STREPTOMYCIN AND PROMANIDE IN EXPERIMENTAL OCULAR TUBERCULOSIS* ·

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It is known that bacteriostatic concentrations of streptomycin can be produced in various tissues of the rabbit's eye after systemic administration (Leopold and Nichols, 1946) ; after application to the conjunctival sac when the cornea is inflamed or abraded, but not otherwise (*Id*, *ibid.*, and Bellows, Burkholder, and Farmer, 1947); after intravitreal injection; and, rather transiently, after subconjunctival administration (Gardiner, Michaelson, Rees, and Robson, 1948). There appear to be no measurements of the penetration of sulphones into the eye, but Steenken, Wolinsky, and Heise (1946) obtained therapeutic results in guinea pigs with experimentally induced tuberculosis of the eye by subconjunctival or oral administration of sodium p-p'-diaminodiphenylsulphone-N-N'-didextrose sulphonate (" Promin " or " Promanide "). The present paper records some observations on the efficacy of streptomycin and Promanide under various conditions in experimentally produced tuberculosis of the iris and ciliary body of the rabbit's eye.

Methods

In order to study the effect of supposedly therapeutic agents, it was necessary to produce a suitable experimental lesion, at least moderately consistently. Such lesions have frequently been produced in rabbits by inoculation of tuberculous material into the eyes (for early references, see Robson, 1944) and rather less reliably by systemic inoculation (Angevine and Huntington, 1941; Bablet and van Deinse, 1943). The number of organisms necessary to produce lesions apparently varies widely and depends on the strain of organism and the precise site of inoculation. In their very thorough study, Woods, Burky, and Friedenwald (1938) obtained irregular results with the standard strain H37, and produced a more consistent lesion by intra-aqueous inoculation of a strain obtained from human sputum, grown on hormone-bouillon containing 5 per cent. glycerine for four to eight weeks, and introduced as a filtered saline suspension, of which a single inoculum apparently contained about 10^5 organisms. Gray (1937)

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used if anything larger numbers of organisms, but infected the posterior chamber. Robson (1944) produced lesions by intra-corneal injection of far smaller number of organisms, about 100 human or as few as about 5 of a bovine strain. His data indicate that a hundred-fold increase in the number of organisms increases only slightly the rate of onset and severity of the lesions. For the present purpose, intra-aqueous inoculation seemed to us most likely to produce lesions approximating to those observable clinically, and after some preliminary experiments, with various strains obtained from human lesions, good lesions were obtained with a dose of about 10^4 organisms of a moderately virulent strain. We failed to produce lesions with inocula containing less than 10^3 organisms of any strain. Larger inocula or more virulent strains rapidly produced severe lesions. There was considerable variation in sensitivity to different strains, as is described below.

Strains of *Mycobacterium tuberculosis*, human type, were isolated from the sputum of consumptive patients and cultured on Loewenstein-Jensen egg medium. Colonies from two to three week old subcultures (at 37°C.) were picked into sterile 0.9 per cent. sodium chloride and ground in a sterile mortar, and the suspensions were matched against standard opacity tubes so that the number of bacilli in 1 ml. might be estimated approximately. Suitable dilutions for inoculation were prepared from these suspensions. As it was difficult to prepare a uniform suspension of bacilli and as it seemed likely that some variation in the lesions produced was due to the injection of aggregates containing variable numbers of organisms, the expedient was tried in one experiment of picking the colonies into sterile saline containing 0.02 per cent. sodium dodecyl sulphate. This concentration of detergent was about the highest expected to be harmless to the organisms during the short period for which they were exposed to it (*cf.* Baker, Harrison, and Miller, 1941) and the final concentration injected into the eyes (0.0002 per cent.) was well below levels found to be injurious with other anionic detergents applied to abraded corneae (Leopold, 1945).

Adult rabbits of both sexes were used. Inoculation was made into the anterior chamber in front of the limbus, under thiopentone anaesthesia (25 mg./kg. body weight). The severity of the lesions produced was assessed by clinical examination of the eyes during life, by post mortem search for dissemination of the infection, and by histological examination. Tuberculin tests were performed, but the responses were weak and erratic, and have been of no value. Streptomycin (Merck) was used by subconjunctival or intravenous injection, and Promanide (Parke Davis) by intravenous injection.

