EXPERIMENTAL TOXOPLASMOSIS OF THE UVEAL TRACT*

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Introduction

THE histopathological studies of Wilder (1952) on eyes removed on account of intractable iritis of unknown origin suggest that toxoplasmosis may have been the cause in some cases. Following on her work, requests were received for the serological investigation of patients suffering from chronic anterior uveitis of undetermined aetiology. Some of these gave weak positive results in the low titre ranges, but were of doubtful significance since Beverley and Beattie (1952) found, in a survey of normal people, that 74 per cent. were negative or $<1:4$, 23 per cent. positive with a titre from $1:4$ to $<1:16$, 2 per cent. with a titre from $1:16$ to $<1:32$, 1 per cent. with a titre from $1:32$ to $<1:64$, and none with a titre of $1:64$ or over. In three patients, however, the levels were high: Case 1, $1:160$; Case 2, $1:64$; Case 3, $1:96$.

In the first case the eye had been removed before the publication of Wilder's work. The eye removed in the second case was unfortunately promptly fixed for histological examination and animal inoculation tests could not be done. Though pseudocysts could not be found in sections of either of these eyes, structures resembling paired vegetative forms could be seen. Unequivocal proof of the latter's identity is not available since recovery of the parasite by inoculation into animals was not attempted, and at the present time there is no staining procedure which is specific for toxoplasms. In the third case the eye was not removed as the condition slowly improved. The possibility of treatment by desensitization on the lines suggested by Frenkel (1949) was discussed and finally rejected. In view of the possibility of recurrence of the uveitis in this case, and bearing in mind future cases, a series of therapeutic experiments were undertaken, the first group of which is now reported.

Experimental Investigations

Production of Toxoplasma Infection of Rabbit's Eye.—Rabbits were anaesthetized with nembutal and open ether. The anterior chamber of the left eye was inoculated with 0.05 ml. of a dilution of peritoneal exudate taken from a mouse inoculated 3 days earlier with the *Rh* strain of toxoplasma. Inoculations were made with a tuberculin type syringe fitted with a Schick test needle which was introduced gently by rotation through the cornea just in front of the limbus and parallel with the

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plane of the iris. The needle was inserted so that its point was at the opposite sector of the anterior chamber before slowly injecting the required dose. This resulted in little of the inoculum escaping when the needle was withdrawn.

Choice of Chemotherapeutic Agents.—In experiments on the treatment of systemic toxoplasmosis in mice and rabbits by chemotherapeutic agents the most encouraging results were obtained from the use of 4: 4'-diaminodiphenylsulphone (hereafter called D.D.S.). This was earlier used by Summers (1949), and two of its soluble derivatives, Diasone and Promin, were used by Cross (1951), in systemic toxoplasmosis of mice produced by intra peritoneal inoculation.

In our experience better results were obtained with D.D.S., which is relatively insoluble, than with four of its more soluble derivatives—Promin, Sulphetrone, Diasone, and 2196 (4: 4'-bis (ethylamino) diphenylsulphone- α : α' -disodium sulphonate).

Estimation of D.D.S. in Blood and Eye Fluids.—The concentration of D.D.S. in the blood and eye fluids of the rabbits was determined by the colorimetric procedure of Francis and Spinks (1950), in which the Sulphone is coupled to N- β -sulphatoethyl-*m*-toluidine to yield a pink-coloured derivative. The intensity of the latter was measured by means of a Hilger Spekker absorptiometer with green filters (Ilford No. 604), and in the case of the eye fluids micro-cells were used. For the eye fluids, the procedure was essentially that described by Francis and Spinks except that weighed amounts were first treated with trichloroacetic acid and the precipitated protein removed by centrifugation. To 0.5 ml. of the protein-free supernatant was added 0.1 ml. NaNO₂ (1 mg./ml.) and, after 5 min., 0.1 ml. N- β -sulphatoethyl-*m*-toluidine (1 per cent. w/v). The mixture was allowed to stand for 20 min. and then 0.9 ml. ethanol was added.

Dosage and Body Fluid Levels of D.D.S.—When the rabbits were given daily doses of 100 mg. D.D.S./kg. in a gelatin capsule, the concentration of the drug in the blood followed the same sequence as that described by Francis and Spinks (1950). A peak concentration of the order 10 μ g. Sulphone per ml. blood was usually attained within 2 hrs and thereafter soon declined, so that 12 hours after dosing the concentration was about 1 μ g./ml. With certain rabbits it was noticed that the peak concentration was not so pronounced and was maintained for a much longer period (about 4 hrs). A concentration of at least 10 μ g. D.D.S./ml. blood could be maintained throughout the experimental period by dosing the animals at 8-hrly intervals with capsules containing 100 mg. Sulphone per kg. body weight, and rabbits receiving such treatment for a fortnight showed no signs of ill health.

