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Therapeutic Effectiveness of Certain Sulfonamides on Infection by an Intracellular Protozoon (Toxoplasma).*

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The fact that many drugs of known effectiveness against other protozoa were toxoplasmacidal *in vitro* without exhibiting any therapeutic action on toxoplasmic infection in mice and rabbits was reported in the preceding communication.[1] It was also pointed out that sulfanilamide, sulfathiazole, and sulfapyridine in concentrations of 25 mg % had no effect on toxoplasma *in vitro*, and that sodium sulfapyridine administered parenterally twice daily failed to modify the course of toxoplasmic infection in mice and rabbits.

In preliminary tests with the commonly used method of administering sulfonamides in the diet, it was found that mice receiving a diet containing 1% sulfathiazole resisted infection with many fatal doses of toxoplasma. A series of experiments designed to test the extent of the therapeutic or curative effect of certain sulfonamides on infection caused by this obligate intracellular protozoon parasite[2,3] were then carried out. The same virulent "R.H." strain of toxoplasma of human origin that was used in the preceding study was also used in the present experiments. The brains of mice succumbing after intracerebral inoculation were ground in a mortar without an abrasive and enough physiological salt solution or Tyrode's solution was added to make a 10% suspension. These suspensions were not centrifuged since toxoplasma are sedimented at relatively low speeds.

Comparative Effect of Sulfanilamide, Sulfapyridine, and Sulfathiazole. Mice weighing approximately 20 g were inoculated intraabdominally with 0.3 cc of various dilutions of toxoplasma-infected mouse brain suspension and divided into 4 groups. One group received a powdered diet without drug and the others were given the same diet containing 1% of sulfanilamide, sulfapyridine, or sulfathiazole. The mice were allowed to consume all they could eat. Random tests on the blood of these mice after they had been consuming these diets for several days indicated levels of 4 to 5 mg % in the sulfanilamide group, 2 to 3 mg % in the sulfapyridine group, and 1 to 2 mg % in the sulfathiazole group. The results shown in Table I indicate that sulfanilamide only prolonged the survival time and prevented death only in mice inoculated with about a single M.L.D. of toxoplasma; however, none of the mice receiving sulfapyridine or sulfathiazole succumbed while they were on the diet—not even those inoculated with 1,000 to 10,000 M.L.D. of toxoplasma. When these drugs were discontinued after 13 days, the mice inoculated with the larger numbers of toxoplasma succumbed, as a rule within 8 to 16 days after removal of the drug. That toxoplasma were the cause of death was established by demonstrating them in large numbers in stained films of the lungs. The mice were observed for 10 to 12 weeks before the experiment was discontinued. Surviving animals were found to be free of toxoplasma as demonstrated by subinoculation of their viscera into other mice.

Influence of Duration of Sulfathiazole Therapy on Extent of Curative Effect. It was apparent from the preceding experiment that sulfapyridine and sulfathiazole were capable of holding in check the proliferation of toxo-

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1 Warren, J., and Sabin, A. B., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 15.

2 Sabin, A. B., and Olitsky, P. K., *Science*, 1937, **85**, 336.

3 Sabin, A. B., Toxoplasmosis, a recently recognized disease of human beings, *Advances in Pediatrics*, Interscience Publishers, Inc., New York, 1942.

TABLE I.
Comparative Effect of Sulfanilamide, Sulfapyridine, and Sulfathiazole in Diet on Toxoplasmic Infection in Mice.

Drug in diet	Duration of therapy, days	Dilution of toxoplasma-infected brain suspension (0.3 cc intraäbdominally) 10–1	10–2	10–3	10–4	10–5
None	—	5, 5, 6* 6, 6, 7	8, 8, 8 8, 8, 9	8, 9, 9 10, 10, 10	10, 11, 11	15, 0
Sulfanilamide 1%	34	6, 7, 7 9, 12, 12	12, 12, 15 16, 16, 21	17, 18, 20 21, 26, 0	28, 0, 0	—
Sulfapyridine 1%	13	*21, 24, 25* *25, 27, 29*	*25, 27, 43* *46*, 0, 0	0, 0, 0 0, 0	0, 0, 0	
Sulfathiazole 1%	13	K 22,† K 22† *26*	*25, 25*, 0	*27*, 0, 0	0, 0, 0	

*Numerals refer to day of death of inoculated mouse.
0 = mouse remained well.
Numerals in italics indicate mice which died of toxoplasmic infection after discontinuance of drug; numbers refer to days after inoculation.
†K 22 = mice were well but were killed on the 22nd day and toxoplasma were demonstrated in their viscera by subinoculation.

