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COMMUNICATIONS

EXPERIMENTAL OBSERVATIONS ON THE INTRA-VITREOUS USE OF PENICILLIN AND OTHER DRUGS*

BY

J. P. DUGUID, M. GINSBERG, I. C. FRASER, J. MACASKILL, I. C. MICHAELSON and J. M. ROBSON

From the Departments of Pharmacology and Bacteriology, University of Edinburgh, the Tennent Institute of Ophthalmology, University of Glasgow and the Department of Pharmacology, Guy's Hospital Medical School.

Introduction

IT is a matter of common experience that infection of the vitreous body is a major catastrophe which results almost inevitably in destruction of the eye. Interest, therefore, has been directed to new therapeutic agents which have been shown to be of value in the treatment of sepsis elsewhere in the body; in the hope that severe intra-ocular infection may also be controlled by them. Unfortunately, while drugs administered either orally or by injection readily reach most tissues in adequate concentrations, the vitreous is exceptional in this respect, in that drugs, present in the blood stream, do not easily diffuse into it. Hence, it is very difficult to produce adequate

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concentration of therapeutic agents in the vitreous by their systemic administration.

It seemed desirable, therefore, to investigate the practicability of injecting these drugs directly into the vitreous, since it is known that they can produce their effect when applied locally to a site of infection. The experiments recorded below were made to this end and may conveniently be described under three headings. First, the effects of the drugs on the normal eye, secondly, diffusion of the drugs within the eye, and lastly, the value of certain drugs in the treatment of experimentally produced infections of the vitreous body.

METHODS

All experiments were performed on rabbits. Under ether anaesthesia the drugs were injected into the vitreous by means of needle puncture of the sclera in a region well behind the ciliary body, the needle being directed backwards to avoid possible injury to the lens. The needle used had a short bevell point, was 7-8 mms long and the amount of solution (0·05 or 0·1 c.c.) delivered by the syringe was accurately controlled by means of a micrometer gauge (see Fig. 1.).

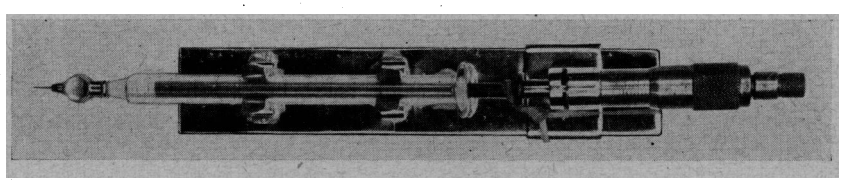

FIG. 1.

In most experiments a single injection was given, in a few the injection was repeated after 48 hours. Thereafter, the eyes were observed over periods of 10 to 128 days by the usual methods, particular attention being paid to the fundi and media. Finally, the eyes were removed and submitted to histological examination. The following drugs were used:—

1. Penicillin: Both commercial preparations (300-700 units/mg) and the pure sodium salt (1600 units/mg). The dose introduced was 2000 or 5000 units.

2. Sodium sulphacetamide: A 30 per cent. solution in pyrogen free water.

3. Marfanil: A 30 per cent. solution of Marfanil hydrochloride neutralised to pH 7·0.

4. V.335: (p-methyl sulphonyl benzylamine)—see Evans, Fuller and Walker (1944)—a 30 per cent. solution of the hydrochloride neutralised to pH 7·0.

Control experiments with normal saline were carried out.

Section 1. The effects of the Drugs on the Eye

METHODS

The effects of the drugs were observed on a total of 52 eyes of which 36 were examined histologically. Eight normal eyes, and 6 injected with saline were also examined histologically. Most of the material was fixed in 10 per cent. formol saline; that selected for examination by Nissl's method in 90 per cent. alcohol. While still in the fixative, the globes were divided equatorially and the posterior halves examined with a binocular microscope and the slit-lamp, and areas for section were selected. By this means, lesions seen and sketched in the living animal were identified and the correlation between clinical and histological findings ensured.

The tissues were embedded in paraffin, excepting those to be stained for fat, which were embedded in gelatin. All tissues were stained with haemalum and eosin; a few were in addition stained with scarlet red, with Nissl's stain, and with Marchi's method.

RESULTS

Great differences exist in the appearances of the fundi in apparently normal eyes of rabbits. While the usual appearance in pigmented animals is a fairly even granular one, not uncommonly pigment patches or clumps are seen, similar to those found in an old choroiditis in man. Histologically, however, such eyes do not show retinal abnormalities of the type to be described as resulting from the intra-vitreous injection of certain drugs. The vitreous opacities resulting from the injection of normal saline were slight and as a rule had almost completely disappeared after a week or two. No fundal changes were noted clinically in these eyes; in some examined histologically there were slight changes in the rod and cone layer which may have occurred post-mortem.

In 15 out of the 52 eyes infection was experimentally produced prior to injection of the drug. Histological changes in these cases were possibly products of both the drug and the infection and their significance was considered in the light of the changes found in those eyes into which only the drug had been injected.

COMMERCIAL PENICILLIN

Eight eyes were observed over a period of 10 to 29 days and 4 were sectioned. In only 1 eye had infection been experimentally produced. Opacities, sometimes dense, appeared in the vitreous body and remained until the end of the experiments. These eyes received a single dose of 2000 units or, in one eye, of 5000 units; or a double dose of 2000 units with an interval of 48 hours. The fundi

suffered damage in all cases. Below the medullated nerve fibre bundle pigmentary changes developed, and, in one case, this progressed to a retinitis proliferans with detachment. Three eyes showed fine new vessel formation with swelling of the medullated nerve fibre bundle, and retinal haemorrhages appeared in one of them. Posterior cortical lenticular opacities appeared once.

