POLYMYXIN E*
PENETRATION INTO THE EYE AND THERAPEUTIC VALUE IN
EXPERIMENTAL INFECTION DUE TO *PS. PYOCYANEA*

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THE polymyxins are a group of five closely allied antibiotics extracted from the *Bacillus polymyxa*. *In vitro* and in the field of general medicine they have been found bactericidal to nearly all Gram-negative bacilli, and the good results obtained in combating *Ps. pyocyanea* infection of burns by the local application of polymyxin (Jackson and others, 1951) suggested the possibility of using them locally in eye diseases.

Early experiences with polymyxins A and D, and later with polymyxin C, showed that they caused damage to the renal convoluted tubules (Stansly and others, 1947; Brownlee and Bushby, 1948) and consequently they were not widely used clinically. Polymyxin B (Brownlee and others, 1949) was found to be almost free from nephrotoxic effect, but it gave rise to neurotoxic signs such as ataxia, and also led to severe irritative reactions at the site of injection.

The most recent preparation, polymyxin E, has a toxicity similar to that of polymyxin B, but does not cause irritation at the site of injection. It therefore seemed probable that polymyxin E would be suitable for sub-conjunctival injection. The general level of antibiotic in the body after this method of administration would be small and thus unlikely to lead to any neurotoxic reaction.

Eye infections with Gram-negative bacilli are not common but may be of the greatest severity. Treatment in the past has been unsatisfactory but recently good results have been reported with streptomycin therapy (Bignell, 1951; Sorsby and others, 1952). Bignell collected ten cases infected with *Ps. pyocyanea* during 1950. In his case notes he emphasizes the severity and rapid spread of the infection; he considers that pyocyanea ulcers can be recognized clinically, showing first as a dead white area in the cornea with central superficial necrosis, and later assuming the form of a white ring with a semitransparent centre. If untreated the whole cornea rapidly becomes disorganized. Sorsby also emphasizes the characteristic clinical appearance of corneal ulceration due to Gram-negative bacilli.

PROPERTIES OF POLYMYXIN

Polymyxin E is prepared as a white crystalline powder which is highly soluble in water. 1,000,000 units dissolve readily in 1 ml. Its molecular weight is in the

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neighbourhood of 1,000, which is about three times the size of the penicillin molecule. It is not readily soluble in lipoids and the ether-water partition ratio is low.

It has been shown by Langham (1951) that water-soluble substances with a low ether-water partition ratio appear to enter the intra-ocular fluid through the inter-cellular spaces of the blood-aqueous barrier, while the lipoid-soluble substances themselves pass *via* the cells. Substances with large molecules cannot pass through the inter-cellular spaces and will not therefore cross the blood-aqueous barrier unless they are lipoid-soluble.

Polymyxin E is thus unsuitable for systemic administration in intra-ocular infection, but fortunately its extreme solubility in water renders it highly suitable for subconjunctival injection, and, as will be seen, direct penetration of the coats of the eye, when given by this method, is very satisfactory. The aqueous solution, even in concentrations of 1,000,000 units per ml., causes little irritation when given subconjunctivally, though it is best to add a local anaesthetic to the solvent. Mydracaine is suitable and has the added advantage of containing adrenaline. Subconjunctival scarring as a result of the injections is minimal.

EFFECTS OF POLYMYXIN INJECTED SUBCONJUNCTIVALLY

A single dose of 500,000 units of polymyxin (0.5 ml. of solution) was injected subconjunctivally in two rabbits. The subconjunctival bleb remained for 3 days but disappeared leaving no trace. Slight corneal oedema developed in both instances but this resolved entirely.

Two further rabbits were injected with 200,000 units (0.2 ml. of solution). The smaller subconjunctival blebs resolved in about 48 hours. The corneae remained bright throughout.

Five rabbits were given a more prolonged course, 330,000 units (0.33 ml. of solution) on 4 successive days, followed by 200,000 units on 2 subsequent days. Large subconjunctival blebs arose but settled down in from 4 to 6 days after cessation of treatment and left no trace. In one rabbit a faint corneal haze developed on the 3rd day but this had cleared entirely 2 days after the cessation of treatment. In another rabbit a small band-shaped opacity developed in the interpalpebral area. The conjunctival blebs in this case were considerably larger than in the others and the opacity appeared to be due to exposure. Had it been possible to pad the eye this would probably have been prevented. As the conjunctival oedema settled the opacity lessened, but a permanent nebula resulted. In the three remaining cases the corneae remained bright throughout.

