated, the right eyes serving as controls. The injections were repeated at 48-hour intervals.

The other rabbits in each group received subconjunctival injections containing exactly the same combination of polymyxin and epinephrine. To promote more rapid infiltration of the antibiotic to the deeper levels of infection, 0.05 ml. of hyaluronidase (containing 10 turbidity-reducing units) was added to the preparation. In these rabbits also, only the left eyes were treated, and the injections were repeated at 48-hour intervals.

2. Results:

In the rabbits treated immediately after inoculation all six control eyes became infected and corneal ulcers developed. These ulcers were easily visible within 24 hours and by 48 hours had extended over the entire cornea. The three eyes treated only with polymyxin plus epinephrine remained clear and showed no evidence of infection of the cornea. Two of the three eyes treated with polymyxin. epinephrine, and hyaluronidase remained clear. In the third eye a moderate infiltration developed in the cornea, which became hazy, although no discharge was noted; this effect subsided somewhat after the third injection.

In the rabbits treated from 24 hours after inoculation, all six control eyes became infected and rapidly progressive corneal ulcers developed. In the other six eyes circumscribed ulcers of the cornea 3 to 5 mm. in diameter developed but remained localized throughout the six-day course of therapy with minimal discharge. No significant difference was noted between the three eyes which had been treated with hyaluronidase and the three which had not.

In the rabbits which received no treatment for 48 hours after inoculation, four of the six control eyes became infected and rapidly progressive corneal ulcers developed, while the other two eyes remained clear. In all six treated eyes rather large corneal ulcers developed before treatment was begun, but by comparison with the controls it was concluded that the treatment checked the further spread of the infection. No significant difference was noted in the eyes treated with hyaluronidase.

It is possible that the action of hyaluronidase contributed to the further spread of the infecting organisms as well as the polymyxin. It is believed that a significant change in the results might be attained by larger doses.

The high concentration of polymyxin in the injections caused a chemical irritation of the conjunctiva, but this subsided in the 48-hour periods between injections.

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