Histologically, in keeping with these findings, all the sectioned eyes show numerous monocytes in the posterior vitreous, large circumscribed areas of complete retinal destruction with glial replacement, and pigment aggregates in the retina resulting from disturbance of the retinal pigment layer. The vessels, which normally lie free on the medullated nerve fibre bundle, are embedded in a non-cellular matrix. This matrix is possibly a factor in producing the swollen appearance in the nerve fibre bundle seen ophthalmoscopically. No infiltrates or transudates are present within the retina, and the choroid is unchanged.

PURE PENICILLIN

Fourteen eyes were examined over a period of 10 to 27 days and 8 were sectioned. These eyes received a singe dose of 2000 units or of 5000 units of crystalline penicillin; or a double dose of 2000 units with an interval of 48 hours. In 4 eyes the penicillin was injected 6 hours after inoculation of the vitreous with haemolytic streptococci. Toxic effects, while present in many of the eyes, were much less than with the commercial preparations. In one eye there was swelling of the medullated nerve fibre bundle and in another opacities in the posterior cortex of the lens. Histologically 3 out of 8 eyes sectioned show areas of retinal destruction. This is in keeping with the findings of Sallmann *et alia* (1944) who found 4 out of 11 eyes so affected. The histological changes are more marked than the clinical appearances had suggested. For example, 2 non-infected eyes in which vitreous opacities were the only ophthalmoscopic changes show circumscribed areas of retinal destruction and in the lower periphery an accumulation of pigment in the rod and cone layer. These eyes received a single dose and a double dose of 2000 units of crystalline penicillin respectively and indicate the difficulty, repeatedly experienced in these experiments, of assessing cellular changes in the retina on the basis of the ophthalmoscopic appearances alone. In one eye there are numerous small pigment aggregates within the retina. The eyes receiving a double dose were more affected than the others. These findings show that crystalline penicillin, in the doses stated, can produce definite toxic effects on the retina. Nevertheless, the changes are not so marked as with the commercial preparation, and unlike the latter, crystalline penicillin appears to have no predilection for the vascular system.

SODIUM SULPHACETAMIDE

Of 16 eyes examined over a period of 10 to 128 days, 13 were sectioned. The eyes received a single dose of 0·1 c.c. of 30 per cent. sodium sulphacetamide or a double dose of that quantity with an interval of 48 hours. In 5 eyes the drug was given at one hour after the inoculation of the vitreous with haemolytic streptococci. Of the 11 eyes in which no infection was introduced the fundi of 6 appeared normal while in the others patchy changes of a mottled appearance developed in the lower part of the fundus. There were no notable changes in the retinal vessels. In all the sectioned globes there are circumscribed areas, varying in size, of complete retinal destruction in the lower part of the fundus, excepting the 5 eyes infected with haemolytic streptococci. As these latter eyes had been removed 10 days after the infection and the minimum period between injection and enucleation in the others was 75 days, it may be presumed that a definite period of probably several weeks is required for the development of areas of complete retinal destruction. The eye which received a double dose of the drug is more affected than the others and shows retinal detachment and cystic formation within the retina as well as an extensive area of destruction. Practically all the eyes (infected and non-infected alike) show fairly widespread changes at the level of the rod and cone layer as indicated by the presence of pale staining detritus and numerous large droplets. These appear to be the product of the rod and cone layer and not of the pigment epithelium as suggested by Koyanagi and Kinnikawa (1937) and von Sallmann *et alia* (1944). This change can be noted particularly in the retina adjacent to the atrophic areas already noted. In assessing the significance of this appearance it must be remembered that it may be noted in a lesser degree as a post-mortem change. In some cases the outer nuclear layer of the portions of the retina affected in this way shows diminution in the number of its cells, and in one case there is complete absence of the outer molecular layer in the area corresponding to the maximum degeneration of the rods and cones. It is noteworthy that the fundi of several of the sectioned eyes showing marked histological changes were clinically normal, as had indeed previously been found by Leopold and Scheie (1943). Beyond 100 days infiltration of the vitreous with monocytes is not a feature of any of the cases.

After 128 days 5 eyes which were stained for Nissl's granules, with Marchi's method and for fat with scarlet red, show no abnormalities in the ganglion cells, in the medullary sheaths or in the nerve fibre bundles. In no case was the choroid found to be affected.

The toxic effects of sodium sulphacetamide on the retina appear to be more severe than those of crystalline penicillin; and further,

there is a difference in that the areas of destruction with sodium sulphacetamide appear only after a delay of several weeks. With both drugs, however, the initial effect is apparently on the outer part of the retina. It is noteworthy that the media, including the lens, were clear in all cases after 128 days.

MARFANIL

Injections of marfanil were made in 5 eyes, 4 of which were sectioned. Each case received a single injection of 0·1 c.c. 30 per cent. solution or two such doses at an interval of 48 hours. In 2 cases the injection was preceded by the inoculation of haemolytic streptococci. The periods of observation were 10 to 17 days.