PENETRATION OF POLYMYXIN INTO THE EYE FOLLOWING SUBCONJUNCTIVAL INJECTION

ASSAY.—The assay of polymyxin in ocular fluids is difficult owing to the small volumes of these fluids which are available. Since an anterior chamber puncture will not yield more than 0.25 ml., it is impossible to use the normal serial dilution methods (Brownlee and Bushby, 1948) using 1-ml. volumes.

Our first assays were made by adding 0.1 ml. aqueous fluid to a filter paper disk and placing this on a plate seeded with the test organism. By this technique small amounts of polymyxin were detected in samples aspirated from 45 minutes to 2 hours after a subconjunctival injection of 500,000 units

of polymyxin. No polymyxin was detected before 45 minutes or after 24 hours had elapsed since the subconjunctival injection. Unfortunately the rings of inhibition of growth were small and could not be compared accurately with rings produced by standard amounts of polymyxin.

To obtain more accurate assays it was decided to use a capillary tube micro method. The technique was that described by Fleming and Smith (1947) for the assay of penicillin, except that the medium used was 1 per cent. glucose digest broth at pH 7.8 and the test organism was a *B. coli* strain S.1. The disadvantages of this technique have been pointed out by May and others (1947). To minimize errors the method was rigidly standardized and with each batch of fluids assayed a control assay of 100 units of polymyxin was included.

TECHNIQUE.—1 ml. of the phenol red-glucose broth medium was inoculated with *B. coli* S1 and incubated overnight. The next morning 5 cmm. of this culture were added to each ml. of medium to be used in the test. Tests were set up using 25 cmm. volumes for the serial dilutions, and it was noted that the aqueous always imparted a slightly more alkaline pH to the first tube of each series. Capillaries were filled, sealed at both ends, and incubated horizontally for 18 hours. In every case eight two-fold dilutions were set up.

Readings were made on the following scale:

Negative	...	...	No colour change
±	...	...	Incomplete change of colour.
+	...	...	Complete change of colour but no gas formation.
++	...	...	Complete change of colour with gas formation.

Five corneae were assayed. These were prepared as follows:

A disk of cornea was removed by means of a cork borer from an eye which had been frozen immediately after its enucleation. The disk was weighed and then ground up in saline, 0.1 ml. of buffered saline (pH 6.8) per mg. of moist weight.

Vitreous fluid was obtained by dividing the frozen eye immediately behind the lens; the vitreous could then be picked out as a solid mass and thawed for use in the assay.

It was found that, with strict adherence to technique, assays of standard solutions of polymyxin gave identical readings.

RESULTS

(a) *Aqueous.*—Twenty-five aqueous fluids were assayed, all being removed between 1 and 3 hours after subconjunctival injection of 250,000 units of polymyxin E in aqueous solution. The whole operation was performed under nembutal anaesthesia. Of the twenty-five fluids, nine showed polymyxin contents of 200 units per ml. or greater, ten from 50 to 100 units per ml., and five from 10 to 50 units per ml. No relationship between time of removal and concentration was noted in specimens aspirated between 1 and 3 hours after giving polymyxin.

(b) *Vitreous.*—Six vitreous fluids were assayed 3 hours after subconjunctival injection. They had a similar range to that of the aqueous fluids, the actual levels being 200 units per ml. in two cases, 50 to 100 units in three cases, and 20 units in the remaining case.

(c) *Cornea*.—Five corneal extracts were assayed 3 hours after subconjunctival injection. They showed a somewhat higher concentration, and, with more prolonged extraction, this might have been still greater. Concentrations were 2,000 units per g. or more in two cases, 100 to 200 units in a further two cases, and 100 units in the remaining case.

TREATMENT OF EXPERIMENTAL CORNEAL INFECTION

It was decided to assess the value of polymyxin in pyocyanus corneal infections produced experimentally since the cornea is the area most commonly found to be infected by this organism in ophthalmic practice.

INOCULUM.—The organism used to produce the eye lesions was a stock laboratory strain No. 32 which had been isolated 12 months earlier. The organism was grown in digest broth for 24 hours before use and then diluted 10^{-3} or 10^{-4} in broth. Estimation of the number of organisms present by the technique of Miles and Misra (1938) gave viable counts of 10,000 organisms at 10^{-4} . When 0.04 ml. of a 10^{-4} dilution (500 organisms) was injected, it was found that about 60 to 70 per cent. of controls were infected. One hundred per cent. of infections could be produced by 0.02 ml. of a 10^{-3} dilution, i.e., 2,000 organisms.

The virulence of the infections produced is shown by their rapid progress in all the control cases. There was no sign of spontaneous resolution.