TABLE II.
Influence of Duration of Sulfathiazole Therapy on Extent of Curative Effect in Mice.

Exp.	Duration of therapy. 1% sulfathiazole in diet	Dilution of toxoplasma-infected brain suspension (0.3 cc intraäbdominally) 10–1	10–2	10–3	10–4	10–5
A	Controls—no drug in diet	—	5, 7, 7	8, 8, 9	9, 10, 10	14, 0, 0
	16 days	*29, 29, 31*	0, 0, 0	0, 0, 0	0, 0, 0	—
B	Controls—no drug in diet	5, 5, 5	7, 8, 8	8, 9, 9	10, 10, 11	0, 0, 0
		5, 6, 6	8, 8, 8	9, 9, 10	11, 11, 0	0, 0, 0
	30 days	*42, 42, 43*	*46*, 0, 0	0, 0, 0	0, 0, 0	*46*, 0, 0
		46, 47, 47	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
	45 "	*62, 65, 66*	*107*, 0, 0	*88*, 0, 0	0, 0, 0	0, 0, 0
		68, 68, 81	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0

Legends as in Table I.

plasma. The question arose as to how long these drugs could hold back this proliferation and whether, if they were administered long enough, the body might finally rid itself of even the larger numbers of toxoplasma. Results of tests in which the 1% sulfathiazole diet was administered for periods of 16, 30, and 45 days to mice infected with various doses of toxoplasma, are shown in Table II. It may be seen that, although somewhat better results were obtained in these groups than in the one given the same diet for 13 days, there is, nevertheless, a limit to the number of toxoplasma, namely about 100 M.L.D., of which the mice can rid themselves, apparently regardless of the duration of therapy. It is of great interest that as long as the mice consume the drug, even those inoculated with the largest amounts remain well. Moreover, among the mice receiving the diet for 45 days a period of 20 to 60 days may elapse after removal of the drug before they succumb to toxoplasmic infection, although even the smallest numbers of toxoplasma require a maximum of only about 14 days to kill normal mice. The changes thus produced in the parasite or the host by the long administration of sulfathiazole would appear to be worthy of further investigation.

Tests on toxoplasma isolated from mice which ultimately succumbed after discontinuance of the drug revealed that they had not become drug-fast since infection produced by them in other mice was affected by sulfathiazole in the usual manner. The surviving mice from the groups treated with sulfathiazole for 30 and 45 days were used for two types of tests 4 months after they were in-

TABLE III.
Effect of Delaying Sulfathiazole Therapy on Toxoplasmic Infection in Mice.

Time after infection 1% sulfathiazole diet begun	Total time on diet, days	No. of mice	% of mice surviving one month	% of mice cured	Day of death of succumbing mice
Immediately	23	9	100	78	*37, 37*
2 days	28	9	67	56	8, 14, 20, *42*
4 "	26	19	47	0	8, 8, 8, 9, 11 12, 13, 13, 13, 21 *40, 40, 40, 42 42, 42, 42, 42, 44*
5 "	Duration of experiment	10	0	0	6, 6, 6, 8, 8 8, 8, 11, 11, 15
6 "	"	10	0	0	7, 7, 8, 8, 8 8, 8, 8, 8, 8
6 " 2 mg t.i.d. by stomach tube on 6th day + diet	"	10	10	0	7, 7, 7, 7, 7 7, 8, 8, 8, *46**
Untreated controls	—	20	0	0	7, 7, 7, 7, 8 8, 8, 8, 8, 8 8, 8, 8, 8, 8 8, 9, 9, 9, 10

Legends as in Table I.
*This mouse was on the sulfathiazole diet for 22 days.
All mice were inoculated intraäbdominally with 0.3 cc of a 1:100 suspension of toxoplasma-infected brain—about 100 M.L.D.

oculated. In one test, one mouse from each group inoculated with a given dilution of toxoplasma was sacrificed and a pool of its viscera was subinoculated in 3 other mice to establish the presence or absence of toxoplasma; 7 sulfathiazole-treated and 3 control mice were thus tested but no toxoplasma were found. The remaining mice were tested for immunity by an intraäbdominal injection of about 100 M.L.D. of toxoplasma, but none was found to be immune. Similar tests on other toxoplasma-infected, sulfathiazole-treated, surviving mice revealed that they were not resistant to reinfection and that immunity did not appear to be a factor in their survival.

