THE EFFECT OF PARA-AMINOBENZOIC ACID ON THE CHEMOTHERAPEUTIC ACTIVITY OF THE SULFONAMIDES IN LYMPHOGRANULOMA VENEREUM AND IN DUCK MALARIA

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It is now well established that para-aminobenzoic acid inhibits the action of sulfonamides on bacteria *in vitro* and *in vivo* (Rubbo and Gillespie, 1940; Selbie, 1940; Woods, 1940). Wiedling (1941) found that para-aminobenzoic acid produced an inhibition of the effect of sulfanilamide, sulfapyridine and sulfathiazole on the fresh water diatom *Nitzschia palea* var. *debilis*. Experiments published by Dimond (1941) showed that para-aminobenzoic acid interfered with the action of sulfanilamide on the fungus *Trichophyton purpureum*. Findlay (1940) has reported that para-aminobenzoic acid inhibited the action of sulfanilamide on a strain of lymphogranuloma venereum.

In this communication experiments will be reported which demonstrate the inhibiting effect of para-aminobenzoic acid on the chemotherapeutic activity of sulfamethyldiazine in *Plasmodium lophurae* infections in Pekin ducklings. Experiments will also be presented in which, contrary to the experience of Findlay (1940), we were unable to show that para-aminobenzoic acid neutralized the action of sulfanilamide and sulfamethyldiazine on a strain of lymphogranuloma venereum.

EXPERIMENTAL

In two sets of experiments a total of thirty-eight Pekin ducklings weighing about 50 grams each were inoculated intravenously with 2,000,000 erythrocytes parasitized by *P. lophurae*.[1] Twelve of the infected birds were given 200 mgm. per kgm. of para-aminobenzoic acid together with 200 mgm. per kgm. of sulfamethyldiazine. Eight birds received 200 mgm. per kgm. of para-aminobenzoic acid alone and six birds served as untreated controls. The drugs were incorporated in the food (Startena Mash) so that a reasonably constant blood level was maintained. Therapy was begun on the day of inoculation and continued for 10 days. Beginning on the fourth day following inoculation, the severity of the infection was followed by daily examination of the blood. The results are recorded in table 1. The course of the malaria in the birds receiving para-aminobenzoic acid alone did not differ from that seen in the untreated controls. As the table shows there was a striking difference between the course of the infection in the ducklings given sulfamethyldiazine alone and the course in the birds receiving the drug together with para-aminobenzoic acid. The ducklings receiving sulfamethyldiazine showed few parasites in the blood while those

[1] We are indebted to Dr. L. T. Coggeshall for this parasite and for information concerning the technique of handling it in the laboratory.

receiving both sulfamethyldiazine and para-aminobenzoic acid developed as severe a parasitemia as the untreated birds. Para-aminobenzoic acid completely neutralized the protective action of sulfamethyldiazine.[2]

In the experiments with lymphogranuloma venereum[3] young Swiss mice were inoculated intracerebrally with 0.02 ml. of a 1:100 saline suspension of a brain of a mouse acutely ill from a regular intracerebral virus passage. In previous experiments it had been found that infection with this strain of lymphogranuloma venereum responded to sulfanilamide, sulfathiazole, and sulfapyridine in much the same fashion as has been reported recently by Jones, Rake, and McKee (1941). The first three experiments were planned to determine the effect of para-aminobenzoic acid on the chemotherapeutic action of sulfanilamide against lymphogranuloma venereum. The fourth experiment was designed to show the effect of para-aminobenzoic acid on the action of another sulfonamide (sulfa-

TABLE 1

Effect of para-aminobenzoic acid on the action of sulfamethyldiazine in P. lophurae infections of Pekin ducklings

NO. OF DUCKS	DRUG	DOSE	AVERAGE NUMBER OF PARASITIZED ERYTHROCYTES PER 10,000 ERYTHROCYTES						
			Number of days						
			4	5	6	7	8	9	10
		mgm. per kgm.							
12	Sulfamethyldiazine	200	3	4	3	3	1	.3	.2
12	Sulfamethyldiazine + para-aminobenzoic acid	200*	20	90	256	648	2267	3545	4738
8	Para-aminobenzoic acid	200	11	58	172	675	1544	3938	5670
6	Control		17	127	263	697	1687	3247	4530

* 200 mgm. per kgm. of each drug.

methyldiazine) in lymphogranuloma venereum. All mice were maintained on a powdered stock diet.[4] Both sulfanilamide and sulfamethyldiazine were added

[2] Since this report was written E. K. Marshall, Jr., J. T. Litchfield, J. E. and H. J. White (J. of Pharm. & Exp. Therapeutics, **75**, 89, 1942) have reported that para-aminobenzoic acid inhibits the antimalarial action of sulfonamides in *P. lophurae* infections of Pekin ducklings and John Maier and Edwin Riley (Proc. Soc. Exp. Biol. & Med., **50**, 152, 1942) have published studies which demonstrate that para-aminobenzoic acid inhibits the action of sulfonamides in *P. gallinaceum* infections of chicks.

