Brit. J. Pharmacol. (1957), **12**, 185.

THE TREATMENT OF EXPERIMENTAL TOXOPLASMOSIS IN RABBITS

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(RECEIVED DECEMBER 21, 1956)

Sulphadimidine alone is of little value in treating experimental toxoplasmosis in rabbits, because most rabbits acetylate the drug very rapidly. Within a short time of dosing such animals, there is little or none of the uncombined drug in the blood. In rabbits which do not acetylate sulphadimidine rapidly, relatively high concentrations of the free sulphonamide can be attained in the blood: in such animals, toxoplasmosis responds to treatment with sulphadimidine. Sulphathiazole is not acetylated so rapidly and is effective even in rabbits which acetylate sulphadimidine quickly. Pyrimethamine, even in doses as high as 50 mg. three times daily, is ineffective; dapsone is effective.

There are few reports upon the action of sulphonamides in the treatment of toxoplasmosis in rabbits. Good results have been reported with sulphathiazole (Sabin and Warren, 1942; Biocca, 1945); toxoplasmosis of the uveal tract in rabbits was found to respond with moderate success to treatment with dapsone, and cures were obtained with a combination of dapsone and cortisone (Beverley, Beattie and Fry, 1954). We have now compared the efficiency of sulphadimidine with that of pyrimethamine and of dapsone in experimental toxoplasmosis in rabbits, and have investigated the way in which the therapeutic effect is influenced by the ability of individual rabbits to acetylate sulphonamides.

METHODS

Comparison of the Efficiency of Sulphadimidine, Dapsone, and Pyrimethamine.—Twenty-seven rabbits, which had been shown to have no detectable complement-fixing antibodies to toxoplasma, were arranged in groups of 3, each group containing rabbits weighing 2.0, 2.5, and 3.0 kg. The rabbits were inoculated intradermally with 0.2 ml. of diluted peritoneal exudate taken from a mouse infected 3 days earlier with the *RH* strain of *Toxoplasma gondii*. The inoculum contained about 80,000 organisms.

Beginning 2 hr. after inoculation, each rabbit was treated 3 times daily for 9 days with sulphadimidine, dapsone, or pyrimethamine. Sulphadimidine was given in doses of 50, 100, or 200 mg./rabbit, dapsone in doses of 25, 50, or 100 mg./rabbit, and pyrimethamine in doses of 12.5, 25, or 50 mg./rabbit (Beverley *et al.*, 1954). Drugs were administered in gelatin capsules. The rabbits were fed on a pellet diet (Diet No. 18, Associated London Flour Millers).

Estimation of Sulphonamides.—Doses of drugs were given in gelatin capsules or suspended in 5 ml. of water and given by stomach tube under pentobarbitone (Nembutal) anaesthesia, or by intravenous injection of an aqueous solution of a soluble salt (*p*H adjusted to 7.0).

Blood was collected from an ear vein, heparinized, and 1 ml. samples transferred to centrifuge tubes containing 2 ml. of 0.2 M-Na_2HPO_4. Protein was precipitated by the addition of 1 ml. 20% w/v trichloracetic acid, and removed by centrifuging. Free and acetylated sulphonamides were determined colorimetrically by the procedure of Bratton and Marshall (1939), as modified by Francis and Spinks (1950). A Unicam spectrophotometer SP 500 was used for the measurement of absorption at 525 mμ.

RESULTS

All three rabbits which were given 100 mg. of dapsone and two of those receiving 50 mg. three times a day survived. Pyrimethamine, even in doses as high as 50 mg. thrice daily, gave no protection. Only two of the rabbits receiving sulphadimidine survived; one of these had been given 200 mg. and the other 50 mg. three times daily. Sulphadimidine was therefore not very active in controlling toxoplasmosis in rabbits, although it had proved more effective than dapsone in infected mice (Beverley and Fry, 1956, 1957).

