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Prophylactic and Therapeutic Effect of Sulfonamide Compounds in Experimental Malaria.

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The treatment of malaria, either in man or experimental animals, with any of the accepted antimalarial drugs is not entirely satisfactory. A high percentage of relapses occurs in spite of vigorous therapeutic measures. In view of this and many other recognized deficiencies, it seemed worth while to try the effect of sulfonamide compounds on controlled experimental malarial infections. The reports in the literature concerning the chemotherapeutic action of the sulfonamide compounds on malaria are very meager. De Leon[1] and Hill and Goodwin[2] used prontosil in acute vivax malaria, and reported beneficial results, while Read and Pino[3] employed sulfanilamide and stated that it was without value. Van der Wielen[4] has also reported the use of prontosil in 2 cases of quartan malaria with a favorable therapeutic result.

The following report concerns the prophylactic and therapeutic effect of 2 sulfonamide compounds, sulfanilamide* (paramido benzene sulfonamide) and sulfanilyl sulfanilate,† in experimental malaria and the action of these drugs on the parasites *in vitro*. Both preparations were injected intraperitoneally and the former was also given by mouth.

Experiment 1. Two rhesus monkeys, Nos. 1 and 2, were each inoculated intraperitoneally with one million *Plasmodium knowlesi* parasites. Monkey 1 was then given 0.25 g of sulfanilamide intraperitoneally, and the same amount of the drug was given on each of 4 successive days thereafter. Monkey 2 served as a control. No infection developed in test Monkey 1 while control Monkey 2 became infected and died 12 days after inoculation.

Experiment 2. Inasmuch as the parasites and the first dose of

[1] De Leon, A. D., Translation of original, *U. S. Pub. Health Rep.*, 1937, **52**, 1460.

[2] Hill, R. A., and Goodwin, M. H., *South. Med. J.*, 1937, **30**, 953.

[3] Read, H., and Pino, J. O., *Arch. Schiffs. u. Tropenhyg.*, 1938, **42**, 132.

[4] Van der Wielen, Y., *Nederl. Tijdschr. v. Geneesk.*, 1937, **81**, 2905.

* Supplied by the Winthrop Chemical Company, New York.

† Made by the Calco Chemical Company and obtained through the courtesy of Dr. A. R. Dochez.

sulfanilamide were injected simultaneously into the abdominal cavity, a direct parasiticidal effect was considered possible. In order to rule out this possibility, 2 monkeys, Nos. 3 and 4, were each inoculated with one million parasites, but treatment was withheld in the test animal until parasites appeared in the blood stream. Circulating parasites were first detected in both animals on the 4th day after inoculation. Monkey 3 then received 0.25 g of sulfanilamide intraperitoneally on that day and 0.5 g on the following day. No drug was administered to Monkey 4, the control. Monkey 3 had a low-grade infection which subsided into a chronic form, while the control, Monkey 4, died of an overwhelming infection on the 9th day.

Experiment 3. The foregoing experiments were repeated on 5 monkeys, Nos. 5 to 9, inclusive. These monkeys were each inoculated intraperitoneally with 20 million parasites, a larger number than previously employed, and at various intervals after inoculation they were given sulfanilamide. The procedure and results are summarized in Table I. It will be seen that Monkey 5, which received 0.5 g of sulfanilamide at the time of inoculation and on the 3rd and 5th days afterwards, never developed an infection. Monkey 6 received 0.5 g on the 3rd day, which was the first day that parasites appeared in the blood stream. Forty-eight hours later the parasites had disappeared. Monkey 7 was given 0.5 g on the 3rd day when the infection was well established and again on the 5th day. The course of the infection was severe but subsided rapidly without further therapy. Monkey 8 was treated with 0.5 g of sulfanilamide on the 4th day upon showing evidence of a rather intense infection with a count of 182 parasites per 10,000 red blood cells. This dose was repeated on the following day because the parasite count was increasing rapidly. After the second dose there was a marked improvement in the animal, and 48 hours later no parasites could be found in the blood smears. Control Monkey 9 died of acute malaria on the 10th day following inoculation. It was noted in the stained blood smears of those monkeys which had rapid declines in the number of parasites following sulfanilamide therapy that many of the parasites were distorted and broken. This observation indicated that the drug had a direct action on the parasites. The results of this experiment show that sulfanilamide in the amounts given was effective in preventing infection as well as in having a marked therapeutic value after the infection was established.

Experiment 4. In the following experiment the prophylactic effects of sulfanilamide were tested in 4 monkeys. Monkeys 10 and 11 each received intraperitoneally 0.5 g of the drug in a 10%

TABLE I.
Effect of Sulfanilamide Given Intraperitoneally at Various Intervals After Inoculation of Parasites.

Monkey	Sulfanilamide injections	No. of parasitized cells per 10,000 red blood cells. Days after inoculation.												
		1	2	3	4	5	6	7	8	9	10	11	12	13
5	.5 g on 1st, 3rd, and 5th days	0	0	0	0	0	0	0	0	0	0	0	0	0
6	.5 " " 3rd day	0	0	+	+	0	0	0	0	0	0	0	0	0
7	.5 " " 3rd and 5th days	0	+	12	143	1037	1188	210	0	0	0	0	0	0
8	.5 " " 4th and 5th days	0	+	8	182	990	25	0	0	0	0	0	0	0
9	Control, no drug given	0	+	16	129	480	980	1464	1500	2516	Dead			

TABLE II.
Prophylactic Effect of Sulfanilamide Given Intraperitoneally and by Mouth.

