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COMMUNICATIONS

SUB-CONJUNCTIVAL PENICILLIN AND INTRA-OCULAR INFECTION*

BY

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THE success of local penicillin in the treatment of certain superficial lesions of the eye has stimulated interest in the possible value of this remedy for deep intra-ocular infection especially since Schu-mine warfare has provided many favourable opportunities for study.

Before it can be stated that any given drug will control infection, two conditions must be fulfilled: (a) the organisms of the infection must be susceptible to the drug, and (b) the drug can be brought to bear on the organisms in effective concentration.

The first condition is difficult to determine so far as penetrating eye wounds of warfare are concerned, but the work of Bentley and Scott Thomson¹ on the bacteriology of somatic war wounds offers a clue as to what bacterial flora may generally be expected within the eye. These observers found that in Italy the *S. pyogenes aureus* was by far the most common pyogenic coccus present and that the *Strept. pyogen. (haemolyticus)* occurred in about 6 per cent. of all wounds. In a series of 100 cases examined before operation at C.C.S. level, 51 cases had pyogenic cocci in the

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wound (Hutchison). It is at this level that definitive ophthalmic surgery commences in modern warfare. The various strains of *S. pyogenes aureus* found were examined for penicillin resistance. It was found that whilst 20 per cent. were resistant to 0.1 unit per c.c. (parenteral therapy concentration) few organisms were able to grow in broth containing 50 units per c.c. indicating the possible beneficial results which could be expected by using the higher concentrations of local penicillin applications.

The author has reported previously² the results of the bacteriological examinations of the aqueous and vitreous in cases of penetrating wounds of the eye, but as these cases were all late and examined some time after the initial injury, when the inflammation was subsiding, they cannot be accepted as valid evidence of the causal organism.

The second condition of effective concentration of a drug in the eye largely depends on the routes by which the drug is administered. All possible routes for penicillin administration have been widely explored by American workers who used rabbits as their experimental animals, and a summary of their recent work follows.

Penicillin may be caused to reach the interior of the eye by the following methods:—

- (1) Local applications such as instillations, ointments, iontophoresis or constant corneal baths.
- (2) Subconjunctival injections.
- (3) Intra-aqueous injections.
- (4) Intra-vitreous injections.
- (5) Intra-venous injections.
- (6) Intramuscular injections.

The bacteriostatic standard adopted in this paper is that of Rammelkampf and Keefer³ and Salter⁴ namely that the maximum bacteriostatic effect against *Staph. aureus* is obtained by 0.1 unit penicillin concentration and against *Strep. haem.* by 0.01 unit penicillin concentration per c.c. serum.

(1) *Local applications.*—The penetration of local penicillin into the aqueous has been studied by Leopold and Lamotte⁵ who state that it does not appear in the anterior chamber of a normal rabbit eye after single or constant instillations of drops (500 O.U. per c.c. isotonic saline) or after the application of ointment (500 O.U. per gramme Carbowax). If however, one third of the corneal epithelium is damaged or if experimental infection of the cornea (*P. leptiseptica*) is present then bacteriostatic quantities of penicillin will be found in the aqueous.

These observations confirmed the work of von Sallmann and Meyer⁶ who also showed that the addition of wetting agents of the aerosol group was without effect on penetration.

Turning to the penetration produced by constant corneal baths

and iontophoresis von Sallmann and Meyer⁶ pointed out that penicillin appeared in moderate quantity in the aqueous after a single corneal bath but that after ionisation the concentration was increased by ten times. Von Sallmann⁷ also compared the amount of penicillin to be found in the aqueous after iontophoresis (2 milli-amp. for 5 minutes) with that of sodium sulphacetamide after similar treatment and the amounts were about equal. When, however, experimental infection of the anterior chamber was present (*S. aureus*) nine of twelve eyes were saved by penicillin iontophoresis whereas only one eye was saved by sodium sulphadiazine iontophoresis.

Dunnington and von Sallmann⁸ later published the results of their work on penicillin iontophoresis and experimental infection (*S. aureus*) of the rabbit lens. After penicillin iontophoresis of these cases infection and shrinkage of the eye was the eventual result but when direct penicillin injections were given into the lens within six hours of inoculation the eyes were saved. Their work showed that penicillin introduced into the eye by iontophoresis does not enter the lens in bacteriostatic concentration and it is likely that this route is more valuable for anterior eye infections.