FIG. 1.—Blood levels in two rabbits after ingestion of 100 mg./kg. at 6 a.m., and 8-hrly for the previous 3 days.

Fig. 1 shows the levels of the Sulphone in the blood of two such rabbits during the 10-hr. period

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after administration of the last of ten capsules given at 8-hrly intervals over the previous 3 days. The concentration of D.D.S. in the blood and the eye fluids of rabbits being dosed at 8-hrly intervals are recorded in the Table. Rabbits K and J were normal, whilst rabbits IA, IB, II, IV, VA, VB, VI, and VII had been inoculated 3 days earlier with approximately 20,000 toxoplasms into the anterior chamber of the left eye. At the time of taking the samples there was an active unilateral iritis.

TABLE
CONCENTRATION OF 4: 4'-DIAMINODIPHENYLSULPHONE IN THE BLOOD AND EYE FLUIDS OF RABBITS

Rabbit No.	Blood Level ($\mu\text{g./ml.}$)	Aqueous Humour ($\mu\text{g./g.}$)			Vitreous Humour ($\mu\text{g./g.}$)		
		Left	Right	Mean	Left	Right	Mean
IB	51.3	8.0	8.4	8.2	8.5	5.6	7.1
IA	34.5	8.6	9.5	9.1	9.0	9.2	9.1
VA	21.7	8.7	9.5	9.1	—	—	—
II	18.0	5.7	6.5	6.1	7.5	8.3	7.9
K	17.8	7.8	—	—	—	—	—
J	14.6	6.5	—	—	—	—	—
VB	12.3	4.8	4.1	4.5	—	—	—
IV	9.2	4.9	5.1	5.1	5.9	4.1	5.0

Rabbits received doses of D.D.S. at 8-hrly intervals. Blood sample taken immediately before anaesthetizing the animal for withdrawal of sample of aqueous humour, or before death when both aqueous and vitreous humour were required.

Since the eye fluids contain only about 0.3 per cent. solids per 100 g. water, it can be assumed that 1 g. of each of the eye fluids occupies 1 ml., and thus that the concentration of D.D.S. in the aqueous humour is between one-sixth and one-half of that in the blood. It is of interest to note that Sorsby (1949) has recorded that, in rabbits given sulphonamides intravenously, the levels of the drug in the aqueous humour were about 50–60 per cent. of those in the plasma. Though the figures for the concentration of the Sulphone in the vitreous and aqueous humour appear to show some variation between the right and left eye, this is no doubt due to experimental error; from the limited data available it would seem that the concentration of the drug in the vitreous humour is the same as that in the aqueous humour, and that there is no significant difference between the levels of the drug in the inflamed (left) and normal (right) eyes.

Preliminary Experiments

The injection of approximately 750 toxoplasms from a 3-day mouse peritoneal exudate into the anterior chamber of a non-immune rabbit resulted in an acute iritis, usually within 72 to 96 hrs. When the number of organisms was increased to 20,000, the delay in onset of signs of infection was only 30 to 40 hrs. Death in both cases occurred on the 8th or 9th day. If treatment with D.D.S. was begun within 4 to 6 hrs of inoculation with the smaller number of organisms, a good functional result could be obtained, but the animal still developed a severe iritis; with the larger inoculum the animal survived, though usually with a useless eye.

If treatment was withheld until the onset of signs of iritis, a useless eye was nearly always the result, but the animal survived; if treatment was delayed until the iritis was well established, the animal developed a panophthalmitis and died between the 8th and 12th day.

Since in these early experiments the results obtained by the use of D.D.S. alone were not satisfactory, it was decided to try the effect of combining D.D.S. with cortisone on the lines of the experiments of Woods and Wood (1952) on tuberculous iritis. Pilot experiments showed that 10 mg. cortisone given daily by subcutaneous injection produced beneficial effects, and this was the dosage used in systemic treatments in the following experiments.

Therapeutic Experiments

(1) *Effect of D.D.S. and Cortisone.*—Nine pairs of non-immune rabbits were inoculated with approximately 20,000 toxoplasms into the anterior chamber of the left eye by the method described: all inoculations were completed within 1 hr. One pair had no treatment, one pair was treated with cortisone alone, two pairs with D.D.S. alone, and six pairs with a combination of both drugs. Some of the pairs were used to study the effects of delaying treatment for varying periods after inoculation.