Vitreous opacities were slight. The most notable clinical features in all cases were constriction and, in places, variation in calibre of the retinal vessels which were often reduced to threads. Two of the cases showed swelling of the medullated nerve fibre bundle. One showed haemorrhages and new vessel formation. In all cases examined histologically there are circumscribed areas of retinal destruction and in many, pigment aggregates within the retina. In 2 cases there appears to be thickening of the retinal vessel walls.

Marfanil is more toxic to the retina than sulphacetamide or penicillin. Like impure penicillin it appears to have an effect on the retinal vessels and to cause pigmentation of the retina.

V.335

Injections of V.335 were made in 9 eyes of which 7 subsequently were sectioned. A single dose, or two doses at an interval of 48 hours, was given and in 2 cases the injection was preceded by experimental infection of the vitreous. The periods of observation were from 10 to 33 days.

Only slight opacities formed in the vitreous. Clinically all eyes had marked changes in the lower part of the fundus with obvious pigment disturbance. The vessels were not notably affected. Histological examination confirms the severity of the fundal changes. In all cases there are extensive areas of retinal destruction and 3 eyes show separation of the retina. In most there are marked disturbances of the rod and cone layer, similar in appearance to those already described. In 3 the choroid shows numerous foci of round cells, though none of these eyes had been infected. Both the clinical and histological findings indicate that V.335 is toxic to the retina and apparently to the choroid. Of all the drugs used and described in this report it appears to have been the most toxic.

Summary of effect of drugs on tissues of rabbits' fundus

(1) A study of these 52 eyes shows that all of the drugs used are capable of producing a toxic effect on the retinal cells. In

Table I the drugs are arranged in the order of the increasing severity of their toxic effects. It is obvious that crystalline penicillin is the least toxic of the 5 substances tested and V.335 the most toxic. Although it is possible to assess the vessel changes clinically, the assessment of retinal cell damage must be based on the eyes histologically examined. The number of eyes showing areas of retinal destruction is therefore expressed as a fraction of the eyes sectioned.

TABLE I

Showing certain toxic effects of drugs arranged in order of their increasing severity.

Drug	No. of eyes observed clinically	No. of eyes showing vessel changes in the fundus expressed as fraction of eyes clinically observed	No. of eyes showing opacity in posterior lens expressed as fraction of eyes clinically observed	No. of eyes sectioned	No. of eyes showing histological areas of retinal destruction expressed as fraction of eyes observed histologically	No. of eyes showing pigment aggregates in retinae expressed as fraction of eyes observed histologically
Crystalline penicillin	14	0/14	1/14	8	3/8	2/8
Commercial penicillin	8	3/8	1/8	4	4/4	4/4
Sodium sulphacetamide	16	0/16	0/16	13	8*/13	0/13
Marfanil ...	5	5/5	1/5	4	4/4	3/4
V. 335 ...	9	0/9	1/9	7	7/7	2/7

* The five eyes which do not show areas of retinal destruction were removed 10 days after injection, a period probably too short for development of these areas in the case of sodium sulphacetamide. Such areas of destruction were present in all eyes sectioned after 73 days.

(2) Although with all these drugs eyes are shown in which there are localised patches of retinal destruction in the lower part of the fundus, the initial effect especially with sodium sulphacetamide, appears to be on the rod and cone layer. This is suggested by the

eyes in which the only retinal changes were in the rod and cone layer, the presence of changes in that layer in the otherwise normal retina adjacent to foci of complete retinal destruction, the presence in several eyes of cystic and other changes in the outer nuclear layer, and the absence of changes in the inner retinal elements in several eyes specifically stained. Sallmann *et alia* (1944) have commented on the early involvement of the outer retinal elements following intra-vitreous injection of penicillin.

(3) Commercial penicillin and marfanil are capable of a toxic effect on the retinal vessels as indicated clinically by vascular constriction, calibre variation, and new vessel formation, and histologically in one or two eyes by changes in the vessel walls.

(4) In no case were there transudative or exudative changes present in the retina.

(5) The choroid was free from change in practically all eyes.

(6) Correlation between ophthalmoscopic changes if present and histological findings should be ensured by identification of the lesion in enucleated eyes with the help of the slit-lamp and the binocular microscope. No absolute statement regarding retinal cell changes of even a gross nature can be based on ophthalmoscopic examination alone.

Section II. The diffusion of the drugs within the eye

METHODS

The drugs were injected into the vitreous as described in Section I. After intervals varying from 1 to 72 hours, the animals were killed and certain tissues of the eye were removed for estimation of their drug content.

Sulphacetamide estimations were made on the aqueous, vitreous, cornea, iris, lens, chorio-retinal tissue and sclera, which were removed in that order immediately after the rabbit was killed. Following dissection, the tissues were washed rapidly in saline, dried on blotting paper and, after weighing, thoroughly ground in glass mortars with 2 ml. of 15 per cent. trichloracetic acid and silver sand. The macerated tissues were allowed to stand for 30 minutes, when the contents of the mortars were washed and filtered into 25 ml. measuring cylinders and the volume made up to 25 ml. Sulphonamide estimations were made by the colorimetric method of Bratton and Marshall (1939) in 10 ml. aliquot portions. Readings were taken on a Klett visual colorimeter and Spekker photo-electric absorptionmeter.