Bellows⁹ investigated the penetration of penicillin into the eye when constant corneal baths (20,000 O.U. per c.c.) and various ointment bases (25,000 O.U. per gramme) were used.

After the ointment was placed over the conjunctiva and cornea of a rabbit for one hour the aqueous was subjected to penicillin assay and it was found that the use of the "vanishing" type stearate base produced most penetration. However, as Bellows points out, a diffuse corneal stain followed the use of this base and probably accounted for the increased penetration.

Leopold and Lamotte⁵ are also inclined to think that corneal damage accounts for the high aqueous concentrations obtained by corneal baths and iontophoresis technique. Von Sallmann¹⁰ had previously pointed out that corneal baths of 0.5 to 1 per cent. sod. penicillin solution did cause damage to corneal epithelium and superficial stroma but that 0.1 per cent. sod. penicillin solution produced no corneal damage even by iontophoresis¹¹.

(2) *Subconjunctival injections*.—Bellows⁹ has compared the aqueous concentration of penicillin after corneal baths with that obtained from subconjunctival injections and he found it to be much less after the injections but still to be of bacteriostatic amount.

Leopold¹² found that penicillin was detectable in the vitreous forty-five minutes after subconjunctival injections of 2,500 O.U. sod. penicillin every 3 hours for 72 hours, but that where experimental inflammation of the anterior eye was present the penicillin vitreous concentration appeared earlier and was higher.

Without previous inflammation the vitreous concentration after

45 minutes was 0.078 O.U. per c.c. whereas when the anterior chamber was infected the concentration was 0.12 O.U. per c.c. after 15 minutes and 0.51 O.U. per c.c. after 45 minutes.

(3) *Intra-aqueous injections*.—Dunnington and von Sallmann⁸ have pointed out that the injection of sodium penicillin into the anterior chamber of the rabbit eye produces only a transient inflammatory reaction of mild degree.

Leopold¹² found that after an injection of 250 O.U. per c.c. into the anterior chamber, penicillin was detected in the vitreous of normal eyes at bacteriostatic concentration fifteen minutes later but that here again the vitreous concentration was much increased if previous inflammation of the anterior chamber was present. In eyes subjected to previous iridectomy the vitreous concentration rose higher than it did in normal eyes suggesting the value of the presence of penicillin in the anterior chamber in inflamed aphakic eyes.

(4) *Intra-vitreous injections*.—Von Sallmann, Meyer and di Grandi¹³ found that when penicillin was injected directly into the vitreous of rabbits it remained in uneven distribution and that the concentration fell sharply for twelve hours and thereafter remained after twenty-four hours at bacteriostatic levels. In only one case did the aqueous show any bacterial inhibition after an intra-vitreous injection. These authors stress the importance of the delayed resorption of penicillin from the vitreous as a therapeutic measure. The author² has described one case in which penicillin was found in the vitreous of a human eye three days after an intra-vitreal injection of 2,000 O.U. sod. penicillin.

Dunnington and von Sallmann⁸ have shown that one intra-vitreous injection of penicillin will constantly control susceptible vitreous infection provided the injection is given within twelve hours of inoculation.

After twenty-four hours the penicillin injection did not stop the inflammation but there was some improvement noticeable. Larger inoculations did not affect the results of treatment nor were multiple injections more beneficial than a single injection.

Von Sallmann, Meyer and di Grandi studied the effects of these injections on the eye structure and took the protein concentration of the aqueous as a measure of the irritation produced. The protein content of the normal rabbit aqueous is from 19 to 51 mg. per 100 c.c.; after one intra-vitreous injection of 0.2 normal saline (control) it rose to an average of 183.2 mg. per 100 c.c. and after 2.5 per cent. sodium penicillin it was 247 mg. per c.c. These levels were much lower than that produced by an intra-vitreous injection of 10 per cent. sodium sulphacetamide.

After one intra-vitreous injection of purified penicillin there was moderate flare and a fine veil in the vitreous which was resorbed in a short period. On section four eyes showed circumscribed retinal atrophy of 1—2 disc diameters.

A crude penicillin injection produced an accentuation of these changes with more extensive retinal damage.

When, however, a second injection was made within twenty-four hours the damage was severe, amounting to lens changes, to heavy vitreous exudates and retinal detachment.