All eyes were examined and recorded twice daily in the early days after inoculation and later once daily. After the conclusion of the experiment the severity of the iritis at each examination was assessed on a points system with the following allocation of points:

Conjunctival injection	Mild $\frac{1}{2}$	Moderate 1	Marked $1\frac{1}{2}$
Pupil contraction	Mild $\frac{1}{2}$	Moderate 1	Marked $1\frac{1}{2}$
Colour difference of iris	Slight $\frac{1}{2}$	Definite $\frac{1}{2}$	Marked 1
Pattern of iris	Alteration $\frac{1}{2}$	Loss of detail 1	
Exudate on iris	Flecks $\frac{1}{2}$	Clumps $\frac{1}{2}$ -1	Woolly aggregates $1\frac{1}{2}$
Intra-ocular haemorrhage	1		
Chemosis	$\frac{1}{2}$ -1		
Turbidity of anterior chamber	$\frac{1}{2}$ -1		
Keratitis	$\frac{1}{2}$ -1		

The graphs in Fig. 2 (a-c) were constructed on this basis and show the effects of the various treatments on the progress or amelioration of the iritis. Because the condition of the eyes in each pair of rabbits ran a very similar course, the graphs were constructed by using the means of the points value for the two animals.

FIG. 2.—Course of iritis

- (a) In rabbits receiving no treatment.
- (b) In rabbits treated with D.D.S. (no delay).
- (c) In rabbits treated with D.D.S. and cortisone (no delay).

After 30-40 hrs the untreated pair (Fig. 2a) developed an iritis which progressed steadily till the animals died on the 8th and 9th days (Fig. 3).

The pair treated with cortisone only, starting at the time of inoculation, showed very little difference from the untreated pair. They had a slightly milder reaction in the early stages, but eventually developed an equally severe iritis and died at the same time (8th and 9th days).

The pair treated with D.D.S. from 5 hrs after inoculation (Fig. 2b), and the pair whose treatment with D.D.S. was delayed until the first signs of iritis were appearing, *i.e.* 53 hrs (Fig. 4), both had an initial iritis which was followed by a partial remission and then a relapse. The two phases of intense iritis (subsequently referred to as a biphasic response) were more obviously separated in the first of these two pairs.

The pair receiving D.D.S. and cortisone from the start (Figs 2c and 5), and the pair receiving D.D.S. from the start and cortisone from the onset of iritis, both developed an iritis of only one phase which corresponded in time with the first phase of the two groups treated with D.D.S. alone. The duration and severity of the iritis were greater in the second pair.

FIG. 3.—Rabbit receiving no treatment

FIG. 4.—Rabbit receiving treatment with D.D.S. (48 hrs delay).

FIG. 5.—Rabbit receiving treatment with D.D.S. and cortisone.

The last three pairs all received D.D.S. from the onset of the signs of iritis (53 hrs), and in addition one pair received cortisone from the outset, another cortisone from the onset of iritis, and the third cortisone at 96 hrs when the iritis was judged to have become well established. All of these, with one exception, had a monophasic response; they showed

general improvement from the peak of the primary phase and were left with eyes showing little damage and good functional result. The exception was one of the eighth pair. This animal developed a generalized bacterial infection with panophthalmitis. Bacteria, but no toxoplasms, were found in the eye after death. The only permanent sequel in the animals treated with both D.D.S. and cortisone was depigmentation of the iris, the intensity of which was proportional to the length of the delay in administering cortisone.

Four of the rabbits receiving cortisone together with D.D.S. died at intervals after the conclusion of the experiment. One died of a generalized bacterial infection 30 days after inoculation and the eye remained quiet throughout its illness. The other three died from acute generalized toxoplasmosis at 19, 17 and 35 days after inoculation, and there was a recurrence of iritis which preceded the death of the animal by 4, 5 and 7 days respectively.

(2) *Route of Cortisone Administration.*—Four pairs of non-immune rabbits were inoculated with 20,000 toxoplasms into the anterior chamber and all were treated with D.D.S. from the 5th hr. The first pair had no other treatment; the second was given 10 mg. cortisone subcutaneously at the time of inoculation and each day afterwards; the third was given 5 mg. cortisone in 0.2-ml. amounts subconjunctivally at the time of inoculation and again at 73 hrs but not afterwards; the fourth pair received cortisone drops thrice daily for 12 days. The drops were made by mixing 1 volume cortisone acetate suspension (25 mg./ml.) with 4 volumes buffered saline diluent containing 4.6 g. NaH_2PO_4 , 4.7 g. Na_2HPO_4 , and 4.8 g. NaCl per litre of distilled water.

The progress of the pair treated with D.D.S. alone was similar to that of the corresponding pair of the previous experiment, and a biphasic response was again noted. The pair treated with systemic cortisone and the pair treated with subconjunctival cortisone behaved similarly, with only mild inflammatory changes corresponding to the first phase and nothing corresponding to the second phase. The fate of the pair treated with cortisone drops resembled that of the pair treated with D.D.S. alone. The first phase was a little milder, and the onset of the second phase was delayed, but it eventually developed to an even greater intensity.