Effect of Delaying Sulfathiazole Therapy. In the preceding experiments the mice were given the sulfathiazole diet immediately after inoculation with toxoplasma. It was of some interest to know how long it would be possible to withhold the drug and still obtain a therapeutic effect. Ninety mice were infected intraäbdominally with about 100 M.L.D. of toxoplasma; one group received no drug and others were given the 1% sulfathiazole diet at different times—immediately, 2 days, 4 days, 5 days, and 6 days after infection. Because the mice were already so sick on the 6th day that they consumed little or nothing of their diet, one group also received 2 mg of sulfathiazole by stomach tube 3 times during that day. The results shown in Table III indicate that when the drug was first given 2 days after infection, 56% of the mice were cured, *i.e.*, survived without relapse after discontinuance of the drug. This result was unexpected since it is known that toxoplasma undergo considerable proliferation during the first 2 days, and the preceding experiments indicated that infection with 1,000 M.L.D. or more could be held in check only while the drug was being administered and for a short time thereafter. When administration of the drug was delayed for 4 days, it was without effect in about 25% of the mice, prolonged the survival time in another 25%, and in the remainder the infection was checked while the drug was being consumed but all the mice died 10 to 14 days after therapy was discontinued. No therapeutic effect was obtained when sulfathiazole was first given 5 days after infection.

Effect of Sulfathiazole in the Diet on Infection by the Intracerebral Route. Severe

TABLE IV.
Effect of Sulfathiazole in Diet on Toxoplasmic Infection by Intracerebral Route in Mice.

Dil. of toxoplasma-infected brain suspension	Controls no drug in diet	1% sulfathiazole in diet for 21 days after intracerebral injection of toxoplasma	
		Mice dying while on drug	Mice dying after removing drug
10–1	4, 5, 5	0, 0, 0	0, 0, *33*
10–2	6, 6, 6	0, 0, 0	*36, 46, 51*
10–3	6, 6, 7	0, 0, 0	0, *54, 54*
10–4	7, 7, 7	0, 0, 0	0, 0, *46*
10–5	8, 9, 0	0, 0, 0	0, 0, 0

Legends as in Table I.

ependymitis, encephalitis and meningitis follow intracerebral injection of toxoplasma in mice. The results, shown in Table IV, indicate that sulfathiazole completely held in check this cerebral infection even when 10,000 M.L.D. were injected, as long as the drug was being consumed and for a considerable time thereafter. About 50% of the mice were cured, seemingly regardless of the size of inoculum, while the others died 12 to 33 days after discontinuance of the drug. When one considers the relatively short time in which the concentration of sulfathiazole in the blood reaches insignificant levels after discontinuance of the drug, and that the longest survival time after the minimal dose of toxoplasma was 9 days, it is rather remarkable that so many of the animals did not die for 25 to 33 days after the drug was removed from their diet.

Effect of Sulfathiazole on Toxoplasmic Infection in Rabbits. Rabbits were infected by the intracutaneous injection of toxoplasma-infected mouse brain suspension. The results shown in Table V indicate that 1% sulfathiazole in their diet had no effect on the infection. Examination of the blood of these rabbits revealed sulfathiazole levels which varied from 0 to 2 mg %, indicating that their irregular eating habits were probably responsible for the failure of the drug diet in the above experiment. In the previous communication it was reported that sodium sulfapyridine given intravenously twice a day was also ineffective. It remained to be determined, therefore, whether if proper levels of sulfonamide could be maintained, it would be possible to influence toxoplasmic infection in rabbits as well as in mice. Preliminary tests showed that after the intraäbdominal injection of 200 mg of sulfathiazole (suspended in 4 cc of H_2O) blood levels of 1 mg % or more were still present at 3 hr but irregularly or not at all later on. When sulfathiazole was administered intraäbdominally as described in Table V, it was possible completely to check the infection in the treated rabbits, even when the drug was first started 3 days after inoculation, at a time when a well-marked local lesion as well as high fever, indicative of widespread systemic involvement, were already present. This development of the local lesions and the fever before treatment was begun, affords good evidence that the rabbits were not spontaneously resistant. Small local lesions but no significant fever occurred in the rabbits treated immediately after infection; after discontinuance of the drug there were several rises of temperature which might have been on a toxoplasmic basis. The rabbits, however, survived and unlike the mice were resistant to reinfection.

