tration of sulfaguanidine used in these experiments was 200 mg %, since solubility is only 220 mg %.

Summary. Studies of the effect of certain media on the action of sulfonamides indicate that a medium which may seem to be inhibitory is one which favors the growth and multiplication of bacteria so that an ultimate large bacterial population results. Our data also indicate that a medium, which by comparison may seem not to be inhibitory, is one which is not favorable to the development of large numbers of the bacteria under study. It is also apparent that the type of medium, time of incubation and number of bacteria inoculated are important factors which should influence conclusions drawn from sulfonamide studies *in vitro*. Prolonged incubation may result in adaptation of the bacteria to unusually high concentrations of the sulfonamide.

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Inhibition of Antimalarial Action of Sulfonamides by p-Aminobenzoic Acid.

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It has been found that p-aminobenzoic acid prevents the inhibitory action of sulfanilamide on the growth of hemolytic streptococci *in vitro*[1, 2] and *in vivo*.[3] This compound also inhibits the therapeutic effect of sulfanilamide on mice inoculated intracerebrally with the virus of lymphogranuloma venereum.[4] It has been suggested[2, 4] that sulfanilamide exerts its action by competing for an enzyme associated with a metabolite similar in structure to p-aminobenzoic acid and essential to the organism.

Coggeshall[5] showed that sulfanilamide had a marked effect against plasmodia (*Plasmodium knowlesi* in rhesus monkeys). Other sulfonamides[6] were shown to be effective against *P. knowlesi* and against *Plasmodium cynomolgi* and *Plasmodium inui* in rhesus monkeys.

1 Woods, D. D., and Fildes, P., *Chem. and Ind.*, 1940, **59**, 133.

2 Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

3 Selbie, F. R., *Brit. J. Exp. Path.*, 1940, **21**, 90.

4 Findlay, G. M., *Brit. J. Exp. Path.*, 1940, **21**, 356.

5 Coggeshall, L. T., *Am. J. Trop. Med.*, 1938, **18**, 715.

6 Coggeshall, L. T., and Maier, J., *J. Inf. Dis.*, 1941, **69**, 108.

Sulfadiazine was shown[7] to be effective against human malaria (*Plasmodium vivax* and *Plasmodium falciparum*). The present paper reports the effect of p-aminobenzoic acid in inhibiting the action of sulfanilamide against plasmodia.

Plasmodium gallinaceum was used as the test infection in Leghorn or Rhode Island Red chicks weighing 50-100 g. Five birds were used for each test and 10 for each control group. The drugs used were mixed with a powdered chick mash so that the stated daily dose of drug was contained in 10 g of mash. This amount of drug-food mixture was consumed over a 24-hour period. Blood concentrations of sulfanilamide, determined by the method of Marshall and Litchfield[8] with a photoelectric colorimeter, were shown to be maintained at a uniform level over a 24-hour period by this method of administration. Drug feeding was continued for 10 days with blood level determinations on the 2nd and 3rd days. On the 2nd day 1,000,000 parasitized red cells suspended in 0.25 cc of sterile normal saline were injected intravenously. With this inoculum in untreated birds, parasites were first found in thin blood smears on the 3rd or 4th day. The parasite density increased rapidly until the 7th or 8th day, when 60 to 80% of the circulating erythrocytes were parasitized. Most birds died from the acute infection on the 8th to 12th day. Those which recovered usually died from the 19th to 23rd day with massive blocking of brain capillaries by exo-erythrocytic forms in capillary endothelial cells.

When known antimalarial drugs such as quinine and atebrin were tested in this manner, no parasites were found until several days after the termination of drug feeding. Following this the infection usually showed the same characteristics, including density and mortality, as those seen in untreated birds. Variations in these characteristics could be correlated with the size of the birds, because of rapid growth during the long incubation period, and not with the relative effectiveness of the drug. Accordingly, the length of the period from inoculation to first appearance of parasites was believed to represent the best criterion of effectiveness of a given drug. Table I shows the effect of quinine, atebrin, and sulfanilamide in increasing this interval.

In Table II are listed the results obtained when p-aminobenzoic acid was fed simultaneously with sulfanilamide, quinine, or atebrin. p-Aminobenzoic acid itself produced no increase in the incubation

[7] Coggeshall, L. T., Maier, J., and Best, C. A., *J. Am. Med. Assn.*, 1941, **117**, 1077.

[8] Marshall, E. K., Jr., and Litchfield, J. T., Jr., *Science*, 1938, **88**, 85.

TABLE I.
Effect of Drugs on *P. gallinaceum*.

Drug	Dose mg/day/ chick	Dose mg/kilo/ day	Blood levels avg mg%	Day of first appearance of parasites (avg) Control group	Day of first appearance of parasites (avg) Drug group	Effect
Quinine SO_4	10	130		3.0	13.4	+++
Atebrin HCl	8	100		3.0	16.8	+++
Sulfanilamide	20	400	3.3	3.2	12.2	+++
Sulfanilamide	40	800	7.0	3.2	17.0	+++

period and no change in the infection. At 2 dosage levels this compound inhibited completely the effect of sulfanilamide. In other experiments using 400 mg kilo of sulfanilamide, the inhibitory effect was completely blocked by 0.25 mg/day of p-aminobenzoic acid and partially blocked by 0.1 mg day, or a PABA/sulfanilamide ratio of 1/400. Similar results were obtained with sulfathiazole and sulfadiazine. p-Aminobenzoic acid failed to inhibit the effect of quinine or atebrin.

These results suggest that the mechanism of inhibition by sulfonamides is similar for bacteria, viruses, and plasmodia. Quinine and atebrin apparently affect plasmodia through an entirely different mechanism than do sulfonamides.

TABLE II.
Effect of p-aminobenzoic Acid on Antiplasmodial Action of Drugs.

Drug	Dose mg/day/ chick	Dose mg/kilo/ day	Blood levels avg mg%	Day of first appearance of parasites (avg) Control group	Day of first appearance of parasites (avg) Drug group	Effect on infection	Inhibition by p-aminobenzoic acid
p-aminobenzoic acid	25	300	3.1*	3.0	3.0	0	
sulfanilamide	20	400	3.3	3.2	12.2	+++	
sulfanilamide	25	300					
+			9.0*	3.0	3.0	0	Complete
p-aminobenzoic acid	25	300					
sulfanilamide	25	400					
+			4.8*	3.0	3.0	0	,,
p-aminobenzoic acid	5	80					
quinine SO_4	8	160					
+			12.2*	3.0	11.7	+++	0
p-aminobenzoic acid	40	800					
atebrin HCl	8	125					
+				3.0	19.2	+++	0
p-aminobenzoic acid	25	400					

*Calculated as sulfanilamide.