Blood Concentrations of Sulphadimidine.—Two rabbits (Nos. 273 and 274) were each given three capsules containing sulphadimidine equivalent to a dose of 400 μg./kg. Both rabbits showed two maxima in the blood concentration of the sulphonamide, one at 1 hr. and the other at 5 hr. after

the dose. A single maximum was expected, and the observed result may have been due to the capsules failing to dissolve at the same time. The most striking feature of the results was that, whereas rabbit 274 reached a peak concentration of 170 μg. sulphadimidine/ml. of blood, the highest concentration in rabbit 273 was only 16 μg./ml. To ensure that all the drug was free in the intestine immediately after giving the dose, 200 mg. of the drug was given by oesophageal tube to the same two rabbits, anaesthetized with pentobarbitone given intravenously. Once again the level of sulphadimidine in 274 was much higher than in 273 (Fig. 1). These results led us to suspect that

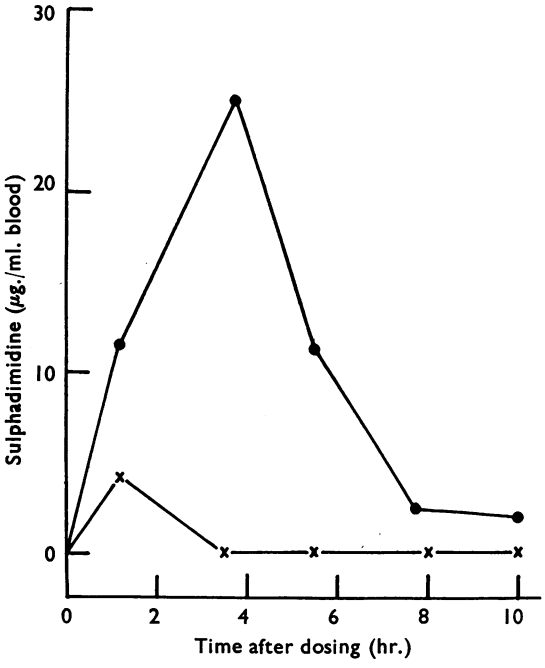

FIG. 1.—Blood concentration of sulphadimidine in rabbits after a single dose of 200 mg. given by oesophageal tube. Rabbits 273 (crosses) and 274 (dots) were anaesthetized with pentobarbitone.

the low activity of sulphadimidine against toxoplasma was due to the inability of those rabbits which died to attain a high enough concentration of the drug in the blood. The two survivors (263 and 267) from the groups of infected rabbits given sulphadimidine were each given one dose of 200 mg. sulphadimidine by oesophageal tube, and, whilst there was an appreciable difference between the two animals (Fig. 2), it was to be noted that rabbit 263 (crosses) with the lower blood concentration of sulphadimidine was the survivor which had received 200 mg. doses of sulphadimidine, whereas rabbit 267 (dots) with the higher concentration had received only 50 mg. doses.

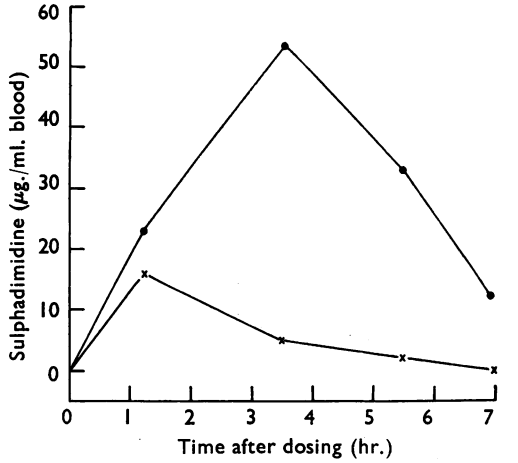

FIG. 2.—Blood concentration of sulphadimidine in two rabbits which were successfully treated with sulphadimidine after being infected with toxoplasma. Rabbits 263 (crosses) and 267 (dots) each received a single dose of 200 mg. sulphadimidine by oesophageal tube under pentobarbitone anaesthesia.