Penicillin estimations were made on the aqueous, vitreous and cornea. These tissues were removed from the rabbit and subsequently handled with aseptic precautions. The cornea was ground in an agate mortar with sand and with a certain measured amount

of nutrient broth; after standing for one hour, the broth containing the macerated cornea was centrifuged and the supernatant fluid taken for testing. The penicillin estimations were made by a serial dilution test; this determined the highest dilution of the eye fluids in nutrient broth which completely inhibited growth of the standard (Oxford) *staph. aureus*. The particular dilutions examined in each instance were chosen according to the penicillin concentration considered likely to be present. The volume of the test mixture was usually 0·4 ml. Each tube was inoculated with a loopful of a 1 in 300 dilution of an eighteen hour broth culture of the standard staphylococcus. After incubation at 37°C. for about eighteen hours, the tubes were examined for growth as shown by the presence of turbidity. The presence or absence of growth was confirmed by a stroke-subculture on a blood agar plate. In control tests, it was found that the lowest concentration of penicillin completely inhibiting growth was between 1/20th and 1/25th of a unit per ml. For the purpose of calculating the penicillin concentrations in unknown fluids in terms of unit per ml., it was assumed that their highest bacterio-static dilutions contained 1/20th of a unit per ml. For instance, if growth of the staphylococcus was inhibited by the fluid diluted 1 in 20, but not by the fluid diluted 1 in 40, the penicillin concentration of the fluid was reported as being between 1 and 2 units per ml. (The eye fluids themselves, in the absence of penicillin, were not found to have any bacteriostatic effect in the dilutions tested).

RESULTS

(*a*) *Injection of sodium sulphacetamide into the normal vitreous.*—Table II shows the concentrations of sulphacetamide found in the vitreous, aqueous, cornea, iris, lens, chorioretinal tissue and sclera, one hour, six hours, one, two, three and four days after the injection of 0·1 ml. of 30 per cent. sodium sulphacetamide.

It was found that the drug rapidly diffused into all the tissues which always contained high concentrations within an hour after injection. In the vitreous, the concentration fell from about 1000 mg. per cent. (*i.e.* per 100 gm.) at one hour after injection to an average value of 2·5 mg. per cent. after four days. Chemotherapeutic concentrations (*i.e.*, over 5 or 10 mg. per cent.) were usually found in all the ocular tissues after 2 days and sometimes, in the vitreous, even after 3 days. In Figs. 2 and 3, the logarithm of the average concentration of sulphacetamide has been plotted against the time after injection. In the vitreous (Fig. 2), the log. of the concentration fell in inverse proportion to the time after injection.

(*b*) *Injection of sodium penicillin into the normal vitreous.*—Table III shows the concentrations of penicillin found in the vitreous, aqueous and cornea, one hour, six hours, one day, two days and

three days, after the injection of 2000 units of impure sodium penicillin into the vitreous. Table IV shows the results for similar experiments with pure crystalline sodium penicillin.

TABLE II

Concentrations of sulphacetamide in the ocular fluids and tissues after the intra-vitreous injection of 0·1 ml. 30% sodium sulphacetamide in the normal eyes of rabbits.

Time	Rabbit No.	Mg. Sulphacetamide per 100 g.						
		Vitreous	Aqueous	Cornea	Iris.	Lens	Choroid and Retina	Sclera
1 hr.	1896 R.E.	1300	232	102	122	21·1	290	213
	1898A „	1300	69·3	51·6	183	28·3	250	212
	1910A R.E.	960	268	177	308	—	391	149
	1910A L.E.	980	342	257	440	—	351	198
6 hrs.	1896 L.E.	670	—	105	138	38·0	120	115
	1898A „	1080	218	180	263	61·7	254	121
	1911A R.E.	550	205	187	221	—	202	125
	1911A L.E.	810	275	203	253	—	287	197
1 day	1888 L.E.	209	92·5	94·0	106	—	—	71·7
	1888 L.E.	353	111	84·2	12·0	—	—	43.7
	1900A R.E.	310	49·9	70·0	36·6	38·5	41·0	—
	1900A L.E.	311	58·2	64·7	85·0	20·2	42·5	45·0
	1912A R.E.	201	20·5	20·7	—	—	—	—
	1912A L.E.	239	63·5	80·1	74·5	—	—	—
2 days	1887 R.E.	115·1	18·9	11·3	19·2	—	—	38·2
	1887 L.E.	70·9	—	7·0	14·0	—	—	10·1
	1901A R.E.	31·1	4·0	6·3	11·1	16·9	10·7	13·5
	1901A L.E.	25·7	3·5	7·4	12·1	13·9	9·6	7·9
3 days	1886 R.E.	23·7	4·9	2·8	3·0	—	—	2·0
	1886 L.E.	13·0	0·85	1·0	1·6	—	—	1·2
	1902A R.E.	1·5	0·5	1·0	1·0	15·2	—	1·0
	1902A L.E.	0·9	1·0	0·5	1·0	9·0	—	1·0
4 days	1903A R.E.	3·4	0·6	0·6	—	—	—	—
	1903A L.E.	6·3	0·9	0·6	—	—	—	—
	1909A R.E.	0·1	0·2	0·5	—	—	—	—
	1909A L.E.	0·5	0·2	0·5	—	—	—	—

