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COMMUNICATIONS

CONTROL OF EXPERIMENTAL INFECTION OF THE VITREOUS BY PENICILLIN*

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ATTEMPTS to control experimental vitreous infections by the use of penicillin involve a clear understanding of the concentrations of penicillin made possible by different methods of application, and also an appreciation of the fact that to be effective treatment must be initiated before the vitreous becomes disorganized. In a series of earlier communications (Sorsby and Ungar, 1946; 1947; 1948a and b) it has been shown that levels of penicillin concentration in the vitreous were low after systemic administration of 50,000, 250,000, 500,000, and 1,000,000 units. The application of concentrated ointments (50,000 U/g.) likewise gave unsatisfactory results. The values obtained by the injection of 50,000 units subconjunctivally were also not high, but became significant

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when adrenaline 1:1,000 was used as the solvent. A high level persisting for 48 hours was obtained by the direct intravitreal injection of 5,000 units, and levels almost as high and as persistent could be obtained by the injection of 1,000,000 units in adrenaline, 1:1,000 subconjunctivally. The subconjunctival injection of 1,000,000 in water, or the retrobulbar injection of 1,000,000 units in either adrenaline or water, gave distinctly lower, though still tangible, levels. The Figure shows the more significant of these findings. On the basis of these studies, the best method of treatment would appear to lie between the direct intravitreal injection of 5,000 units and the subconjunctival injection of 1,000,000 units in adrenaline.

FIGURE.—Levels of penicillin in the vitreous reached by different dosages and methods of administration (after Sorsby and Ungar, 1948a, Graph 2).

Experimental studies (Sorsby and Ungar, 1946; 1948a, c) have shown that :

- vitreous infections do not respond well to intramuscular injections, and still less to the application of concentrated ointments, and although subconjunctival injections of 50,000 units gave satisfactory results, these were not uniform;
- direct intravitreal injections likewise gave inconstant and on the whole unsatisfactory results;
- preliminary trials with subconjunctival injections of 1,000,000 units gave promising results, but emphasized the need for early treatment. Treatment begun 24 hours after infection prevented panophthalmitis but left the vitreous as a grey organized mass.

Present Investigations

In the present study an attempt has been made to assess the value of retrobulbar and subconjunctival injections of penicillin, treatment being initiated either immediately after infection or 3 hours later.

Treatment by Retrobulbar Injection of 1,000,000 Units.—Rabbits weighing about 2 kg. each were used; 100,000 organisms of *Staph. aureus* 663 suspended in 0.05 ml. saline were introduced centrally into the vitreous.

The injection was made by the means of a tuberculin syringe into the right eye of each animal. Treatment consisted of retrobulbar injection of 1,000,000 units penicillin in 1 ml. water or adrenaline 1:1000. Two animals were used as controls, and four further batches of two animals each were first treated (a) immediately, (b) at 3 hours, (c) at 6 hours, and (d) at 12 hours after infection respectively.

The controls developed panophthalmitis within 48 hours and were destroyed. The course of events in the treated animals is recorded in Table I.

TABLE I

Retrobulbar Injection of 1,000,000 Units Penicillin in 1 ml. Water or Adrenaline

Infection: Intravitreal injection of 100,000 organisms *Staph. aureus* 663

Time of Treatment in Relation to Infection	Penicillin 1,000,000 units in 1 ml. water	Penicillin 1,000,000 units in 1 ml. adrenaline 1 : 1,000
Immediately and 24 hours after	<i>Rabbit A</i> died 2 days after	<i>Rabbit E</i> at 7 days cornea hazy, suppurative conjunctivitis at 17 days cornea opaque, suppurative conjunctivitis at 12 weeks eye normal except for small corneal scar
3 and 24 hours after	<i>Rabbit B</i> died 5 days after	<i>Rabbit F</i> at 7 days slight conjunctivitis, otherwise normal at 17 days normal at 12 weeks normal
6 and 24 hours after	<i>Rabbit C</i> at 7 days normal at 17 days normal at 12 weeks normal	<i>Rabbit G</i> at 7 days conjunctivitis, otherwise normal; died on 13th day, eye normal
12 and 30 hours after	<i>Rabbit D</i> at 7 days conjunctivitis, otherwise normal at 17 days thin vitreous veil at 12 weeks normal	<i>Rabbit H</i> at 7 days conjunctivitis, otherwise normal; animal very ill, died the following day

