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Effect of Certain Antiprotozoal Drugs on Toxoplasma *In Vitro* and *In Vivo*.*

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The recent demonstration that toxoplasma are pathogenic for human beings[1] stimulated the search for a chemotherapeutic agent that might be effective against this protozoon infection. Toxoplasma behave like obligate intracellular parasites with affinities for a great variety of fixed tissue cells and thus far it has not proved possible to cultivate them in media devoid of living, susceptible cells.[2] The effect on toxoplasma of certain compounds, known for their activity against various protozoa or other infectious agents, was, therefore, studied in two ways: (1) by incubating *in vitro* toxoplasma-containing exudate with the various drugs and then testing for survival of the toxoplasma by injecting the mixtures in mice, and (2) by administering the compounds under investigation to mice and rabbits infected with known amounts of toxoplasma.

The "R. H." strain of toxoplasma isolated in 1939 from a fatal case of human encephalitis[3] was used in these tests. This strain has been maintained in the laboratory by regular intracerebral passages in mice. Its virulence was such that 0.03 cc of a 1:10 suspension of infected brain inoculated intracerebrally killed all mice in 4 to 6 days. The minimal lethal dose (M.L.D.) was as a rule contained in the 10^{-4} to 10^{-5} dilution of infected brain, the mice inoculated with the smallest numbers of toxoplasma usually dying in 10 to 12 days. About 10 times as many toxoplasma were required to produce fatal infection by the intraperitoneal route, since the M.L.D. by this route was usually 0.3 cc of the 10^{-4} to 10^{-5} dilution. There was no evidence that this strain of toxoplasma gave rise to infection when less than lethal amounts were used in mice. Peritoneal exudate is abundant during the terminal stages of infection in mice inoculated with toxoplasma by the intraäbdominal route. This exudate diluted with heparinized (1:5,000) Tyrode's solution until there were 4 to 6 toxoplasma per high power field (×400), contained at least 10,000 M.L.D. per 0.3 cc administered by the intraäbdominal route, and was used in the *in vitro* tests.

In Vitro Tests. The procedure was as follows: 1.5 cc of diluted, heparinized, toxoplasma-containing, peritoneal exudate was mixed with 1.5 cc of various concentrations of drug in Tyrode's solution (pH 7.8) and incubated in a water bath at 37°C. One cc portions were removed at intervals of 3, 6, and 24 hr, and 0.3 cc was inoculated intraabdominally into each of 3 mice. For control, similar mixtures of toxoplasma and Tyrode's solution without drug were incubated and tested simultaneously at the same intervals. This procedure of testing the *in vitro* effect of various drugs is somewhat similar to that employed by Yorke and Murgatroyd[4] in their studies on trypanosomes.

The results of several such tests, summarized in Table I, indicate that quite a number of the compounds tested were able to destroy toxoplasma *in vitro*. This toxoplasmacidal effect was not altogether expected since, while most of the toxoplasma in the exudate were extracellular, many were intra-

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

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

3 Sabin, A. B., *J. A. M. A.*, 1941, **116**, 801.

4 Yorke, W., and Murgatroyd, R., *Ann. Trop. Med.*, 1930, **24**, 449.

TABLE I.
Effect of Certain Compounds on Toxoplasma *in Vitro*.

Drug	Conc. of drug in mixture × 1000	Infectivity of mixture Toxoplasma and drug inoculated intra-abdominally into mice after incubation of mixtures at 37°C for 3 hr	6 hr	24 hr
Atabrin	1:10	0, 0, 0	0, 0, 0	0, 0, 0
,,	1:50	0, 0, 0	0, 0, 0	0, 0, 0
,,	1:100	7, 8, 9	10, 10, 11	0, 0, 0
Trypaflavin	1:10	9, 9, 9	0, 0, 0	0, 0, 0
Pot. Antimony Tartrate	1:10	11, 12, 12	0, 0, 0	0, 0, 0
Rivanol Lactate	1:10	10, 10, 12	0, 0, 0	0, 0, 0
Optochin	1:10	7, 9, 9	0, 0, 0	0, 0, 0
Mapharsen	1:20	7, 7, 7	0, 0, 0	0, 0, 0
Neosalvarsan	1:20	6, 6, 6	6, 7, 7	0, 0, 0
Tryparsamide	1:10	7, 7, 7	6, 6, 7	0, 0, 0
Quinine HCl	1:10	6, 6, 7	7, 8, 8	0, 0, 0
Bayer 205	1:10	7, 7, 8	7, 8, 9	12, 0, 0
Stibosan	1:10	6, 7, 7	7, 8, 8	13, 14, 14
4:4′ Diamidino Stilbene	1:10	7, 7, 7	8, 9, 9	12, 12, 12
Sulfanilamide	1:4	6, 6, 6	6, 6, 7	11, 11, 13
Na Sulfathiazole	1:4	6, 6, 6	6, 7, 7	10, 10, 10
Na Sulfapyridine	1:4	6, 6, 7	6, 6, 7	10, 10, 10
		6, 6, 8*	8, 8, 9	9, 9, 10
		6, 6, 9	7, 7, 7	8, 9, 9
Controls		6, 6, 6	6, 6, 6	9, 9, 10
Toxoplasma + Tyrode's solution		6, 6, 7	7, 7, 8	10, 11, 14
		7, 7, 7	6, 7, 7	9, 9, 10