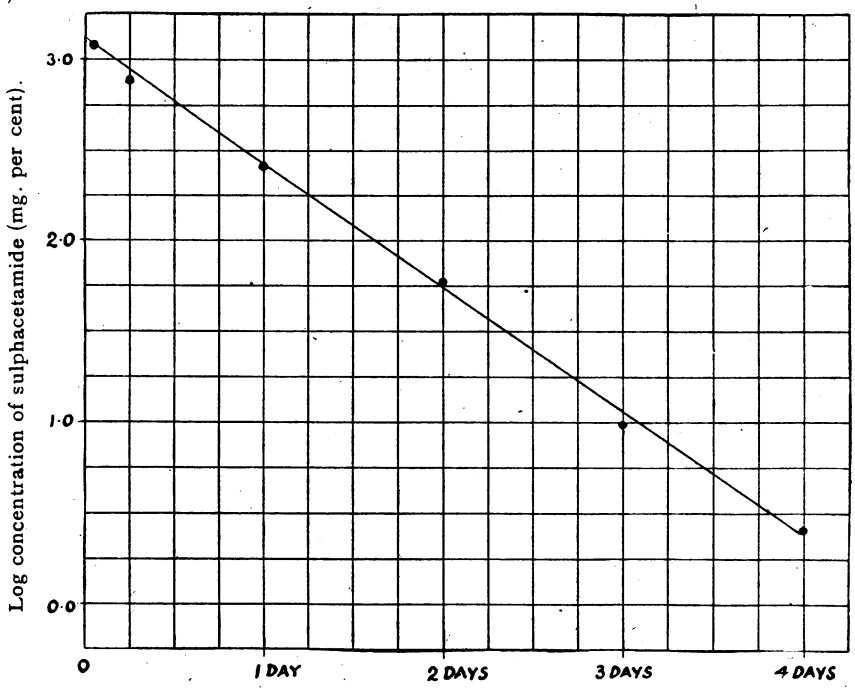

FIG. 2.

Persistence of sulphacetamide in the vitreous following intra-vitreous injection of 30 mg. sodium sulphacetamide.

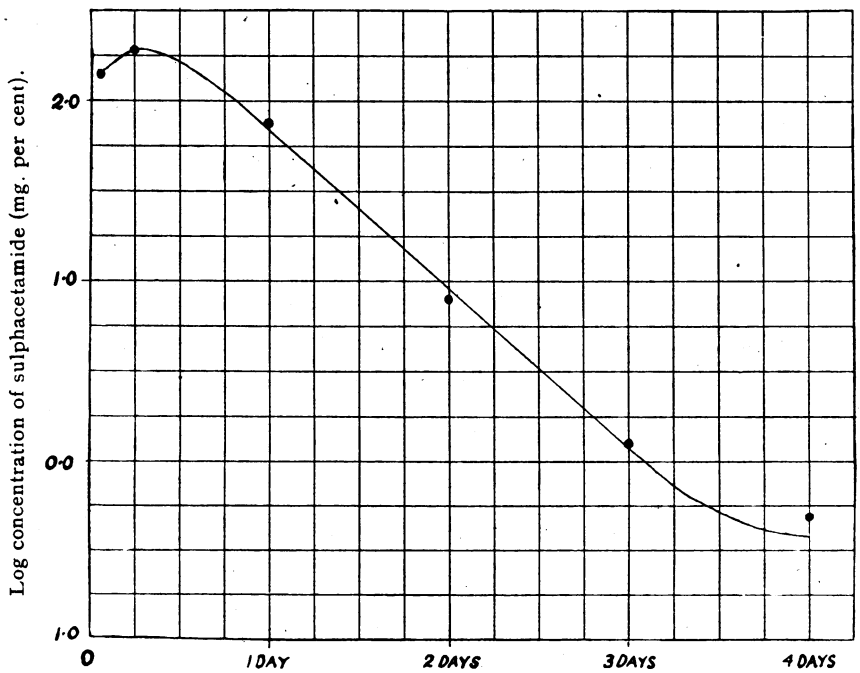

FIG. 3.

Persistence of sulphacetamide in the cornea following intra-vitreous injection of 30 mg. sodium sulphacetamide.

TABLE III

Concentration of penicillin in the aqueous, vitreous and cornea, 1 hour, 6 hours, 1, 2, and 3 days after the intra-vitreous injection of 2000 units of impure sodium penicillin.

Time	Rabbit No.	Concentration of Penicillin (units per ml.)		
		Vitreous	Aqueous	Cornea
1 hour	1892 R.E.	500-1000	20-40	16-32
6 hours	1892 L.E.	500-1000	5-10	8-16
1 day...	1873 R.E.	100-300	—	—
	1873 L.E.	100-300	—	—
	1891 R.E.	100 200	3-6	2-4
	1891 L.E.	200-400	6 12	4-8
	1893 R.E.	200-400	1-2	2-4
	1893 L.E.	200-400	1-2	2-4
2 days	1874 R.E.	6-10	—	—
	1874 L.E.	6-10	—	—
	1890 R.E.	<1/5	<1/10	<1/2
	1890 L.E.	1-2	1/10-1/5	<1/2
	1894 R.E.	5-10	1/10-1/5	<1/4
	1894 L.E.	1-2	1/10	
3 days	1889 R.E.	<1/10	<1/10	<1/2
	1889 L.E.	<1/10	<1/10	<1/2
	1895 R.E.	1/10-1/5	<1/10	<1/4*
	1895 L.E.	1/10-1/5	<1/10	<1/4*

*Tissues from both eyes analysed together.