It will be seen that four of the eight animals died during the course of the experiment. A post-mortem examination revealed no definite cause for these deaths. There was in particular no evidence of any generalized infection; as two of these deaths occurred early they prevented any assessment of the therapeutic effect of the injection on the vitreous infection. The results, as far as they went, suggested the following conclusions:

(i) Injections of 1,000,000 units penicillin in water retrobulbarly, given to one animal 6 hours after infection and 18 hours later, and to another animal 12 hours after infection and 18 hours later, were effective in controlling a heavy vitreous infection.

(ii) A similar dose of penicillin in adrenaline was effective in controlling the infection in the surviving animals in which it had been employed, irrespective of whether the first treatment was given immediately, or 6 hours after infection.

In this group of animals there was a severe conjunctival reaction and in one the cornea became opaque. These reactions proved transient and were apparently irritative in nature. These irritative phenomena are discussed more fully below.

Treatment by Subconjunctival Injection of 1,000,000 Units.

(a) Fifteen rabbits were used. The same infecting agent and mode of injection was employed but the dose was increased to 250,000 organisms suspended in 0.05 ml. saline. Three animals were used as controls. Six animals were treated by injections of penicillin at 3 hours and 24 hours after infection, and six received the same treatment with a third subconjunctival injection at 48 hours. The three control animals developed panophthalmitis within 36 hours and were destroyed. The course of events in the twelve treated animals is shown in Table II. It will be seen that six of the twelve animals had to be destroyed on the tenth day owing to severe conjunctival and corneal reactions, with which were associated considerable irritative lesions on the skin of the lids and the surrounding areas. Clinically, the impression was gained that the ocular infection was under control, but the state of the animals did not warrant their being kept alive. Two possibilities had to be considered: that the severe intra-ocular infection was not controlled and produced heavy corneal reactions; or that the penicillin injections had proved irritative. The latter possibility proved to be true, as was shown by independent tests with another apparently pure sodium penicillin preparation. The significant finding is that all the six animals that were kept alive ultimately did well; three infected eyes were completely normal by the ninth week and the remaining three infected eyes were functionally good organs. By the 13th week four eyes were completely normal and the remaining two had minimal lesions of no great significance. It will be noted that of these six animals

TABLE II

*Subconjunctival Injection of 1,000,000 Units Penicillin in 1 ml. Water*Infection: Intravitreal injection of 250,000 organisms *Staph. aureus* 663

Rabbit No.	At 4 days	At 24 days	At 9 weeks	At 13 weeks
<i>Treatment at 3 hours and 24 hours after infection</i>				
1	Hypopyon, eye quiet, but shows grey reflex	Destroyed on 10th day	—	—
2	Cornea hazy	Dense central corneal opacity, good red reflex	Slight corneal haze, vitreous clear, penicillin retinitis	Very slight corneal opacity and penicillin retinitis
3	Leucoma, otherwise normal	Small corneal opacity, vitreous clear, small area of organization in periphery of vitreous below	Slight corneal scar, penicillin retinitis	Normal
4	Died on 3rd day	—	—	—
5	Heavy conjunctivitis, cornea hazy	Corneal opacity, good red reflex, vitreous probably normal	Normal	Normal
6	Upper third of cornea hazy	Small corneal opacity, fairly heavy central vitreous opacity, penicillin retinitis	Normal	Normal
<i>Treatment at 3 hours, 24 hours, and 48 hours after infection</i>				
7	Upper cornea hazy	Cornea clear, central vitreous opacity	Normal	Small post-cortical lens opacity
8	Eye normal, but conjunctiva inflamed	Cornea and vitreous clear, penicillin retinitis	Slight penicillin retinitis	Normal
9	Cornea hazy	Destroyed on 10th day	—	—
10	Cornea hazy	Destroyed on 10th day	—	—
11	Cornea opaque	Destroyed on 10th day	—	—
12	Cornea opaque	Destroyed on 10th day	—	—

treated to conclusion four had received only two injections, one at 3 hours and the other at 24 hours after infection.