Experiments

Two sets of experiments have been performed. In the first, four dilutions of four different strains of tubercle bacilli were introduced into the sixteen eyes of eight rabbits. In these rabbits an endeavour was made to introduce a fixed amount of the suspension of tubercle bacilli into the anterior chamber. However, on withdrawal of the needle a variable amount of fluid leaked away, and as it was doubtful whether this fluid was aqueous humour or inoculum or a mixture of both, the accuracy of the dose was uncertain. Four of these rabbits were treated with streptomycin subconjunctivally, as described below; and, as severe lesions were

produced rapidly, this experiment was terminated after three weeks. In the second set of experiments a suspension of tubercle bacilli of uniform concentration was used. With approximately 0.1 ml. of the suspension in a syringe, the contents of the syringe and the aqueous were well mixed by depressing and withdrawing the piston of the syringe several times while the needle was in the anterior chamber, and the syringe was finally removed containing 0.1 ml. of the mixed fluid, so that the final volume of fluid in the anterior chamber was not increased. Less leakage occurred than in the first set of experiments. Sixteen rabbits were used: the right eyes were all inoculated with a suspension containing 0.002 per cent. sodium dodecyl sulphate and the left eyes with a suspension which contained no detergent. When iritis was present in nearly all the rabbits, they were divided into four groups according to the severity of their lesions, and were treated, as described in the section on results, for one week. After treatment they were sacrificed at various times up to forty-five days for post-mortem and histological examination. The severity of the lesions was assessed for about six weeks from the time of inoculation mainly by the extent and degree of inflammation of the iris. Mild, moderate and severe lesions were scored one, two and three respectively, and intermediate scores were used in ambiguous cases. Later, the extent of caseation was assessed and scored similarly. The observer was at all times unaware of the previous score and of the treatment which the rabbit had received, and was occasionally shown the same rabbit twice in the same series of observations: in such circumstances the scores usually agreed exactly and rarely differed by more than one half unit.

The second set of experiments was factorially designed, and the arbitrary scores were analysed on lines described by Fisher (1942). Results were deemed significant when the estimated probability of their occurring by chance was less than one in a hundred, and insignificant when they might have occurred more often than once in ten times. Intermediate probabilities were not in fact met.

Results

The first experiment (Table I) was designed partly in order to find an optimal dose and strain of tubercle bacilli for the present purpose, and the wide variation in the lesions was expected. There were substantial differences in the progress both of lesions produced by the same strain at different dilutions and of lesions produced by different strains at the same dilution. However, the relative rates of progress of untreated lesions were reasonably constant, and sufficiently so to allow therapeutic trial in a proportion

TABLE I

The severity of ocular lesions in rabbits of the first series of experiments.

Days from inoculation			7	12	17	21	Treated on days	Remarks
Rabbit 48, strain 3798,	10^4 orgs./ml.	...	0	0	$\frac{1}{2}$	$\frac{1}{2}$	13-17	—
	10^6 orgs./ml.	...	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$		
Rabbit 49, strain 3798,	10^7 orgs./ml.	...	$\frac{1}{2}$	1	$\frac{1}{2}$	—	None	Died on 19th day
	10^4 orgs./ml.	...	2	2	$2\frac{1}{2}$	—		
Rabbit 326, strain 3253,	10^4 orgs./ml.	...	0	0	$\frac{1}{2}$	1	None	—
	10^6 orgs./ml.	...	0	1	1	1		
Rabbit 335, strain 3253,	10^7 orgs./ml.	...	0	$1\frac{1}{2}$	$1\frac{1}{2}$	2	7-17	—
	10^4 orgs./ml.	...	0	$\frac{2}{2}$	$\frac{2}{2}$	$\frac{2}{2}$		
Rabbit 340, strain 3800,	10^4 orgs./ml.	...	0	0	$\frac{1}{2}$	1	None	—
	10^6 orgs./ml.	...	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$		
Rabbit 341, strain 3800,	10^7 orgs./ml.	...	$2\frac{1}{2}$	$\frac{2}{2}$	—	—	7-12	Killed on 12th day
	10^4 orgs./ml.	...	3	$\frac{3}{3}$	—	—		
Rabbit 343, strain 3808,	10^4 orgs./ml.	...	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{2}{2}$	$2\frac{1}{2}$	13-17	—
	10^6 orgs./ml.	...	1	$1\frac{1}{2}$	$\frac{2}{2}$	$2\frac{1}{2}$		
Rabbit 344, strain 3808,	10^7 orgs./ml.	...	$2\frac{1}{2}$	2	$2\frac{1}{2}$	—	None	Killed on 18th day
	10^4 orgs./ml.	...	$2\frac{1}{2}$	$2\frac{1}{2}$	3	—		
Mean score, untreated, 10^4 and 10^6 orgs./ml.			$\frac{1}{2}$	$\frac{5}{8}$	1	$1\frac{3}{8}$	None	—
Mean score, treated, 10^4 and 10^6 orgs./ml.			$\frac{1}{2}$	$\frac{5}{8}$	$1\frac{1}{2}$	$1\frac{1}{8}$	13-17	—
Mean score, untreated, 10^7 and 10^4 orgs./ml.			$1\frac{1}{4}$	$1\frac{1}{4}$	$2\frac{3}{4}$	—	None	—
Mean score, treated, 10^7 and 10^4 orgs./ml.			$1\frac{1}{2}$	$2\frac{1}{2}$	—	—	7-12 or 17	—