One of the two rabbits receiving systemic cortisone gradually lost weight, developed weakness of the hind limbs, and was killed on the 19th day after inoculation. One of the two rabbits receiving subconjunctival cortisone remained well for 4 weeks, but then lost its appetite and became thin, and was killed on the 37th day after inoculation. There had been no recurrence of the iritis in either of these animals. The two animals receiving cortisone drops remained fit but developed glaucoma and were killed on the 19th day. The other animals used in this experiment remained well for 63 days.

(3) *Subconjunctival Cortisone.*—To confirm the efficiency of subconjunctival cortisone with D.D.S., three more non-immune rabbits were inoculated with 20,000 toxoplasms into the left anterior chamber. They were given 5 mg. cortisone subconjunctivally at the time of inoculation and 100 mg./kg. D.D.S. 8-hrly. The iritis which followed was generally of a more severe nature than that which occurred in the third pair of Experiment 2, and it was considered necessary to give the subconjunctival cortisone at 48-hr intervals for a total of five doses. All these animals had an iritis of one phase only: the eyes were completely quiescent on and after the 10th day.

Discussion

The biphasic response in the animals treated with D.D.S. alone suggests that two different reactions were involved. The period of least intensity was at the 7th day. We know that serum antibody is detectable on the 10th day in rabbits, and from other experiments with toxoplasmic peritonitis in mice

we have grounds for believing that antibody is produced at the primary site of infection and is detectable there one or two days before it is detectable in the blood serum. We tentatively suggest that the first phase of intense iritis is due to the toxic effect of the organisms and that the second phase may be "reversed allergy". In the untreated animal these two phases merge into one another, because it is not until the animal develops an antibody mechanism that the organisms are eliminated and the toxic effect ceases, and it is at this phase that the "reversed allergy" mechanism operates. The premature suspension of the toxic effect brought about by the chemotherapeutic action of D.D.S. allows a gap to appear between the two responses, the interval being larger, as would be expected, in the animals receiving earlier treatment. We have noticed similar biphasic responses in the size of skin lesions in rabbits being immunized to toxoplasmosis by intradermal inoculation followed by D.D.S. therapy.

Some support for this theory is provided by the examination of smears of ocular fluids, exudates, and tissues of the animals which died in the preliminary experiments. They showed very few free toxoplasms and those that were seen showed the bloated, faintly staining, reticulated appearance that we associate with antibody-affected organisms. However, "cysts" or terminal colonies were readily seen in sections.

If our surmise is correct, the possibility of reversed allergy in many acute as well as subacute and chronic infections opens up a large field of experimental work, and may perhaps require a revision of some of our conceptions of inflammatory responses.

Cortisone, when given from the start, would also appear to have some effect in reducing the primary toxic effect. Why it should do so is not clear, but there are three possible explanations. It may facilitate passage of D.D.S. into parasitized cells, or it may prevent the interaction of non-specific antibody with toxoplasma antigens, or it may prevent the products of interaction of non-specific antibody with toxoplasma antigens from acting on tissue cells and thus lessening vasodilations, the formation of inflammatory exudates, and the dissemination of parasites.

It is possible that in three of the cortisone-treated animals relapses may have been prevented by a longer course of D.D.S. therapy to cover the period of retardation of antibody formation and/or to eradicate more completely the toxoplasms from intracellular and possibly terminal colony sites. We feel that a further course of treatment with D.D.S. at the onset of the recurrence of iritis would have prevented the death of the animals. Whether a further period of either systemic or local cortisone should have been given as well is a matter for speculation.

The success obtained with subconjunctival cortisone together with the absence of systemic infections and recurrences of the iritis would suggest that this method of administration is superior to the subcutaneous route in early and in mild cases.

Summary

4: 4'-diaminodiphenylsulphone (D.D.S.) proved of value in the treatment of experimentally produced toxoplasmic infection in the anterior chamber of the eyes of rabbits. It was not completely successful, in as much as, though it caused a regression of the primary iritis, a secondary iritis developed after a further 2 or 3 days. It is suggested that the effect of the D.D.S. was to kill the toxoplasms (or most of them) and thus to cut short the primary toxic effects. It did not abolish the antigenic stimulus which after about 7 days resulted in the production of antibodies and a reversed allergy which was responsible for the secondary iritis.

Cortisone, although ineffective by itself, seemed to act by abolishing the secondary allergic iritis, and hence complete, or nearly complete, cures were obtained by the use of a combination of D.D.S. and cortisone. Best results were obtained when the drugs were given within a few hours of infection, but good results were obtained when they were withheld for as long as 53 hrs.

It is suggested that this treatment might be tried in toxoplasmic infection of the eyes in man. It is unlikely that it could be started so soon, but it is also unlikely that so many parasites would be involved in a natural infection.

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