Further, in ten untreated animals, nine gave positive cultures of *Ps. pyocyanea*, and one a mixed growth of *Ps. pyocyanea* and *Staph. albus*.

EXPERIMENTS.—These were divided into two groups: in Group I the effect of an immediate subconjunctival injection of polymyxin on the prevention of infection was investigated, and in Group II treatment was not started until there was already an established lesion.

GROUP I. Immediate Treatment

Preliminary Trial.—The right corneae of four rabbits were infected with *Ps. pyocyanea* (500 organisms in 0.04 ml.). Two, untreated, developed severe infection, and within 48 hours the whole cornea was involved. These two rabbits were killed, and culture of the corneae revealed a profuse growth of *Ps. pyocyanea*.

The two remaining animals received 500,000 units of polymyxin E subconjunctivally within 30 minutes of the infecting intra-corneal injection of *Ps. pyocyanea*; the same dose was given 24 hours later, and again 24 hours after that. After the first 24 hours moderate corneal oedema developed in both cases but this disappeared within 7 days. There was no sign of infection in either instance. Faint nebulae along the needle tracks were all that remained.

Series 1.—The right corneae of seven rabbits were injected with *Ps. pyocyanea* (500 organisms in 0.04 ml.), and three were kept as controls.

In all three controls corneal infiltration rapidly progressed and there was advanced infection after 48 hours. In one the whole cornea was involved after 72 hours and the rabbit was killed. Culture revealed a heavy growth of *Ps. pyocyanea*. The two other controls also showed marked and increasing infiltration after 48 hours and each was given 500,000 units of polymyxin subconjunctivally. This was done as a preliminary trial for the delayed treatment series to follow. In both, infiltration was very advanced

at the time of injection, but 24 hours later there was less discharge and there had been no further extension of the lesion. There was, however, marked ulceration and the rabbits were destroyed.

The cases treated immediately received 500,000 units of polymyxin E subconjunctivally within 30 minutes of the intra-corneal injection of *Ps. pyocyanea*. To three of the rabbits a further 500,000 units were given on the 2 subsequent days and 250,000 on the fourth day. Moderate corneal haze developed in all, but there was no sign of infection. After 14 days two showed very faint corneal nebulae while in the third the nebula was more marked and there was slight corneal vascularization.

In the fourth instance 500,000 units were given on the first 3 days but on the fourth day the rabbit appeared very weak and was destroyed. The cornea appeared free from infection. It was cultured and there was no growth. One other rabbit in this series became weak and ataxic on the fourth day but rapidly recovered. It is probable that this was a toxic manifestation and this is discussed below.

Series 2.—The right corneae of five rabbits were infected with *Ps. pyocyanea* (500 organisms in 0.04 ml.). One remained as control and developed a rapidly extending infection, the whole cornea being involved in 72 hours. Culture revealed *Ps. pyocyanea* in large quantity.

The four remaining animals each received 200,000 units of polymyxin subconjunctivally within 30 minutes of the initial infection and then further doses at 24-hour intervals. A very slight corneal haze developed in all cases but there was no sign of infection. All that remained after 10 days was very slight nebula formation at the position of the needle puncture in the cornea.

Series 3.—The right corneae of eight rabbits were infected (500 organisms in 0.04 ml.). Two served as controls, and the treatment given to the remaining six was as in Series 2. Again the controls rapidly became infected while all the treated animals remained free from infection.

This series was photographed at intervals. A typical case is shown in Figs 3–6. Figs 1 and 2 show a control rabbit at 24 and 48 hours. The rapid progress of the infection is clearly shown.

Thus, in Group I, all six control rabbits became rapidly infected while the fourteen which received prophylactic polymyxin were protected in every instance.

GROUP II. Delayed Treatment

Series 1.—The right corneae of seven rabbits were infected with *Ps. pyocyanea* (500 organisms in 0.04 ml.) but three did not develop infection. The remainder developed marked ulceration.

Of the four which became infected, one was left as a control and ulceration proceeded rapidly, the whole cornea becoming involved in 48 hours. The animal was killed, and culture of cornea showed a heavy growth of *Ps. pyocyanea*.

After 24 hours the three other cases showed infiltration with ulcers 6 to 7 mm. in diameter, and 330,000 units of polymyxin were given.

Two received a total of four injections at daily intervals after which time, as infection appeared to be overcome, treatment was stopped. There was no recurrence and the eyes settled down and became entirely quiet in 10 to 12 days. Moderate nebulae with slight vascularization remained after 28 days.