The importance of purity in relation to toxic effects has also been stressed by Florey and Jennings¹⁴ and later by Herrell and Nichols¹⁵. Von Sallmann, Meyer and di Grandi conclude therefore that a single intra-vitreous injection of purified potent penicillin is a reasonable safe procedure and is justified in the treatment of exogenous infections of the vitreous along with other adjuvant methods.

Leopold¹² using intra-vitreous injections of various concentration from 2,500 O.U. to 5 O.U. in infected eyes showed that six eyes treated with 2,500 O.U. penicillin were quiet after four weeks and that all eyes receiving more than 1,000 O.U. per injection were favourably influenced. Even in those which received amounts as low as 5 O.U. per injection the inflammation was never as severe as it was in the control eyes.

(5) *Intra-venous injection*.—The distribution of penicillin in the eye after intravenous injection has been shown by Struble and Bellows¹⁶ to be present in decreasing quantities in the extra ocular muscles, sclera, conjunctiva, aqueous, vitreous and cornea.

Leopold¹² using intra-venous injections of 4,000 O.U. sodium penicillin per kilogram of rabbit body weight was unable to detect the drug in the vitreous of normal eyes after 180 minutes and only a trace in the vitreous of those where there was previous infection of the anterior chamber. This is not surprising in view of the experiments of Abraham and others¹⁷ who have demonstrated the rapid excretion in high concentration in the urine of cats after intra-venous penicillin injection.

Leopold¹² investigated 12 eyes with artificial vitreous inflammation (*S. aureus*) and found that all the eyes were lost in spite of two hourly intra-venous injections of sod. penicillin (4,000 O.U. per kilogram of body weight) for seventy-two hours.

(6) *Intra-muscular injections*.—Similarly Leopold¹² studied the effect of intra-muscular injections of 4,000 O.U. sod. penicillin per kg. of rabbit body weight, but even with the addition of vasoconstrictors no more than a trace of penicillin reached the vitreous.

Leopold and Lamotte⁵ using intra-muscular injections of 1,500 O.U. sod. penicillin per kilogram of body weight were unable to detect penicillin in the aqueous of normal eyes later, and only small quantities in those with inflammation of the anterior chamber in amounts from 0.4 unit per c.c. after fifteen minutes to 0.15 unit per c.c. after 105 minutes (average of four eyes).

Elsewhere² the author has reported parallel cases in man—both with regard to the presence of penicillin in the aqueous and vitreous

after intra-muscular injections and also the inability of penicillin to control intra-ocular infection when given by this route.

Seven normal eyes were removed from battle casualties as soon as possible after death. Five of these casualties had previously received a massive dose of intra-muscular penicillin on an average nine hours before. Two eyes were used as controls but the aqueous and vitreous from each of the seven eyes showed no bacterial inhibition.

In order to reduce the time interval between injection and assay, eight casualties, who were due to have a hopeless eye removed, were given massive doses of intra-muscular penicillin before operation. The enucleated eye was then subjected to assay within half an hour of removal, but again no inhibition was detectable in any of the examples.

A survey of this experimental evidence allows certain conclusions to be reached so far as rabbits are concerned, namely :—

(1) Direct intra-vitreous injections of penicillin are the most effective means of obtaining high concentrations and bacteriostasis in the vitreous.

(2) That a single injection of purified penicillin does little harm and will control susceptible infection if given within twelve hours and will maintain bacteriostasis up to at least 24 hours. Multiple injections cause serious intra-ocular damage.

(3) That local application of drops or ointment will not pass penicillin into the aqueous in normal eyes but do so where the cornea is inflamed or traumatized.

(4) That iontophoresis, constant corneal baths and subconjunctival injections will maintain bacteriostatic levels in the aqueous especially in the presence of inflammation.

(5) That intra-muscular and intra-vitreous injections of penicillin do not produce bacteriostatic levels in the aqueous or vitreous and will not control experimental susceptible inflammation there.

In applying the lessons of these experimental results to the treatment of intra-ocular infection in man it was decided to determine the value of subconjunctival injections, for this was a method which appeared to be a useful adjuvant to local penicillin therapy and at the same time less dangerous than direct intra-ocular injection.

The value of the time factor and the presence of previous inflammation on the absorption of penicillin should be borne in mind.

It was necessary to prove that penicillin could be caused to reach the interior of the human eye in bacteriostatic amounts after subconjunctival injections before this method could logically be employed in therapy.