It was found that penicillin diffused rapidly from the vitreous into the aqueous and cornea which always contained high concentrations within an hour after injection. The concentration of penicillin in the vitreous remained, however, much greater than the concentrations in the aqueous and cornea; the vitreous thus acts as a depot replenishing the drug lost from the other tissues. The concentration of penicillin fell from the high levels at one hour after injection to levels approaching or below the limits of detection after two days in the case of the vitreous. Chemotherapeutic concentrations (*i.e.*, about 1/20th of a unit per ml. and over) were maintained in the vitreous for 2 to 3 days, and in the aqueous and cornea for 1 to 2 days.

TABLE IV

Concentrations of penicillin in the aqueous, vitreous and cornea, 1 hour, 6 hours, 1, 2 and 3 days after intra-vitreous injection of 2000 units of pure sodium penicillin.

Time	Rabbit No.	(Concentration of Penicillin (units per ml.)		
		Vitreous	Aqueous	Cornea
1 hour	1922 R.E.	1000-2000	4-8	4-8
	1922 L.E.	2000-4000	2-4	8-16
	1934 R.E.	500-1000	2-4	1-2
	1934 L.E.	500-1000	2-4	2-4
	1938 R.E.	—	25-50	—
	1938 L.E.	—	—	—
6 hours	1921 R.E.	500-1000	8-16	4-8
	1921 L.E.	1000-2000	2-4	4-8
	1935 R.E.	1000-2000	8-16	8-16
	1935 L.E.	500-1000	8-16	8-16
	1939 R.E.	—	1/2-3	—
	1939 L.E.	—	3-6	—
1 day	1920 R.E.	64-128	<1/4	<1/2
	1920 L.E.	32-64	1/4-1/2	1/2-1
	1936 R.E.	16-32	1/4-1/2	1/2
	1936 L.E.	32-64	1/2-1	1/2-1
	1940 R.E.	—	1/2-1	—
	1940 L.E.	—		—
2 days	1919 R.E.	1/2-1	<1/10	<1/4*
	1919 L.E.	1/2-1	<1/10	
	1937 R.E.	1-2	1/10-1/5	<1/4*
	1937 L.E.	1-2	1/10-1/5	
3 days	1918 R.E.	<1/10	<1/10	<1/4*
	1918 L.E.	<1/10	<1/10	
	1938A R.E.	<1/10	<1/10	<1/4*
	1938A L.E.	<1/10	<1/10	

* Tissues from both eyes analysed together.

In Figs. 4 and 5 the logarithm of the average concentration of penicillin has been plotted against the time after injection. It will be noted that, in the case of pure penicillin, except for a short period immediately after the injection, the logarithm of the

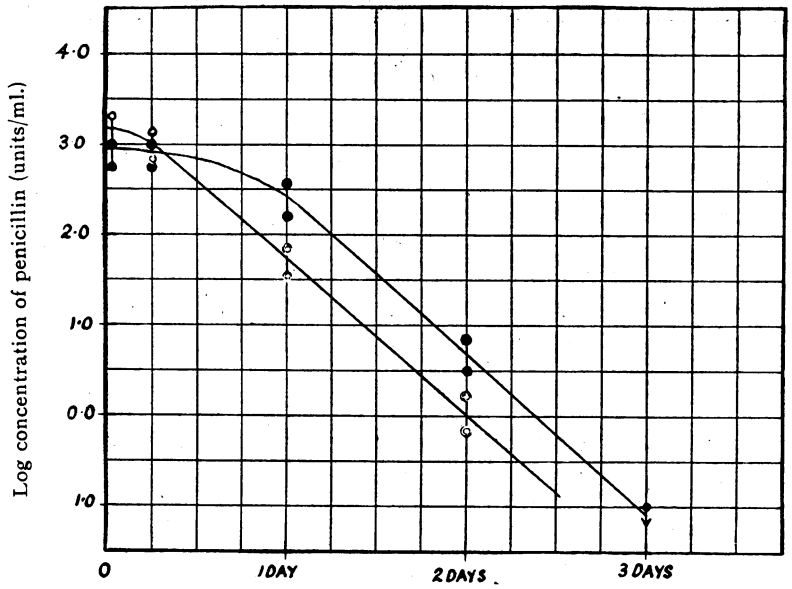

FIG. 4.

Persistence of penicillin in the vitreous following the intra-vitreous injection of 2000 units of penicillin.

● Impure penicillin. O Pure sodium penicillin.