The third treated case had a 3-mm. hypopyon after 4 days' treatment, but, as the ulcer was improving, treatment was continued for a further 2 days, when the hypopyon disappeared and the cornea healed. A dense nebula resulted but the eye became quiet.

FIG. 1.—Control case. Ulcer on day after infecting dose.

FIG. 2.—Same control case one day later. Note discharge and involvement of whole cornea.

FIG. 3.—Appearance of a case in immediate treatment series on day after infecting dose. No discharge—moderate corneal oedema.

FIG. 4.—Same case one day later.

FIG. 5.—Same case 6 days after initial injection. FIG. 6.—Same case 9 days after initial injection. (All six photographed cases in this series gave identical results.)

Series 2.—The right corneae of eight rabbits were infected, 2,000 organisms in 0.02 ml. being given. Ulcers varying from 4 to 7 mm. in diameter were obtained after 24 hours. Treatment was started in six cases and two were left as controls.

In the controls infiltration spread rapidly during the next day, and the rabbits were killed. Culture of the corneae revealed *Ps. pyocyanea*.

The six cases treated each received 330,000 units of polymyxin E subconjunctivally for 6 days and 150,000 units on the seventh. In three instances infiltration rapidly decreased and after 15 days slightly vascularized nebulae were all that remained.

In the fourth case the cornea healed well but the rabbit died on the 12th day, when treatment had already been stopped for 5 days. Culture of the cornea revealed no *Ps. pyocyanea* but a few colonies of *B. coli*.

In the two remaining cases, moderate infiltration was still visible on the 11th day, and in one, definite discharge had restarted. Both animals were killed; in the case with discharge the corneal culture grew *Ps. pyocyanea*, but in the other culture only a few colonies of staphylococci were found.

Series 3.—Eight corneae were injected (2,000 organisms in 0.02 ml.) and 24 hours later all were found to be infected. In two rabbits left as controls, infection advanced rapidly, and they were both killed after 48 hours. The six treated rabbits all received 330,000 units of polymyxin E for 4 days, followed by 200,000 units for 2 days. Photographs were taken of this series, a typical result being shown in Figs 7–12.

The infection was overcome in all the treated animals in this series. In five the recovery was uneventful, and after 10 days the eyes appeared quiet though there were dense nebulae. The sixth rabbit died from intercurrent infection on the 8th day (two days after cessation of treatment). The cornea was excised and cultured but no *Ps. pyocyanea* was found. Clinically the ulceration appeared to be entirely overcome.

Series 4.—The right corneae of seven rabbits were infected with *Ps. pyocyanea*. On the next day corneal ulceration and infiltration had occurred in every case.

The rabbit showing the least infiltration was left as a control, and the remaining six had surface cultures taken. In one case there was no growth but in the other five *Ps. pyocyanea* was grown. Polymyxin (100,000 units) was given 24 hours after the initial infection and at this time the corneal infiltration varied from 3 to 6 mm. in diameter.

Treatment was continued, giving 100,000 units of polymyxin subconjunctivally each day. After 48 hours of treatment surface cultures were clear in every case, even though there was still marked infiltration. After 72 hours five of the rabbits were progressing satisfactorily, but in one there was still considerable ulceration and the animal was killed. Culture from the excised cornea, however, revealed no *Ps. pyocyanea*. In the remaining five animals, treatment was continued daily for a total of 6 days. After 96 hours all the surface cultures were still free from *Ps. pyocyanea*, though one gave a moderate growth of *Staph. aureus*, and one a profuse growth of diphtheroids.

In four instances uneventful healing occurred, leaving small nebulae moderately vascularized. The remaining case appeared free from infection on the 11th day but the nebula was bulging slightly and the rabbit was killed. The cornea was excised and found to be thinned in the centre. It seemed to be free from infection and culture gave no growth.

In the control rabbit infection had spread rapidly and the animal was killed after 48 hours. Culture gave a profuse growth of *Ps. pyocyanea*.

In Group II, therefore, the ulceration was entirely overcome in fifteen (71 per cent.) of the 21 cases treated. There was a marked improvement in five instances (24 per cent.), and in these, although some infiltration and ulceration remained, culture of the excised corneae in no case revealed

FIG. 7.—Cornea on day after intra-corneal injection of *Ps. pyocyanea*. Treatment with subconjunctival polymyxin was started immediately after this photograph was taken.

FIG. 8.—Same cornea 2 days later. Note absence of discharge.

FIG. 9.—Same cornea on seventh and last day of treatment. Infiltration and oedema still present.