Experimental technique

Selected eyes were either hopelessly blind and due for removal or required conservative operation.

Four thousand Oxford units of sodium penicillin in normal saline were injected beneath the conjunctiva in the infero-lateral or infero-medial intermuscular quadrant about 8—10 mm. from the limbus. Previous anaesthesia was by 4 per cent. cocaine drops but these did not entirely prevent transitory stinging sensations in certain cases. Skin preparation was by 1:2,000 neutral proflavine application and after the injection the eye was irrigated with warm saline to remove any penicillin which might have leaked back along the track of the needle. After the desired interval of 15, 30 or 60 minutes the eye was again irrigated and aspiration of the media was carried out by a fine (23 S.W.G.) hypodermic needle passed obliquely through the cornea or sclera or sclerotomy wound; 0.12 c.c. aqueous or vitreous was then withdrawn and immediately subjected to penicillin assay by the following method:—

Penicillin assay by Major G. D. Cunningham, M.B.E., R.A.M.C.

An approximate assay of the amount of penicillin present in the aqueous and vitreous was performed by the "agar-cup" method described by Garrod and Heatley²¹. Blood agar plates were poured with agar bases. Subsequent to inoculation with a 6 hour broth culture of the Oxford strain of staph. aureus, holes were made in the agar with a cork-borer. The blood agar was lifted out with a platinum loop, leaving a thin layer of agar in the base of the "cup." The fluids for examination were introduced into the cups by means of a syringe fitted with a hypodermic needle and in every case the amount introduced was about 1/30 c.c. In each case the penicillin which had been used for subconjunctival injection was also checked for potency by the same method.

For the purpose of assay varying dilutions of a freshly prepared solution of penicillin were tested by the same method and the width of inhibition measured. The dilutions were made with a phosphate-buffer solution of pH 6.8. In all, three batches of penicillin were tested, two manufactured by Pfizer and one by Squibb and the estimation was performed in duplicate.

A graph was drawn from an average of the above results and the concentration of penicillin in the aqueous and vitreous calculated directly from the graph. Proflavine was found to have a very slight inhibitory action on the Oxford type of staph. aureus, but when mixed in equal quantities with penicillin did not significantly reduce the inhibitory action of the latter.

Discussion

The series is of necessity small, owing to the fact that suitable human material is not readily available. From the data, however, it would appear that penicillin can be constantly absorbed into the aqueous following subconjunctival injection. Absorption into the

vitreous has occurred in 3 cases out of 6. Of the remaining two it is possible that the short time elapsing between the subconjunctival injection and the removal of the vitreous (15 minutes) was responsible for the failure of absorption in one case. In this connection it is interesting to note that in his series of rabbit eyes Leopold¹² obtained no absorption into the vitreous under 30 minutes. In the other case (case 8) there was considerable disorganisation of the eye and this may account for the lack of absorption.

The following table summarises the results obtained.

Observations on experimental results

It will be seen from the table that after a subconjunctival injection of 4,000 O.U. sod. penicillin the drug penetrated to the aqueous humour in seven of the eight cases after an interval of fifteen minutes. The only negative case was probably due to the short interval of aspiration after injection.

Assay of the vitreous was carried out in six cases of which three cases showed bacteriostasis after forty-five minutes. Two of the three negative cases were examined 30 minutes or less after injection and this may account for the lack of inhibition.

All amounts of penicillin detected in aqueous or vitreous after subconjunctival injections were above bacteriostatic level.

Conclusion

(1) Subconjunctival injections of 4,000 O. units sod. penicillin will produce bacteriostasis of susceptible organisms in the aqueous humour of the human eye within half an hour of injection. It is probable that the same state of affairs is later attained in the vitreous humour and is likely to remain so for at least twenty-four hours.

(2) Subconjunctival injections of penicillin represent a practical method of causing penicillin to reach the interior of the eye. They have been widely employed in this Theatre without harmful effects and the earlier they are employed, the better the results.

(3) Large doses should be employed not only because of the slow rate of diffusion but also to avoid the risk of producing penicillin resistance (18) (19).

Florey²⁰ has written "Penicillin must be kept continuously in contact with all infected tissues until the natural body defences have had time to deal with the infection." It is therefore probable that the best results of penicillin therapy for deep intra-ocular infection will be obtained by a combination of routes and of these the subconjunctival method is practicable and harmless. Experimental evidence stresses the importance of bringing the drug in contact with the invading organism at the earliest possible stage.