These preliminary experiments indicated that there might be considerable variation in the ability of rabbits to attain significant concentrations of sulphadimidine in their blood. The results of an experiment in which eleven rabbits were each given 200 mg. sulphadimidine by oesophageal tube supported this conclusion (Table I). In eight of the rabbits the blood concentrations of sulphadimidine were no more than 4 μg./ml. an hour after dosing, and only two had concentrations greater than 10 μg./ml. Such results might reflect differences in the ease with which sulphadimidine is absorbed by different rabbits. Four rabbits were, therefore, each given 200 mg.

TABLE I

BLOOD CONCENTRATION OF SULPHADIMIDINE IN RABBITS

Each rabbit was anaesthetized with pentobarbitone intravenously and given 200 mg. sulphadimidine by oesophageal tube. Blood samples taken at 1, 3, and 5 hr. after dosing.

Rabbit No.	Type	Weight (kg.)	Conc. of Sulphadimidine (μg./ml. Blood)		
			1 hr.	3 hr.	5 hr.
295	English	3·20	<0·5	0	0
296	Sable	2·62	14·4	13·0	5·4
297	Dutch	1·76	1·8	2·8	0
298	English × Dutch	1·66	3·4	0	0
299	English	2·30	31·8	14·4	5·8
301	Agouti	3·80	0	0	0
302	,,	3·26	<0·5	0	0
303	Dutch	1·60	4·0	2·2	0
304	Blue	2·50	<0·5	0	0
305	English	3·00	1·0	<0·5	<0·5
306	Dutch	2·30	3·4	2·6	4·0

of sulphadimidine by oesophageal tube and then kept in metabolism cages. Analysis of the blood 2 hr. and 4 hr. after dosing showed that, whereas in rabbits 263 and 267 the blood concentration of the free sulphonamide was relatively high and there was only a small amount in the acetylated state, in rabbits 301 and 302 there was no free sulphadimidine although there were considerable amounts of the acetylated form (Table II). Analysis of the urine excreted within 24 to 30 hr. after

TABLE II

CONCENTRATION OF FREE AND ACETYLATED SULPHADIMIDINE IN BLOOD AND URINE OF RABBITS

Each rabbit was anaesthetized with pentobarbitone intravenously and given 200 mg. sulphadimidine by oesophageal tube. The animals were kept in metabolism cages and blood samples taken 2 and 4 hr. after dosing.

Rabbit No.	Blood: Sulphadimidine (μg./ml.) 2 hr. Free	2 hr. Acetylated	4 hr. Free	4 hr. Acetylated	Urine: Total Vol. (ml.)	Period (hr.)	Sulphadimidine Free (μg./ml.)	Sulphadimidine Acetylated (μg./ml.)	Total (mg.)
263	37·1	6·1	28·0	5·3	133	24	274	721	132
267	46·1	3·8	27·5	4·8	156	21	276	616	139
301	0	28·5	0	21·3	188	30	9·5	761	145
302	0	29·1	0	19·7	205	30	3·8	406	84

dosing revealed that all the rabbits had absorbed at least 80 mg. (40% of the dose of sulphadimidine), and in three of them about 130 mg. (65% of the dose) had been absorbed. It was concluded that all four rabbits absorbed sulphadimidine easily and the differences in the blood concentration of free sulphadimidine were the outcome of the speed with which rabbits are able to

TABLE III

CONCENTRATION OF FREE AND ACETYLATED SULPHADIMIDINE IN BLOOD OF RABBITS AFTER INTRAVENOUS INJECTION OF THE DRUG

Each rabbit received 10 ml. of solution containing 1.2 mg./ml. over a period of 4 min.