(b) As these results were inconclusive a further batch of eight rabbits was infected and six were treated at 3 and 24 hours, the remaining two being kept as controls. The controls developed panophthalmitis within 36 hours and were destroyed. The results obtained in the treated animals are set out in Table III. It will be seen that in no case was there a complete recovery, and that in only two cases could the result be regarded as reasonably satisfactory.

TABLE III

Subconjunctival Injection of 1,000,000 Units Penicillin in 1 ml. Water
Infection: Intravitreal injection of 250,000 organisms *Staph. aureus* 663
Treatment at 3 hours and 24 hours after infection

Rabbit No.	At 10 days	At 5 weeks	At 8 weeks
13	Some corneal haze, vitreous clear, localized opacity of retina below (? needle injury)	Slight corneal haze above, penicillin retinitis, good functioning eye	Vitreous opacity in needle track, good functioning eye
14	Cornea clear, vitreous organized	Organized vitreous, use-eye	No change
15	Extensive corneal reaction, upper part of vitreous organized	Died, but eye condition apparently satisfactory	—
16	Cornea clear, vitreous opacities. (? good functioning eye)	Steel-grey organized vitreous	No change
17	Vitreous hazy, two white organized opacities	Traumatic detachment of retina	No change
18	Cornea normal, fairly dense vitreous opacities with one organized opacity	Needle track in vitreous with surrounding opacities, fairly good functioning eye	Vitreous opacities above, penicillin retinitis below, fairly good functioning eye

(c) On the assumption that a less virulent infection might give more consistent results a further batch of ten rabbits was infected with 100,000 organisms. Into two of these, dead organisms (heat-killed) were injected, and two further rabbits injected with living organisms were used as controls. The controls ran their usual destructive course, and the two rabbits which had received dead organisms showed no tangible reaction—in one case the eye was completely normal 7 days later, and in the other there was a

circumscribed vitreous opacity in the track of the injecting needle. The results in the remaining six treated at 3 and 24 hours, are set out in Table IV. It will be seen that here again no complete recovery is recorded, but that in two eyes the result could be regarded as reasonably satisfactory.

TABLE IV

Subconjunctival Injection of 1,000,000 Units Penicillin in 1 ml. Water

Infection: Intravitreal injection of 100,000 organisms *Staph. aureus* 663

Treatment at 3 hours and 24 hours after infection

Rabbit No.	At 7 days	At 28 days
19	Localized corneal vitreous opacities, functioning eye, fundus normal	Localized vitreous opacity, good functioning eye
20	Vitreous haze, fundus just visible	Eye grossly infected (relapse one week previously)
21	Vitreous haze, fundus just visible	Slight penicillin retinopathy, otherwise normal
22	Marked iritis, occluded pupil	Eye grossly inflamed and useless, cornea opaque
21	Organized vitreous	Eye grossly inflamed and useless, cornea opaque
22	Vitreous haze with some organization	Organized vitreous, some inflammation

Treatment of Less Severe Infection by Subconjunctival Injection of 250,000 Units.—In these cases the inconclusive results suggested two possibilities. In the first place, there was the possibility that the heavy infection by 250,000 or 100,000 organisms was in itself a limiting factor in obtaining adequate results. The second and more likely possibility was that, unlike human patients, rabbits do not tolerate subconjunctival injection of 1,000,000 units. It was observed repeatedly that the rabbits tended to scratch their eyes and it was felt that much of the reaction seen in these experimental eyes was the result of reinfection of mechanical injury. In the last series of experiments the infecting dose was therefore reduced to 50,000 organisms and the therapeutic dose to 250,000 units. The first treatment was given a quarter of an hour after infection and the second 24 hours after infection. Two rabbits were used as controls and developed panophthalmitis within 24 hours. To prevent them from scratching their eyes with their claws, all animals were fitted with cardboard collars which were removed after 48 hours.