*Numerals refer to day of death of inoculated mouse. 0 = mouse remained well.

cellular and presumably protected from the action of the drugs. The acridine derivatives, *atabrin, trypaflavin,* and *rivanol lactate* were all toxoplasmacidal, with atabrin being by far the most effective member of the group as well as the best of all compounds tested. In a concentration of 1:50,000 atabrin killed toxoplasma in 3 hr or less, while more than 6 and less than 24 hr were required for the same effect when the amount was reduced to 1:100,000. Among the antimony compounds *potassium antimony tartrate* in a concentration of 1:10,000 destroyed the toxoplasma in 3 to 6 hr while *stibosan* in the same concentration had no effect in 24 hr. Of the arsenic compounds tested *mapharsen* acted more rapidly than *neosalvarsan* or *tryparsamide.* In the quinine group, optochin was more effective than *quinine hydrochloride. Sulfanilamide, sodium sulfathiazole* and *sodium sulfapyridine* in concentrations of 1:4,000 (25 mg %), and *4:4′ diamidino-stilbene* in a concentration of 1:10,000 were completely without effect under the conditions of this test.

In Vivo Tests. Since the effect of the various drugs *in vitro* could be judged only by inoculating the toxoplasma-drug mixtures in animals, it was obviously impossible, solely on the basis of the preceding tests, to be certain that the action occurred either *in vivo* or *in vitro.* The following experiment illustrates, however, that even atabrin, which was the most potent toxoplasmacidal drug under the conditions of the so-called *in vitro* tests, was completely without effect *in vivo.* In the preceding tests atabrin, in a concentration of 2 mg % (*i.e.,* 1:50,000) was found to be capable of killing about 10,000 M.L.D. of toxoplasma contained in peritoneal exudate in the test tube in less than 3 hr. However, when the toxoplasma were injected intra-abdominally in mice weighing 14 to 16 g, and 2 mg of atabrin were injected intramuscularly simultaneously and daily thereafter, there was no apparent effect on the course of the infection even among the mice inoculated with as little as 1 to 10 M.L.D. (Table II). Twenty-one different compounds listed in

TABLE II.
Lack of Effect *in Vivo* of Drug (Atabrin) That Is Highly Toxoplasmacidal *in Vitro.*

Diluation of toxoplasma-infected mouse brain (0.3 cc intraäbdominally)	Untreated mice	Atabrin 2 mg intramuscularly daily beginning immediately after inoculation
10^{-1}	5, 5, 6*	5, 5, 5
10^{-2}	7, 7, 8	8, 8, 8
10^{-3}	9, 9, 10	9, 9, 10
10^{-4}	10, 10, 10	10, 10, 11

*Numerals refer to day of death of inoculated mouse.

TABLE III.
Compounds Without Effect on Toxoplasmic Infection in Mice.

Group	Compounds tested	Therapeutic effectiveness of group against other protozoa
Arsenic	Neosalvarsan Silver salvarsan Tryparsamide	Trypanosomes
Antimony	Potassium antimony tartrate Fouadin Stibosan	,, Leishmania
Bismuth	Bismuth subsalicylate	
Gold	Sodium aurothiomalate	
Iodine	Sodium iodide	Amoebæ
Quinine and related compounds	Quinine HCl Quinine SO_4 Optochin	Plasmodia
Acridine derivatives	Atabrin Rivanol lactate Trypaflavine	,, Trypanosomes
Aniline dyes	Methylene blue	,,
Urea derivative	Bayer 205 (Germanin)	,,
Aromatic diamidines	4:4′ Diamidino-stilbene 4:4′ Diamidino-diphenoxy pentane 4:4′ Diamidino-diphenoxy propane 4:4′ Diamidino-diphenyl ether	,, Leishmania Plasmodia Babesia

