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INTRAVITREOUS STREPTOMYCIN: ITS TOXICITY AND DIFFUSION*

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THE discovery of the new antibiotic, streptomycin, by Waksman and his colleagues in 1944, has put at our disposal a substance capable of producing a chemotherapeutic action on organisms not particularly susceptible to other agents. Gram negative organisms and *M. tuberculosis* are the organisms for which streptomycin is particularly effective. Previous work has shown that streptomycin does not easily penetrate through the cornea (Leopold and Nichols, 1946) and it was decided to investigate the concentrations of the

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drug attained in ocular fluids following its administration (1) into the vitreous and (2) subconjunctivally. It is known that drugs injected into the vitreous may produce damage, sometimes severe, to the retina (Duguid *et al.*, 1947) and an investigation of the action of streptomycin on the eye tissues following its intravitreal administration was therefore also undertaken. The results of Bellows and Farmer (1947) published in the meantime would suggest that streptomycin is non-toxic to the retina and can safely be administered by the intravitreal route.

Methods

Two samples of streptomycin were used in these experiments and we are greatly indebted to the Antibiotics Study Section of the U.S. Public Health Service (through Dr. Seger) for supplying these. The pure sample was used for the intravitreal toxicity tests; it was streptomycin hydrochloride calcium chloride double salt (Pfizer lot X 76), and its biological activity against *B. subtilis* was 719 u/mg. For the diffusion experiments the second sample of commercial streptomycin (Merck) was used.

For the diffusion experiments 2,000 μ g. of streptomycin was injected into the vitreous in 0.1 ml. of sterile saline by the method previously described (Duguid *et al.*, 1947). For the toxicity experiments two doses were used, *viz.*, 1.2 mg., and 3.0 mg., injected in 0.1 ml. saline. In these latter experiments the eyes were observed at regular intervals for periods of 12 to 112 days and the changes noted. The eyes were then removed and examined histologically.

The method for assaying streptomycin in the aqueous and vitreous was based on the measurement of the diffusion of streptomycin through agar. The melted nutrient agar (Stebbins and Robinson, 1945) was seeded with a 24 hour culture of the Mayo Clinic strain of *staph. aureus* to a final dilution of 1/1000 and added to a series of glass tubes. After the tubes had cooled for five minutes small quantities of the eye fluids for assay were added above the agar. Four tubes were used for each eye fluid. The tubes were incubated at 37° C. for 24 hours and the zone of inhibition between agar-eye fluid and line of growth was measured in millimetres. The measurements were made by fixing a tube on to a slide, placing it on the mechanical stage of a microscope, and viewing the zone of inhibition through a 2/3 inch objective with a pointer attached. The transverse millimetre scale gave readings to the nearest 0.1 mm. With suitable controls of 2 and 64 μ g. per ml. a graph was constructed from which the unknown fluids were estimated.

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It was shown that streptomycin control solutions made up in saline gave identical zones of inhibition to those made up in buffered aqueous or vitreous and so the former were used for convenience throughout. The method is much more reliable if the fluids for assay are kept alkaline. All ocular fluids were therefore diluted with an equal quantity of m/10 phosphate buffer at pH 7.8 before assaying.

The method is accurate to within ± 20 per cent. and gives a reading down to $0.5 \mu\text{g.}$ per ml. Even small quantities of 0.2 ml. of aqueous are sufficient for four estimations by this method.

The concentration of streptomycin in aqueous and vitreous was measured, by the above method, 24, 48 and 72 hours after the injection of $2,000 \mu\text{g.}$ streptomycin in 0.1 ml. sterile saline into the vitreous (see Table I).

TABLE I
Concentration of streptomycin after intravitreal injection of $2,000 \mu\text{g}$ streptomycin.

Time after injection, hours	Rabbit Number	Concentration of streptomycin ($\mu\text{g. ml.}$)	
		Aqueous	Vitreous
24	36* R. Eye	41	—
	L. Eye	<0.5	—
	37* R. Eye	84	—
	L. Eye	<0.5	—
	38 R. Eye	43	340
	39 R. Eye	46	200
	40 R. Eye	86	215
48	41 R. Eye	20	—
	42 R. Eye	34	—
	43 R. Eye	24	—
72	44 R. Eye	12	—
	45 R. Eye	13	—

* Right eye only received streptomycin injection.

In another smaller series the concentration of streptomycin in the aqueous was measured 3, 6 and 24 hours after subconjunctival injection of $10,000 \mu\text{g.}$ streptomycin made up to a total volume of 0.2 ml. in $1/1000$ adrenalin (see Table II).

TABLE II

Concentration of streptomycin after injection of 10,000 $\mu\text{g.}$
in 1/1,000 adrenalin subconjunctivally.

Time after injection, hours	Rabbit Number	Concentration of streptomycin ($\mu\text{g. per ml.}$) in aqueous
3	1	20
	2	8
6	3	0.8
	4	0.5
24	5	<0.5
	6	<0.5

FIG. 1.

Concentration of streptomycin in aqueous after intravitreal injection of 2,000 $\mu\text{g.}$ of streptomycin. Aqueous-vitreous ratio at 24 hours = 0.25.