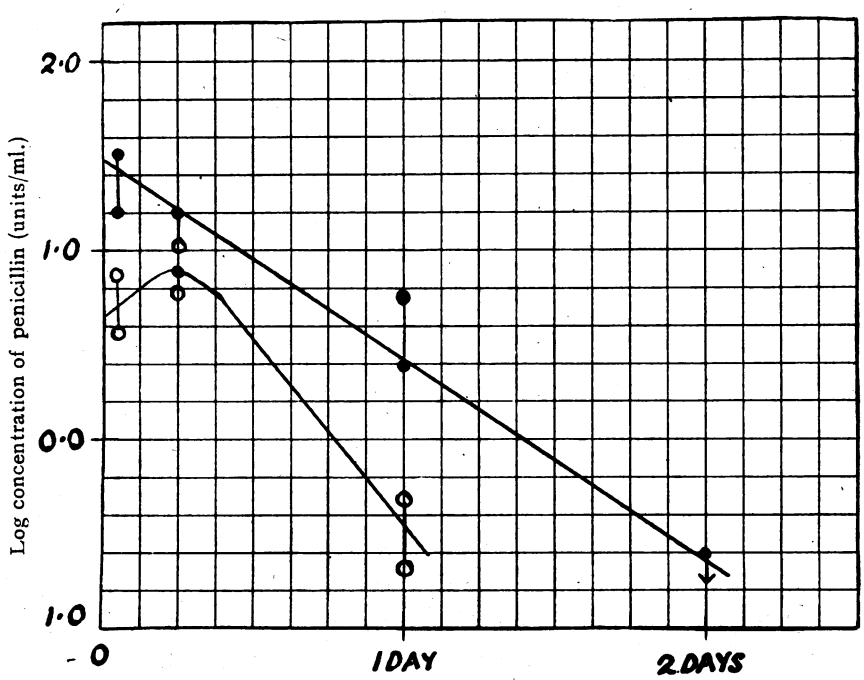

FIG. 5.

Persistence of penicillin in the cornea following the intra-vitreous injection of 2000 units of penicillin.

● Impure penicillin. O Pure sodium penicillin.

concentration diminishes in inverse proportion with the time after injection. In the case of impure penicillin, the fall in concentration is somewhat delayed during the first twenty-four hours.

(*c*) ***Injection of sodium sulphacetamide and sodium penicillin combined into the normal vitreous.*** Experiments *in vitro* showed that incubation of sodium penicillin with sodium sulphacetamide for 24 hours at 37°C. did not destroy any appreciable amount of penicillin.

Table V shows the results of experiments in which 0·1 ml. of 30 per cent. sodium sulphacetamide containing 2000 units of pure

TABLE V

Concentrations of penicillin and sulphacetamide in the ocular fluids and tissues after the intra-vitreous injection of 2000 units pure sodium penicillin, and 0·1 ml. 30 per cent. sodium sulphacetamide combined, into normal eyes.

Time	Rabbit No.	Concentration of penicillin (units/ml.)			Concentration of sulphacetamide (mg./100s.)				
		Vitreous	Aqueous	Cornea	Vitreous	Aqueous	Cornea	Iris	Sclera
1 day	1941 R.E.	16-32	½-1*	1	145	43·5	47·8	61·1	17·9
	1941 L.E.	16-32		1-2	165		55·3	75·2	107
2 days	1942 R.E.	<⅛	<1/10*	<½*	3·9	2·5*	—	—	8·0
	1942 L.E.	½-1			37·7		0·2	0·9	1·8
	1946 R.E.	6-12*	<1/10*	<½*	55·1	2·9*	2·1*	6·4	—
	1946 L.E.				61·4			6·1	—

* Tissue of both eyes analysed together.

sodium penicillin was injected into the vitreous. Except in the case of one rabbit (1942) which was examined at 2 days after injection, when low drug concentrations were found, the results were similar to those obtained when the drugs were injected singly. The combined administration of sulphacetamide and penicillin by intra-vitreous injection does not seem, therefore, to be contra-indicated by any increased rate of disappearance of either drug.

(*d*) ***Injection of sodium sulphacetamide and sodium penicillin into the infected vitreous.*** Table VI shows the results of experiments in which the drugs were injected both together and singly into the vitreous of the eyes of rabbits infected by intra-vitreous injection of haemolytic streptococci. Rabbit 1945, at 2 days after injection with penicillin and sulphacetamide combined, showed drug concentrations in the ocular tissues similar to those found in experiments with non-infected eyes. Penicillin alone was injected into the eyes,

TABLE VI

Concentration of penicillin and sulphacetamide in the ocular fluids and tissues after the intra-vitreous injection of 2000 units pure sodium penicillin and 0·1 ml. 30 per cent. sodium sulphacetamide combined, and singly, into infected eyes.

Time	Rabbit No.	Condition	Concentration of penicillin (units per ml.)			Concentration of sulphacetamide (mg/100 g.)			
			Vitreous	Aqueous	Cornea	Vitreous	Aqueous	Cornea	Iris
1 day	1943 R.E.	Infected	½-1	<1/5	<1 perforated	9·3	5·7	2·5	1·9
	1943 L.E.	Infected							
	1951 R.E.	Infected	50-100	1/5-½	1-2	—	—	—	—
	1951 L.E.	Normal	50-100		contaminated	—	—	—	—
2 days	1960 R.E.	Infected	—	—	—	56·6	5·4	2·0	8·1
	1960 L.E.	Normal	—	—	—	322	52·3	46·3	62·2
	1945 R.E.	Infected	2-4*	1/10*	<½*	112	20·7*	19·2*	32·2
	1945 L.E.	Infected				8·2			21·0

* Tissues from both eyes analysed together.

one infected and one normal, of rabbit 1951; after one day the penicillin concentrations in the vitreous fluids of the two eyes were similar. On the other hand, in rabbits 1943 (injected with penicillin and sulphacetamide combined), and 1960 (injected with sulphacetamide alone) the drug concentrations at one day after injection were considerably lower in the infected eyes than in normal eyes. It appears that in some cases of infection, disappearance of the drug from the eye is accelerated, but even so, chemotherapeutic concentrations are maintained for about one day after intra-vitreous injection.