RESULTS OF 10 CASES

SUB-CONJUNCTIVAL PENICILLIN AND INTRA-OCULAR INFECTION

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No.	Name	History	Date	Penicillin Subconjunctival Injection	Aspiration Interval after Injection	Aqueous Assay of Penicillin		Vitreous Assay of Penicillin		Control	Comment
						Area of Inhibition	O. Units per c.c.	Area of Inhibition	O. Units		
1	Y.	Old complete detachment of retina. R.E. A.C. aspiration	May 2, 1945	4,000 O.U. in 0.9 per cent. sterile saline	15 minutes	5 mm.	3	Not taken	Not taken	17 mm.	Aqueous inhibition after 15 minutes
2	O'H.	Sarcoma of the choroid. L.E. enucleation. Aqueous and vitreous aspiration	May 6, 1945	ditto	60 minutes	7 mm.	10	11 mm.	>20	16 mm.	Both aqueous and vitreous inhibition after 60 minutes
3	P.	Absolute glaucoma due to childhood injury. R.E. enucleation. Aqueous and vitreous aspiration	May 10, 1945	ditto	30 minutes	3 mm.	1	Negative. No inhibition	Nil	17 mm.	No vitreous inhibition after 30 minutes
4	B.	Acute bilateral iridocyclitis and secondary glaucoma due to multiple penetrating wounds of both eyes. (Schu mine). R.L. aqueous aspirations	May 10, 1945	R.E. 4,000 O.U. in 0.9 per cent. sterile saline. L.E. control (nil)	30 minutes	R. E. 8 mm. L. E. nil	R.E. 15 L.E. nil	R.E. not taken. L.E. not taken	Nil Nil	17 mm.	R. E. aqueous inhibition. L.E. was control and was negative
5	C.	Penetrating transverse wound of both orbits. Loss of right eye. Avulsion of L. optic nerve. L.E. aqueous aspiration	May 9, 1945	4,000 O.U.	30 minutes	3 mm.	1	Not taken	Not taken	13 mm.	Aqueous inhibition after 30 minutes

RESULTS OF 10 CASES—continued

No.	Name	History	Date	Penicillin Subconjunctival Injection	Aspiration Interval after Injection	Aqueous Assay of Penicillin		Vitreous Assay of Penicillin		Control	Comment
						Area of Inhibition	O. Units per c.c.	Area of Inhibition	O. Units		
6	S.	Absolute glaucoma R.E. Aqueous aspiration	May 14, 1945	4,000 O.U.	30 minutes	4 mm.	2	Not taken	Not taken	16 mm.	Aqueous inhibition after 30 minutes
7	K.	Penetrating wound L.E. G.M. extraction of I.O.F.B. Vitreous aspiration through scleral incision	May 20, 1945	ditto	45 minutes	None	None	8 mm.	15	14 mm.	Marked vitreous inhibition after 45 minutes
8	V.	Penetrating wound R.E. Phthisis bulb. R.E. enucleation. Aqueous and vitreous aspiration	May 31, 1945	ditto	90 minutes	9 mm.	20	Negative. No inhibition	—	14 mm.	Aqueous inhibition but no inhibition of vitreous after 90 minutes
9	P.	Penetrating wound R.E. G.M. extraction of I.O.F.B. Vitreous aspiration through sclerotomy incision	May 31	ditto	90 minutes	None taken	—	7 mm.	10	14 mm.	Vitreous inhibition after 90 minutes
10	H.	Absolute glaucoma from old penetrating injury. R.E. enucleation. Aqueous and vitreous aspiration	May 18	ditto	15 minutes	Negative	—	Negative. No inhibition	—	15 mm.	This case was completely negative for aqueous and vitreous inhibitions after 15 minutes

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PENICILLIN IN OPHTHALMOLOGY*

An interim review

BY

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LONDON

1.—Ocular pharmacology of penicillin

Tolerance

PENICILLIN in current clinical use is adequately standardised as regards potency, but is still largely an impure product so that a final assessment of the ocular tolerance to this drug as distinct from the available mixtures, is not possible at the present. The following data must therefore be treated with some reserve,

* Based on a lecture delivered at the Royal College of Surgeons, May 29, 1945.