Effect of Toxoplasmacidal Drugs in the Diet. Since in the unsuccessful therapeutic tests with the toxoplasmacidal drugs reported in the previous communication,[1] the drugs were all administered parenterally, it was desirable to repeat these tests with some of the same compounds incorporated in the diet. Groups of mice infected with 1, 10, 100, or 1,000 M.L.D. of toxoplasma by the intraabdominal route, were given diets containing 1% of either atabrin hydrochloride or quinine hydrochloride. However, no therapeutic effect of any kind was observed.

Discussion. The results presented in this communication are the first to demonstrate the inhibitory and, under certain conditions,

TABLE V.
Effect of Sulfathiazole Parenterally and in the Diet on Toxoplasmic Infection in Rabbits.

Exp.	Mode of administration of sulfathiazole	Time after infection drug started	Result
A	Controls—no drug	—	8,* 10, S
	1% in diet	Immediately	9, 9, S
		3 days (1st day of fever)	8, 9, 9
		5 days	6, 8, 12
B	Controls—no drug	—	8, 10, 11
	1.5-2 g per day intraäbdominally, divided doses every 3 hr between 6 A.M. and 6 P.M. and every 6 hr between 6 P.M. and 6 A.M.—for 12 days. Then 5% sulfathiazole in diet for 7 days	Immediately	S, S, S
	2 to 3 g per day intraäbdominally in divided doses as above—for 9 days. Then 5% sulfathiazole in diet for 7 days	3 days (1st day of fever)	S, S

*Numerals refer to day of death of each inoculated rabbit.
S = rabbit survived for 6 weeks or longer.
All rabbits infected by intracutaneous injection of 10% suspension of toxoplasma-infected mouse brain.

curative effect of certain sulfonamides on an infection by an apparently obligate intracellular protozoon parasite capable of multiplying in a great variety of fixed tissue cells. The behavior of sulfathiazole and sulfapyridine in this infection, particularly their capacity to hold in check the proliferation of large numbers of toxoplasma as long as the animal consumes the drug and, under certain conditions, also for long periods thereafter, appears to be different from the effects of these drugs observed in bacterial infection and from the reported action of sulfanilamide on *Plasmodium knowlesi* in monkeys.[4] Sulfanilamide killed these plasmodia *in vitro* and several daily injections, or as little as one dose of 1 g of sulfanilamide by mouth, sufficed to eradicate completely both acute and chronic infections in monkeys.

Summary. Sulfathiazole and sulfapyridine, although without effect on toxoplasma *in vitro,* have been found to have a complete inhibitory and under certain conditions, curative action on toxoplasmic infection in mice when administered in 1% concentration in the diet. Sulfanilamide did not have a similar effect. Mice infected with 100 M.L.D. or less were cured when 1% sulfathiazole was given for 16 days or longer. Although mice infected intraäbdominally with a single M.L.D. had a maximum survival time of about 14 days, animals infected with 1,000 to 10,000 M.L.D. remained well as long as they were consuming the drug diet and for variable periods thereafter, but ultimately succumbed to toxoplasmic infection. It seemed remarkable that many such animals which had received the 1% sulfathiazole diet for 45 days did not succumb for 1 to 2 months after discontinuance of the drug. Sulfathiazole in the diet was equally effective when the toxoplasma were injected intraäbdominally or intracerebrally. Apparently because of the irregular eating habits of rabbits, 1% sulfathiazole in the diet was without effect on toxoplasmic infection in them. However, when large doses of sulfathiazole were administered parenterally at regular intervals, day and night, a distinct curative effect was obtained even when the drug was first administered 3 days after infection by the intracutaneous route, at a time when well developed local lesions and fever, indicative of a generalized systemic infection, were present. Atabrin and quinine hydrochloride which were previously found to be toxoplasmacidal in *in vitro* tests, were without effect on toxoplasmic infection in mice when administered in the diet.

4 Coggeshall, L. T., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 768; *Am. J. Trop. Med.*, 1938, **18**, 715; *J. Exp. Med.*, 1940, **71**, 13.