Section III. Effect of Drugs on experimentally produced infections of the Vitreous Body

METHODS

In all animals both eyes were infected, one eye in each animal was treated, while the other served as control and received an injection of saline. Intra-ocular infection was produced, by the injection into the vitreous, of virulent haemolytic streptococci, the strain (1c) being that previously used in the production of corneal lesions (Robson & Scott, 1944); 0·02 c.c. of a broth culture diluted 1:1000 was the standard dose and was followed by the therapeutic agent, after an interval of 1, 6 or 24 hours. In a few experiments a second injection of the drug was given 48 hours later. The dose was always

0·1 c.c. of a 30 per cent. solution, except for penicillin, when the concentration was 20,000 units c.c. At the end of ten days the animals were killed, cultures were taken from the vitreous and some of the eyes were sectioned.

RESULTS

In untreated eyes signs of a severe reaction were evident within 24 hours of inoculation. There was a severe uveitis, shown by exudates into the vitreous and acute iritis. Twenty-four hours later, the whole vitreous was usually opaque and hypopyon was frequently present. At the end of the experiment there was a vitreous abscess in most of these eyes, and in the remainder the vitreous was completely opaque. The effects of treatment are shown in Table VII.

TABLE VII

Showing the effect of various drugs on the development of vitreous infections.

Drug	Number of Animals	Interval between inoculation and treatment	Result treated eyes
		Hours	
Penicillin	3	1	No infection
Penicillin	1	6	No infection
Pure penicillin ...	2	6	No infection
Pure penicillin ...	3	24 and 72	As controls
Sodium sulphacetamide	4	1	No infection
Sodium sulphacetamide	2	1	Infection delayed
Pure penicillin and sodium sulphacetamide	3	24 and 72	As controls
Marfanil	2	1	No infection
Marfanil	2	6	No infection
V.335	2	1	No infection

It will be seen that penicillin prevented the development of infection when injected up to 6 hours after inoculation but had no effect when the treatment was delayed for 24 hours, even if the drug was combined with sodium sulphacetamide.

Sodium sulphacetamide alone injected 1 hour after inoculation prevented development of infection in some of the cases—merely delaying it in others.

Marfanil and V. 335 gave favourable results, but in view of their obvious toxicity to ocular tissues, no further attention was given to them.

The cultures made from the vitreous of the untreated eyes showed growth of haemolytic streptococci in about 50 per cent. of cases but

in the remainder the abscess had apparently become sterile and the cultures were negative. No haemolytic streptococci were recovered from any of the treated eyes.

In a number of the experiments in which the infection had obviously been controlled, certain effects were noted in the vitreous and retina of treated eyes which, in the light of the experiments described in Section I, could, with confidence, be attributed to the toxic effects of the drugs.

Discussion

An attempt has been made to investigate the value and the practicability of injecting drugs intra-vitreously.

Ophthalmologists, understandably, are hesitant to use this method on account of the possible damage to the vitreous gel, the retina, etc. Experimentally, using no surface diathermy, a small needle could be introduced into the vitreous and a small quantity of saline injected without any ill effects. With the exception of pure penicillin, all the drugs introduced into the vitreous, including impure penicillin, were highly damaging to the retina, to a degree which excludes their use clinically. Pure penicillin in some cases was shown to cause small areas of retinal damage—but slight by comparison with impure penicillin or any of the other drugs. Under favourable conditions penicillin introduced by this means is highly effective in the control of infections of the vitreous body, and the diffusion experiments demonstrate that the drug diffuses but slowly from it, so maintaining a chemo-therapeutic concentration for 2-3 days after a single injection, in contrast to its rapid disappearance when injected into most other tissues in the body. In cases in which an eye is seriously endangered by infection in the vitreous it appears justifiable to consider the use of pure penicillin by intra-vitreous injection—the control of the infection more than outweighing the possibility of small areas of retinal damage. If doses comparable to those used in the experiments are given, it would appear sufficient to repeat the injections at intervals of two to three days; the slow diffusion from the vitreous permitting a therapeutic level of the drug to remain for that time.

Summary

The toxic effects and rate of diffusion of certain drugs introduced into the vitreous of rabbits have been investigated.

The therapeutic value of these drugs in experimentally produced vitreous infections is recorded.

Impure penicillin, sodium sulphacetamide, marfanil and V.335 do not appear to have a clinical value by intra-vitreous injection because of the damage they do to the retina.

Pure sodium penicillin sometimes damages the retina slightly when injected intra-vitreously, but its use by this means seems indicated and justifiable in certain cases of infection.

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A CASE OF THE LAURENCE-MOON-BIEDL SYNDROME SHOWING ATYPICAL RETINITIS PIGMENTOSA ASSOCIATED WITH MACULAR DYSTROPHY*

BY

CHARLES TAYLOR

LONDON

IN their comprehensive studies on the Laurence-Moon-Biedl syndrome, Cockayne, Krestin and Sorsby (1935), and Sorsby, Avery and Cockayne (1939) have shown that the fundus lesion may be an atypical rather than typical retinitis pigmentosa, and that macular dystrophy with optic atrophy may replace either of these, an observation also made more recently by Lyle (1946). The present case report is of interest in that it shows the combination of atypical retinitis pigmentosa with macular dystrophy. The great rarity of the association of retinitis pigmentosa and macular dystrophy and its hereditary character have been brought out by Sorsby (1940 and 1941).

Case report

Past History. T. S., aged 24 years, was fat at birth. There was some loss of weight following illness at 3 weeks (said to be "threatened with meningitis"). No attempt was made at talking

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