The underlined scores are those for eyes during or after treatment with streptomycin (30 mg. to each eye subconjunctivally daily).

of the animals while the rest served as controls. Two of the four rabbits which had received large inocula were treated from the seventh day onwards, and two of the four which had received small inocula were treated from the twelfth day. The second pair were those which had been inoculated with the same strains as the control pair of the large inocula. The treatment consisted of the subconjunctival injection of streptomycin (30 mg. in 0.3 ml. sterile 0.9 per cent. sodium chloride solution to each eye) once daily, and was continued until the seventeenth day from inoculation unless the animal died sooner. The control rabbits were injected similarly with 0.9 per cent. sodium chloride solution.

The rabbits which had large inocula developed very severe lesions. Two of them were sacrificed when they had developed panophthalmitis, and one died. Except in the mildest cases, all these animals showed an acute inflammatory reaction in the iris and ciliary region in a few days. The reaction was accompanied by a mild conjunctivitis with a thin purulent discharge, and at

first had no typically tuberculous features. Between the second and third week small patches of caseation developed. The milder cases showed less or no initial inflammation, and sometimes slight oedema of the iris and irregularity of the pupil were the only signs which preceded the appearance of areas of caseation. This experiment was terminated after three weeks because of the number of animals which had died and because of the severity of the lesions in the survivors.

No benefit whatever was observed either clinically or on subsequent histological examination of these eyes from subconjunctival streptomycin treatment, and the rate of progress of all the lesions was apparently unaffected.

In the second experiment, in which a standard dose of tubercle bacilli was used in all the rabbits, the lesions were much milder and slower in onset. Mild inflammatory reactions developed in the irides between the seventeenth and twenty-seventh days, and the first signs of caseation a few days later. In untreated animals the iritis was maximal about the thirty-fifth day, and then quiesced, while the centres of caseation extended and coalesced to form areas of various size, sometimes involving much of the anterior chamber, or invading the cornea and other surrounding structures. Twenty-eight days after inoculation the rabbits were divided into four

TABLE II

The severity of lesions in rabbits of the second series of experiments.

	Severity of iritis (arbitrary scale)			Severity of caseation (arbitrary scale)		Lesions in animals killed		
	Mean $\pm$ Standard Error			Mean $\pm$ Standard Error		Lungs		Other Organs
Days from inoculation	28	37	52	56	72	56	72	72
Number of rabbits in group	4	4	3	3	2	1	1 or 2	1 or 2
Group O ...	0.56 $\pm$ 0.17	0.86 $\pm$ 0.20	0.79 $\pm$ 0.26	2.17 $\pm$ 0.38	2.00	++	+	0
Group P ...	0.42 $\pm$ 0.11	0.61 $\pm$ 0.16	0.75 $\pm$ 0.26	1.53 $\pm$ 0.33	1.38	+++	+++	Liver (2) Kidney (1)
Group S ...	0.58 $\pm$ 0.23	0.48 $\pm$ 0.23	0.36 $\pm$ 0.42	0.79 $\pm$ 0.47	0.12	0	0	0
Group PS	0.37 $\pm$ 0.12	0.42 $\pm$ 0.16	0.21 $\pm$ 0.15	0.50 $\pm$ 0.17	0.31	0	0	0

One animal in each of these groups died before the date indicated. The figures given are calculated from the latest available scores for the rabbits concerned. As the lesions in these rabbits were if anything progressing, the scores tend to be underestimated. The animals were treated with promanide or streptomycin on the 28th to the 34th days from inoculation.

groups of four, according to the severity of the lesions, and in each group the four rabbits were allotted to different treatments by a random procedure. The treatments consisted of:—

Group O—No treatment.