FIG. 10.—Same cornea 14 days after initial injection. Firm nebula forming—vascularization commencing.

FIG. 11.—Same cornea on 21st day.

FIG. 12.—Same cornea seventh week. Eye quiet. Firm nebula—superficial vascularization has shown no change for two weeks.

Typical example of a successfully treated case.

Ps. pyocyanea. The rabbit which died only 2 days after the cessation of treatment has been included here, since there was still a possibility of recurrence, although the corneal infection appeared to have been overcome.

In the remaining case the infection was delayed but was not overcome, and culture of the excised cornea revealed *Ps. pyocyanea*. In every instance there was an infection of considerable severity before treatment was started.

CLINICAL USE OF POLYMYXIN

We have had the opportunity of trying the subconjunctival injection of polymyxin clinically in only two cases. In these, no untoward local or systemic reaction occurred even though daily injection of 200,000 units was given for 5 days.

The injection, if made with mydracaine as the solvent for the polymyxin, is not painful, though a varying amount of aching is to be expected, starting after half an hour and lasting for about 45 minutes.

(1) One case when seen had already had penicillin and was having streptomycin but the ulceration and hypopyon were still increasing. The ulceration had followed a corneal foreign body. The clinical appearance was that of a *Ps. pyocyanea* ulcer but a surface culture did not give a growth.

The subconjunctival injection of 200,000 units of polymyxin led to the disappearance of the hypopyon within 2 days and rapid improvement. After 21 days, however, there was an increase of corneal infiltration, the eye again became irritable, and the hypopyon recurred. Subconjunctival polymyxin (200,000 units daily) was again given and within 2 days the hypopyon had gone and the corneal infiltration was much improved. Injections were continued for a further 3 days and recovery was uneventful.

(2) In the second case marked endophthalmitis was already present when first seen and culture revealed *Bacillus coli*. Polymyxin (200,000 units) were given daily for 3 days. The eye quietened but too much disorganization had occurred to give hope of useful vision and the eye became soft.

DISCUSSION

Polymyxin can be injected subconjunctivally in doses sufficient to allow therapeutic amounts to penetrate the globe without causing any serious local toxic effects.

In the case of two rabbits given 500,000 units of polymyxin subconjunctivally (Group I, Series 1), weakening of the legs, particularly the hind legs, developed. There is little doubt that this was a manifestation of the neurotoxic effect of polymyxin E, but there was no other instance of this symptom where smaller and apparently adequate doses were used.

Further, a dose of 500,000 units in a rabbit corresponds to a systemic dose of 250,000 units per kg. bodyweight, whereas a similar injection in man would correspond only to about 10,000 units per kg., which is approximately the recommended systemic dose. Ten thousand units (1 mg.) of polymyxin E

per kg. bodyweight have been administered 4-hourly to human subjects for as long as 2 weeks without apparent toxic effect (Swift and Bushby, 1951). It appears from our experimental findings that, so far as corneal infections are concerned, it is unnecessary to give a dose as large as 500,000 units. This is, in fact, inadvisable as it leads to greater corneal oedema than does a smaller dose.

The experimental results suggest that polymyxin should overcome corneal infections with *Ps. pyocyanea* in man in a high proportion of cases if the infections are seen and diagnosed early. We are anxious to obtain such cases for treatment.

Ps. pyocyanea was chosen as the infective organism in these experiments since it is particularly resistant to treatment by other means. It is probable that polymyxin would be at least equally effective against infection with other Gram-negative organisms.

SUMMARY*

(1) Polymyxin E is highly soluble in water. It is relatively non-irritant and therefore suitable for subconjunctival injection.

(2) In the rabbit an injection of 250,000 units of polymyxin subconjunctivally led to therapeutic levels in the cornea, aqueous, and vitreous after 1 hour.

(3) Intracorneal injection of *Ps. pyocyanea* failed to produce infection in fourteen rabbits given subconjunctival polymyxin E immediately after the infecting dose. Rapidly extending infection developed in six control rabbits.

(4) Twenty animals with experimentally produced *Ps. pyocyanea* ulcers were treated with subconjunctival polymyxin E. Infection was overcome in all but one case. In the six untreated controls infection spread rapidly.

(5) No proven cases of pyocyanous corneal ulcers have been treated clinically. Subconjunctival injections of 200,000 units of polymyxin have been given to adults without untoward effects. In the two treated cases of corneal ulceration of which we have experience the results were encouraging.

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* Since this article was written a few cases of *Ps. pyocyanea* corneal infection in humans have been treated with polymyxin, and the results have been promising.