The results in ten treated animals are shown in Table V, from which it can be seen that three of the ten experimental eyes were completely normal at six days and that no substantial lesion was present in any of the remainder. Three days later, one of the animals had died and of the remaining nine only two had minor corneal lesions. At 17 days, one eye showed considerable vitreous disturbances and the rest could be regarded as satisfactory. At

TABLE V

Subconjunctival Injection of 250,000 Units Penicillin in 0.5 ml. Adrenaline

Infection: Intravitreal injection of 50,000 organisms Staph. aureus 663

Treatment at $\frac{1}{2}$ hour and 24 hours after infection

Rabbit No.	At 6 days	At 9 days	At 17 days	At 34 days
23	Eye normal	Normal	Normal	Normal
24	Eye normal	Normal	Normal	Normal
25	Eye normal	Normal	Large vitreous opacity above, inflammatory mass below, functioning eye	Infection flared up, animal killed, eye shows panophthalmitis
26	Slight conjunctival re-action	Normal	Some organized vitreous opacities, functioning eye	Normal
27	Cornea hazy, red reflex	Corneal haze less	Normal	Normal
28	Cornea hazy, conjunctiva swollen, good red reflex	Corneal haze less	Normal	Normal
29	Conjunctiva swollen, good red reflex	Normal	Normal	Normal
30	Normal	Normal	Normal	Normal
31	Found dead, eye excised and found normal	—	—	—
32	Upper lid swollen, good red reflex	Normal	Normal	Normal

34 days, all but one eye were normal and the eye that at 17 days had shown vitreous disturbances was now definitely grossly infected and at post-mortem examination showed extensive panophthalmitis. As the animal that had died on the 6th day had shown histologically a normal eye, the results of this series

may be taken as nine complete recoveries in ten infected eyes. In four of the nine eyes that were fully recovered, the track of the infecting needle could be seen in the vitreous.

It should be noted that in this experiment treatment was begun within a quarter of an hour of infection and maintained by a second injection of 250,000 units penicillin in 0.5 ml. adrenaline.

Discussion

In the light of the findings recorded in Table V, two things are clear.

(1) Vitreous infection can be controlled adequately by subconjunctival injection of penicillin.

(2) In the experimental animal results are limited by the amount of penicillin given and by the time within which treatment is instituted.

It is impossible to apply experimental findings uncritically to clinical conditions. An experimental animal with an infecting dose of either 250,000, 100,000, or 50,000 organisms has obviously no close parallel to clinical infections. What is of significance clinically, is that with an infecting dose of 50,000 organisms panophthalmitis develops within 48 hours in the experimental animal. It is therefore likely that most clinical infections are initially far less severe. It is also of relevance clinically that experimental infections of this type can be fully controlled by subconjunctival injections of penicillin, if the nine successful results out of ten recorded in Table V may be taken as evidence. In man injections of 1,000,000 units in 1 ml. solvent are relatively well tolerated. This does not apply to the rabbit, so that if any deductions may be drawn from these observations the prospects of controlling vitreous infection clinically are as good as these experimental results suggest.

The time factor is all-important. Nothing can be expected from a vitreous that already consists of a collection of pus. How soon such a condition develops in man will obviously depend on the virulence of the organism and possibly on the number of organisms introduced initially. In all cases it is obvious that treatment must begin early and that the classical sign of vitreous infection — a yellow vitreous reflex — is not an indication for treatment but evidence that the eye is already beyond treatment.

On the basis of the results recorded here the following clinical procedure is suggested :

(1) Treatment should be instituted at the earliest possible moment when infection of the vitreous is suspected or may become a possibility.

(2) Such treatment should be energetic and maintained beyond the point when all danger is past. It consists of giving 1,000,000 units crystalline penicillin

dissolved in 1 ml. water and 5 minims adrenaline 1:1,000. The injection should be repeated after 24 hours and maintained at the same interval for some two or three days after the vitreous is completely clear. In most cases this will mean maintaining treatment for 5 to 7 days.

(3) Nothing is to be gained by giving smaller doses, and the intravitreal injection of penicillin is clearly neither so desirable nor so effective as subconjunctival injection.

Summary

(1) Experimental infection of the vitreous by 50,000 organisms *Staph. aureus 663* leads to panophthalmitis within 48 hours.

(2) In nine out of ten experimental eyes, complete recovery was obtained by subconjunctival injection of 250,000 units crystalline penicillin in 0.5 ml. adrenaline 1:1,000 injected subconjunctivally at $\frac{1}{4}$ hour and 24 hours after infection. In the one eye that ended with panophthalmitis there was initial control with subsequent relapse.

(3) Heavier infections and heavier doses of penicillin gave inconsistent results, partly because the rabbit does not tolerate large subconjunctival doses of penicillin.

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