Group P—Promanide, 150 mg./kg. intravenously at twelve-hour intervals.

Group S—Streptomycin, 30 mg./kg., intravenously, at twelve-hour intervals.

Group PS—Promanide and Streptomycin as above.

Treatment was continued for seven days. The rabbits which had been grouped as most severe before treatment were sacrificed two days after the end of treatment, the next group twenty-eight days

FIG. 1.—The progress of the iritis in rabbits of the second series of experiments. The points represent the mean severity of the iritis assessed on an arbitrary scale, in groups of four rabbits which were inoculated with tubercle bacilli in the aqueous humour on April 20 and were treated with promanide and streptomycin as indicated from May 18 to 24.

FIG. 2.—Eye of streptomycin + promanide treated rabbit 44 days after end of treatment.

FIG. 3.—Eye of control rabbit 44 days after end of treatment.

and the survivors forty-five days after the end of treatment for post-mortem and histological examination. The results are shown in Table II and Figs. 1, 2, and 3, and require little comment. All the treated groups did better than the controls, but the improvement was significant only in the streptomycin groups. The difference was equally observable in the milder iritis and lesser caseation, both macroscopically and histologically (Figs. 4-7), and

FIG. 4.—Group O. No treatment.

FIG. 5.—Group P. Promanide only.

FIG. 6.—Group S. Streptomycin only.

FIG. 7.—Group PS. Promanide and Streptomycin.

FIGS. 4-7.—Comparison of extent of caseation in cases of average severity from each of the four groups.

in the diminished spread of the infection to other organs. The difference between the Promanide-Streptomycin group and the Streptomycin alone group was quite insignificant, and no difference was observed between the behaviour of the eyes which had been inoculated with the suspension containing detergent and those not so inoculated.

Discussion

Therapeutic results have been obtained with streptomycin under suitable conditions, but not more than mitigation of lesions has occurred: in none of our rabbits was a definitely established tuberculous infection of the eye eradicated. Our results appear somewhat more favourable than those of Grignolo (1948) in guinea-pigs: he obtained benefit from streptomycin only when it was given before lesions had developed, but he was probably dealing with a heavier infection than was used in our second series. The present data are inadequate as a basis for comparison of the efficacy of different routes of administration, as the lesions were not comparable in the two series. The beneficial effect, if any, of Promanide is clearly limited and no appreciable synergism with streptomycin has been detected. The schedules of dosage were perhaps not optimal: they were designed to make the most prolonged use of a limited quantity of material, and extensive alternatives are possible. In Woods' extensive experimental work on ocular tuberculosis he investigated the efficacy of streptomycin used systemically, on animals rendered immune-allergic by previous systemic tuberculous infection. Using slightly bigger daily dosage than ourselves and for a period of treatment of $2\frac{1}{2}$ months, he found that groups treated by streptomycin were dramatically better than the control group. As none but the mildest forms of tuberculous disease of the uvea in man are amenable to present forms of treatment the systemic use of streptomycin appears worthy of trial.

Summary

Tuberculous iridocyclitis has been produced experimentally in rabbits by intra-aqueous inoculation of human tubercle bacilli. The severity of the lesions produced was assessed by clinical examination, and histologically.

In one set of experiments, in which severe and rapidly progressive lesions were produced, no benefit was observed from treatment with streptomycin subconjunctivally (30 mg. to each eye daily for five or ten days).

In a second set, in which milder lesions developed, streptomycin

intravenously (30 mg./kg. twice daily for seven days) substantially ameliorated the lesions but did not completely eradicate infection in any case.

Promanide intravenously (150 mg./kg. twice daily for seven days) either alone or in conjunction with streptomycin, appeared to be slightly beneficial, but the results were quite insignificant.

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