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For the toxicity experiments streptomycin was injected into the vitreous of the right eyes of five animals while as a control 0.1 ml. of normal saline was injected into the vitreous of the left eye in each case. One animal received seventeen doses of streptomycin subconjunctivally over a period of 20 days, when the globe was examined histologically.

Results

1. Diffusion of streptomycin.

The results are given in Tables I and II and shown graphically in Figs. 1 and 2. It will be seen that after the intravitreous injection of 2,000 μ g. of streptomycin, this acts as a depot and chemotherapeutic concentrations are maintained in the ocular fluids for over three days. The diffusion of streptomycin is therefore appreciably slower than that of penicillin, the data for which (previously described by Duguid *et al.*, 1947) are also shown in Fig. 1, A.

On the other hand the subconjunctival injection of a larger dose

FIG. 1A.

Concentration of penicillin in aqueous after intravitreous injection of 2,000 units of penicillin. Aqueous-vitreous ratio at 24 hours = 0.01.

of the drug (10,000 $\mu\text{g.}$) combined with adrenalin to delay the rate of absorption produced a chemotherapeutic concentration in the aqueous for only a short period (Fig. 2).

FIG. 2.

Concentration of streptomycin in aqueous after subconjunctival injection of 10,000 $\mu\text{g.}$ of streptomycin in 1/1000 adrenaline.

2. Toxicity of streptomycin.

Five animals were injected with streptomycin. Two of them (128 and 129) received 1.2 mg. in 0.1 ml. saline into the right eye and 0.1 ml. saline into the left (control) eye. The periods of observation were 77 and 112 days. The other three (130, 131 and 132) received 3.0 mg. in 0.1 ml. saline into the right eye and 0.1 ml. saline into the left eye. The periods of observation were 12, 77 and 112 days.

The retinal changes following the intravitreal injection of streptomycin were pronounced.

The two eyes which received 1.2 mg. showed only trifling and transitory immediate oedema but pigment stippling was extensive after one week in one and after two weeks in the other. Both

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showed patchy retinal atrophy in the region of the disc and medullated fibres, about a week after the pigment stippling appeared. The globes after fixation were bisected in front of the ora serrata and the lesions noted clinically identified with the help of the slit-lamp. The affected portions of the fundus were excised, embedded in paraffin and the sections stained with haemalum and eosin. In one fundus there can be noted a gross loss of the cellular elements of the retina over a large area with migration of pigment into it from the hexagon pigment layer. In the other eye there is over a large area a disturbance and loss of the outer retinal elements. In neither case does the choroid appear to be affected. The larger dose (3.0 mg.) produced even more distinctive changes. In one (131) the whole inferior retina became atrophic after a period of oedema followed by pigment changes. The other two animals (130 and 132) showed deep oedema immediately after the injection and shortly afterwards widespread pigment reticulation began to be noticeable. Histological examination of the three eyes receiving the larger dose shows in all of them extensive loss of all retinal elements over large areas with massive pigment infiltration of the retina. In one eye the retina was almost completely detached.

In the retina of the eye which received subconjunctival injections of streptomycin no changes were found, either clinically or histologically.

One control eye showed some exudate in the central third of the inferior periphery of the retina. The vitreous of the other eye had been injected with 3.0 mg. streptomycin.

Summary of the toxicity experiments

It is clear that streptomycin has a deleterious effect on the retina when it is introduced into the vitreous in doses of 1.2 or 3.0 mg. It would not appear to have any toxic effect on the fundus when given subconjunctivally.

Discussion

After a single intravitreous injection of 2,000 μ g. of streptomycin an effective therapeutic concentration was maintained in the ocular fluids for at least three days. This slow rate of diffusion of streptomycin is similar to that obtained by Bellows *et alii* (1947) using 25 to 100 μ g. of streptomycin per injection. From this point of view a single intravitreous injection of streptomycin might well be sufficient to deal with certain ocular infections in man.

On the other hand the present findings show clearly that even

1.2 mg. (863 μ g. of activity) of pure streptomycin produces serious and permanent retinal damage. These observations are at variance with those of Bellows *et al* (1947), who showed that intravitreal streptomycin in amounts of 25 to 1000 μ g. per injection failed to give rise to any permanent damage to the fundus. Their series of experiments was done using solutions of various commercial lots and a purified sample of streptomycin and were also followed by ophthalmoscopic and histological examinations. Our toxicity series was done on a pure sample of streptomycin (Pfizer X 76) and we have seen similar changes with the ophthalmoscope in eyes injected with commercial streptomycin. The difference between these findings may well be due to differences in the toxicity of the streptomycin used, but the data suggest that at present great caution should be used in the intravitreal use of streptomycin.

Summary

(1) Intravitreal injection of 2,000 μ g. of streptomycin acts as a depôt from which streptomycin diffuses away slowly, chemotherapeutic concentrations still being present in the ocular fluids after three days.

(2) The toxic effects of streptomycin introduced into the vitreal in dosages of 1.2 and 3.0 mg. result in marked retinal damage.

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