Time after Dose (min.)	Sulphadimidine Conc. in Blood (μg./ml.) Rabbit 263 Free	Rabbit 263 Acetylated	Rabbit 267 Free	Rabbit 267 Acetylated	Rabbit 295 Free	Rabbit 295 Acetylated	Rabbit 301 Free	Rabbit 301 Acetylated
0	0	0	0	0	0	0	0	0
7	10·1	0	10·5	0	4·9	4·6	3·7	4·5
20	7·9	0·7	8·2	1·3	1·9	5·2	1·5	5·6
40	5·4	0	5·2	0	0·8	3·1	0·6	3·3
70	4·2	0·5	4·1	1·1	0·6	2·2	0	3·7
120	2·1	0·7	1·9	0·2	—	—	—	—

acetylate the drug. This was confirmed by an experiment in which four rabbits were each injected intravenously with 12 mg. of sulphadimidine dissolved in water. In the blood of rabbits 263 and 267 described as "slow acetylaters" on the basis of the results of the previous experiments, there were at least 4 μg./ml. of uncombined sulphadimidine in the blood even 1 hr. after dosing, and little of the drug was acetylated (Table III). On the other hand, with rabbits 295 and 301, suspected to be "quick acetylaters," very little of the sulphadimidine in the blood was in the free state after the first 20 min.; most was in the acetylated form. The rapidity with which acetylation of sulphadimidine takes place in some rabbits was shown by giving 1 g. by oesophageal tube to rabbit 302. The maximal concentration of free sulphadimidine in the blood was only 3.4 μg./ml.; this concentration was maintained for about 5 hr. Within 1 hr. of dosing the blood contained 61 μg./ml. of acetylsulphadimidine.

Blood Concentrations of Sulphathiazole.—In early reports of the successful treatment of toxoplasmosis in rabbits the sulphonamide used was sulphathiazole (Sabin and Warren, 1942; Biocca, 1945). It was, therefore, decided to determine whether it is possible to obtain greater blood concentrations of sulphathiazole than of sulphadimidine. Two rabbits, 295 and 305, suspected to be "quick acetylaters" of sulphadimidine (Table I) were given 200 mg. sulphathiazole by mouth. The blood concentrations of sulphathiazole, although relatively low, were greater than those attained with sulphadimidine (Table IV).

TABLE IV

CONCENTRATION OF FREE AND ACETYLATED SULPHATHIAZOLE IN BLOOD OF RABBITS AFTER GIVING THE DRUG BY OESOPHAGEAL TUBE

Each rabbit was anaesthetized with pentobarbitone intravenously and given 200 mg. sulphathiazole.

Time after Dose (Hr.)	Sulphathiazole Conc. in Blood (μg./ml.) Rabbit 295 Free	Rabbit 295 Acetylated	Rabbit 305 Free	Rabbit 305 Acetylated
1	12·5	20·3	5·9	16·8
3	2·7	10·1	2·4	6·4
5	1·3	2·7	1·1	1·6

Comparative Therapeutic Efficiency of Sulphathiazole and Sulphadimidine.—Three pairs of rabbits were inoculated intradermally with toxoplasma; one pair (301 and 302, "quick acetylaters") received sulphadimidine, another pair (295 and 305, "quick acetylaters") received sulphathiazole, and the remaining pair (296 and 299, "slow acetylaters") received sulphadimidine. The drugs were given in capsules (200 mg./rabbit) at four-hourly intervals (omitting one dose at 4 a.m. for nine days). On the eighth day rabbits 301 and 302 died. These were both "quick acetylaters" and known to be unable to attain significant concentrations of free sulphadimidine in the blood (Table I). The treatment of the other two pairs of rabbits was stopped on the

ninth day and they were still alive and well three weeks later. It was concluded that sulphathiazole is likely to be superior to sulphadimidine in the treatment of toxoplasmosis in rabbits. It appears from the results of these experiments that rabbits vary in their abilities to acetylate sulphonamides, and that different sulphonamides are acetylated to different extents in the same rabbit. To test this conclusion, doses of 200 mg. of sulphadiazine, sulphamerazine, and sulphanilamide were given in separate experiments by oesophageal tube to two rabbits. One (295) was a " quick acetylater " and the other (296) a " slow acetylater," on the basis of the experiments with sulphadimidine shown in Table I. Rabbit 295 acetylated sulphadiazine, sulphamerazine and sulphanilamide at a rapid rate (Table V) and it could be reasonably predicted that in this animal sulphamerazine and