Monkey	Sulfanilamide injections	No. of parasitized cells per 10,000 red blood cells. Days after inoculation.											
		2	4	6	8	10	12	14	16	18	20	22	24
10	0.5 g in 4 daily doses*	0	0	0	0	0	0	0	0	0	0	0	0
11	0.5 " " 4 " " *	0	0	0	0	+	18	372	1575	1280	Dead		
12	2.0 " by mouth†	0	0	0	0	0	0	0	0	0	0	0	0
13	2.0 " " " †	0	0	0	0	0	0	0	0	10	32	780	86
14	Control, no drug given	0	3	840	1261	Dead							

*Final dose administered 24 hours before inoculation.
†Administered 24 hours before inoculation.

solution on 4 successive days before inoculation with malaria parasites, while Monkeys 12 and 13 each received 2.0 g by mouth in one dose. Twenty-four hours after the last administration of the drug these 4 monkeys and also Monkey 14, which received no drug and served as control, were each inoculated intraperitoneally with 20 million parasites. This represented approximately 10 million minimal infective doses.

The results of this experiment are shown in Table II, and it will be seen that of the 2 monkeys, Nos. 10 and 11, which received sulfanilamide intraperitoneally before inoculation of parasites, Monkey 10 failed to develop an infection. Monkey 11 became infected but did not show circulating parasites until 10 days following inoculation. It appeared to be recovering but finally succumbed on the 20th day with a marked anemia. Almost identical results were obtained in the 2 monkeys, Nos. 12 and 13, in which a single 2.0 g dose of sulfanilamide was given by mouth. Monkey 12 failed to become infected while Monkey 13 first had demonstrable parasites on the 17th day but recovered. Control Monkey 14 died of an overwhelming infection 9 days after inoculation of parasites. The outcome of this experiment indicates that sulfanilamide in the amounts given was responsible for preventing infection in Monkeys 10 and 12 and appreciably prolonging it in Monkeys 11 and 13.

Experiment 5. The *in vitro* action of sulfanilamide on the parasites was tested in 2 monkeys. Twenty million parasites were incubated with 0.1 g of the drug for 30 minutes at 37°C. This mixture was then injected into Monkey 15. Monkey 16 received a similar mixture except that the parasites were incubated with 0.05 g of sulfanilamide. Both monkeys failed to develop infections, indicating that the parasites had been completely inactivated *in vitro* by the drug.

Experiment 6. The therapeutic and prophylactic effect and the *in vitro* action of sulfanilyl sulfanilate were next studied. The method of administration and the results are summarized in Table III. It is seen from this table that sulfanilyl sulfanilate has some therapeutic value when given early in the course of an acute infection. No prophylactic effect is apparent, however, and *in vitro* the drug apparently has no deleterious effect upon the parasites comparable to that of sulfanilamide.

Experiment 7. Sulfanilamide and sulfanilyl sulfanilate were given to 4 monkeys with chronic infections in an attempt to effect a cure. Each drug was used separately in 2 monkeys which had previously been protected from death with immune serum and the duration of

TABLE III.

Prophylactic and Therapeutic Effect of Sulfanilyl Sulfanilate Given Intraperitoneally and Its Action upon Parasites *in vitro*.

Monkey	Sulfanilyl sulfanilate injections	No. of parasitized cells per 10,000 red blood cells. Days after inoculation.													
		2	4	6	8	9	10	11	12	14	16	18	20	22	24
17	0.5 g 1st to 5th days, incl.; 1.5 g on 9th day	0	0	0	0	12	180	102	82	210	300	80	40	20	12
18	0.5 g on 4th and 5th days	0	1	30	0	0	0	0	0	32	223	282	600	220	102
19	0.5 g on 1st, 3rd and 6th days	0	0	22	48	52	86	128	144	36	14	60	22	12	32
20	Control, no drug given	+	129	220	980	1440	1500	2536	Dead						
21	0.5 g in 4 daily doses*	0	+	150	855	1895	3596	Dead							
22	0.5 " " 4 " " *	0	+	812	1872	4560	Dead								
23	0.1 g incubated with parasites 30 min. at 37°C	0	0	2	32	324	2334	Dead							
24	Control, no drug given	0	3	840	1261	2582	Dead								

*Final dose administered 24 hours before inoculation.

their infection varied from 22 to 135 days. Both drugs were given in 0.5 g amounts for 6 successive days. Forty-eight hours after the last dose the animals were bled and 3 cc of blood from each monkey was subinoculated intravenously into separate normal monkeys. The blood from the 2 monkeys which received sulfanilamide was not infectious for normal monkeys while the blood from the sulfanilyl sulfanilate treated monkeys produced an infection in the normal animals.

Treatment of avian malaria. Sulfanilamide and sulfanilyl sulfanilate had no prophylactic effect or therapeutic value in the treatment of *P. cathemerium* infections in canaries or *P. lophuræ* infections in young chicks.

Summary. Sulfanilamide was found to have a prophylactic value and a marked therapeutic effect on acute *P. knowlesi* infections in rhesus monkeys. It had, in addition, a direct action upon the parasites *in vitro*.

Sulfanilyl sulfanilate proved an ineffective prophylactic agent; it had no apparent *in vitro* action on the parasites, but some therapeutic value in acute *P. knowlesi* infection was noted.

In chronic infections 3.0 g of sulfanilamide given intraperitoneally was sufficient to render blood non-infectious for susceptible monkeys, while the same amount of sulfanilyl sulfanilate was without effect.

Sulfanilamide and sulfanilyl sulfanilate were ineffective for prophylaxis and had no therapeutic value when tested against infections produced by 2 different species of avian plasmodia.

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Effect of Splenectomy on Response to Pituitary Material and the Question of the Antihormone.

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Although there is unanimity among investigators in the field that inhibitory substances appear in the blood following continuous treatment with various endocrine extracts, controversy still exists regarding the nature of these antihormone principles, and the mechanism responsible for their production. This controversy, in general, is confined to two schools of thought. The first adheres to the