Table III were tested in this manner, most of them against about 1,000 M.L.D. of toxoplasma. Most of the compounds were administered in maximal dosage, *i.e.,* in amounts that were close to or within the range of toxicity; they were injected subcutaneously, intramuscularly, or intravenously daily beginning immediately after inoculation of the mice with the toxoplasma. Whenever there was any question regarding the cause of death, stained films of the lungs and viscera were examined for the presence of toxoplasma. The aromatic diamidines (kindly supplied by Dr. D. F. Robertson of Merck and Co.) were tested against 1 to 100 M.L.D. of toxoplasma because they were reported to exert a good therapeutic effect on leishmania and trypanosome infections (Lourie and Yorke).[5] None of the drugs listed in Table III had any effect on the course of toxoplasmic infection in mice. Prontosil administered intramuscularly daily in doses of 5 mg and sodium sulfapyridine twice daily in doses of 10 mg, subcutaneously at first and then intravenously, were also without therapeutic effect in mice.

Neosalvarsan, tryparsamide, stibosan, potassium antimony tartrate and acriflavine were also tested for possible prophylactic activity in mice. These drugs given 4 days, 2 days and immediately before infection with about 1,000 M.L.D. of toxoplasma, failed to modify the course of the experimental disease.

When toxoplasma are injected intracutaneously in rabbits there is an interval of

[5] Lourie, E. M., and Yorke, W., *Ann. Trop. Med.,* 1939, **33**, 289.

TABLE IV.

Effect of Several Drugs on Toxoplasmic Infection in Rabbits.

Rabbits received 0.3 cc of 10% suspension of toxoplasma-infected mouse brain intracutaneously, and all drugs were given intravenously immediately after injection and thereafter as indicated.

Drug	Dose and mode of administration	Result	
		Test 1	Test 2
Atabrin HCl	3 mg daily	10, 13*	—
Sodium sulfapyridine	50 " twice daily	10, 11	—
Neoarsphenamine	100 " daily	9, 10	
Silver arsphenamine	50 " "	S, S†	7, 7, 10, 10
Tryparsamide	100 " "	9, S	7, 7, 8
Untreated controls	—	11	8, 8, 9

*Numerals refer to day of death of each inoculated rabbit.

†S = rabbit survived for more than a month and exhibited neither a local skin lesion nor fever, and upon retest was found to be immune. The probability is that these rabbits were spontaneously immune at the time of the first test.

3 to 4 days before the local lesion develops and fever appears. There is then a phase of generalized infection with fever for 4 to 8 days, which almost invariably terminates in death with the strain of toxoplasma used in these studies. Atabrin, neoarsphenamine, silver arsphenamine, tryparsamide, and sodium sulfapyridine, administered as indicated in Table IV, were without effect on this type of toxoplasmic infection in rabbits.

Summary. In a search for a chemotherapeutic agent against toxoplasma a large number of drugs of known effectiveness against other protozoa were tested *in vitro* and *in vivo*. In the *in vitro* tests, heparinized peritoneal exudate, containing about 10,000 M.L.D. of toxoplasma, was mixed with certain concentrations of the compounds under investigation and after incubation at 37°C in a water bath for periods of 3, 6, and 24 hr, aliquot portions were injected intraäbdominally in mice to test for the survival of the toxoplasma. In this type of test atabrin in a concentration of 1:50,000 yielded a noninfective mixture in less than 3 hr; trypaflavin, rivanol lactate, potassium antimony tartrate, optochin (in concentrations of 1:10,000) and mapharsen (1:20,000) required more than 3 but less than 6 hr, and neosalvarsan (1:20.000), tryparsamide (1:10,000), and quinine HCl (1:10,000) required more than 6 but less than 24 hr to produce the same effect. Stibosan (1:10,000), 4:4′ diamidino stilbene (1:10,000), sulfanilamide (1:4,000), sodium sulfathiazole (1:4,000) and sodium sulfapyridine (1:4,000) were without effect on the toxoplasma even after 24 hr. All these and a number of other compounds had no therapeutic effect when they were administered parenterally to mice and rabbits immediately after injection of the toxoplasma and once or twice daily thereafter.