TABLE V

CONCENTRATION OF FREE AND ACETYLATED SULPHADIAZINE, SULPHAMERAZINE, AND SULPHANILAMIDE IN THE BLOOD OF RABBITS

Each rabbit was anaesthetized with pentobarbitone intravenously and given 200 mg. of the sulphonamide by oesophageal tube.

Drug	Time after Dose (Hr.)	Sulphonamide Conc. in Blood (μg./ml.)			
		Rabbit 295		Rabbit 296	
		Free	Acetylated	Free	Acetylated
Sulphadiazine..	1	13·6	29·6	28·2	3·5
	3	14·7	20·2	44·7	5·9
	5	10·7	13·1	47·0	5·2
Sulphamerazine	1	3·5	28·6	18·4	5·6
	3	0·8	23·0	39·0	21·5
	5	2·7	35·5	35·2	18·2
Sulphanilamide	1	12·0	15·7	18·4	1·2
	3	3·7	16·6	28·6	3·4
	5	1·6	11·5	27·5	3·5

sulphadimidine would be of little therapeutic value against toxoplasma. On the other hand, the blood concentrations of sulphanilamide (Table V) and of sulphathiazole (Table IV) for at least an hour after dosing and of sulphadiazine for 5 hr. (Table V) were such that these drugs might be of some value in combating infection. In rabbit 296, a " slow acetylater " of sulphadimidine, high concentrations of free sulphadiazine, sulphamerazine and sulphanilamide were readily attained (Table V).

DISCUSSION

In the above experiments, the concentration of free sulphadimidine in the blood has been taken as an index of the amount of the drug available to combat infection. Sulphadimidine is successful in curing toxoplasmosis in mice, but its value in treating rabbits is doubtful because of the great variation in the concentration of free sulphadimidine which can be attained in the blood of these animals. The evidence presented in this paper supports the view that the wide range of blood concentrations of free sulphadimidine in rabbits is due to differences in the speed with which different rabbits can acetylate sulphadimidine and thus destroy its therapeutic properties. The enzymatic mechanism for the acetylation of sulphonamide has already been investigated in detail (Lipmann, 1945) and occurs, in the rabbit, only in the liver (van Winkle and Cutting, 1940). It will be of interest to establish whether or not this quantitative variation in the ability of rabbits to acetylate sulphadimidine can be related to differences in the amount of the enzyme system/unit weight of liver or to the size of the liver in proportion to the whole animal. Furthermore, the nutrition of the animal may affect its acetylating abilities, because coenzyme A is an important co-factor in the acetylation of sulphonamides and the amount of coenzyme A present in the liver will be related, in part at least, to the amounts of pantothenic acid present in the animal's diet. Sulphathiazole appears to be more effective than sulphadimidine in the treatment of experimental toxoplasmosis in rabbits: this is probably because it is not acetylated as quickly, and an effective therapeutic concentration of sulphathiazole can therefore be maintained for a longer period. Sulphanilamide, sulphathiazole, and sulphadiazine are already known to be acetylated at different speeds by the acetylase system of pigeon liver (Lipmann, 1945), and there is some evidence that the same applies in man (Janeway, 1942).

We gratefully acknowledge the advice and encouragement of Professor C. P. Beattie and the technical assistance of Miss G. Calton and Mrs. M. Fox during the course of these experiments. The work was aided by a grant from the Medical Research Council.

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