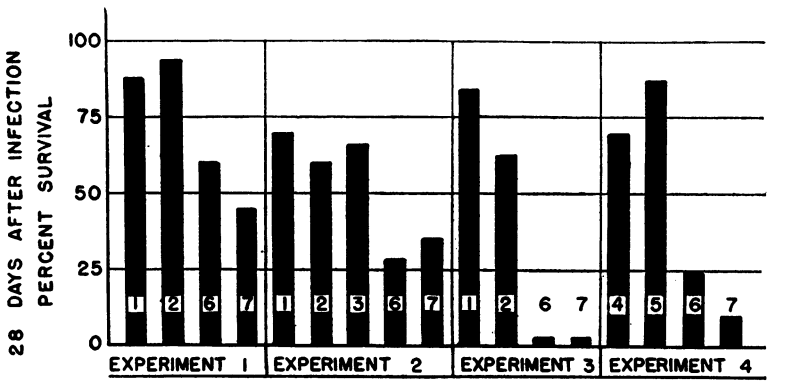
[3] We are indebted to Dr. Murray Sanders for this strain of lymphogranuloma venereum and for information concerning the technique of handling it in the laboratory.

[4] Whole milk powder (Klim) ..... *per cent* 21
Yellow corn meal 33
Whole wheat, ground 33
Linseed oil, meal 7
Alfalfa Leaf meal 2
Sodium chloride 0.5
Calcium carbonate 0.5
Cod liver oil 1.0

to the diet in such a percentage (0.33 per cent) that the mice would receive approximately 10 mgm. per mouse per day. In each experiment one group of infected mice served as untreated controls and a second was given para-aminobenzoic acid (.33 per cent of the diet). A third group of mice received sulfonamide treatment and a fourth group was given both drug and para-aminobenzoic acid in equal amounts. In experiment 2 an additional group of mice received one tenth as much para-aminobenzoic acid as sulfanilamide. Drug treatment was started two days before infection in experiments 3 and 4 and on the day of inoculation in experiments 1 and 2.

TABLE 2

Effect of para-aminobenzoic acid on the action of sulfonamides in lymphogranuloma venereum in mice



1. Sulfanilamide..... .3%
2. Sulfanilamide..... .3% and para-aminobenzoic acid.... .3%
3. Sulfanilamide..... .3% and para-aminobenzoic acid.... .03%
4. Sulfamethyldiazine..... .3%
5. Sulfamethyldiazine..... .3% and para-aminobenzoic acid.... .03%
6. Para-aminobenzoic acid.... .3%
7. Controls

Each column represents a group of 40–50 mice. All mice were inoculated intracerebrally with a 10^{-3} dilution of lymphogranuloma. The drugs were incorporated into a powdered stock diet. In experiments 1 and 2, therapy was begun on the day of infection while in experiments 3 and 4 the mice were placed on drug-containing diet 2 days before they were infected.

The results of these experiments (table 2) show that para-aminobenzoic acid has little if any inhibitory effect on the protective action of sulfanilamide and sulfamethyldiazine on this strain of lymphogranuloma venereum. In experiments 1 and 4, para-aminobenzoic acid itself seemed to afford the mice some protection and the combination of para-aminobenzoic acid and drug seemed somewhat more effective than the drug alone. However, in experiments 2 and 3 para-aminobenzoic acid did not show this slight protecting action and, in 3, actually seemed to inhibit the action of sulfanilamide though not to a significant degree. There was little difference in the action of para-aminobenzoic acid in

the one series in which it was exhibited in doses both equal to and $\frac{1}{10}$ of the sulfanilamide dose.

DISCUSSION

The experiments reported in this communication show that para-aminobenzoic acid can completely inhibit the action of sulfamethyldiazine in *P. lophurae* infections of Pekin ducklings. Wood (1940) in his studies on the mode of action of sulfanilamide worked on the hypothesis that the drug interfered with some substance essential to the bacterial cell. When he found that para-aminobenzoic acid inhibited the action of sulfanilamide he suggested that para-aminobenzoic acid or a compound of similar structure was essential to the bacterial cell. Rubbo and Gillespie (1940) isolated the benzoyl derivative of para-aminobenzoic acid from yeast and furthermore were able to demonstrate that para-aminobenzoic acid stimulated the growth of *Clostridium acetobutylicum* thus affording direct evidence to support Wood's suggestion that para-aminobenzoic acid is essential to the bacterial cell. Although there is no direct evidence that para-aminobenzoic acid is essential for the metabolism of *P. lophurae*, the fact that para-aminobenzoic acid inhibits the action of sulfamethyldiazine suggests that para-aminobenzoic acid or some similar compound may be needed by the parasite.

The experiments reported in this communication do not confirm those of Findlay (1940) who found that para-aminobenzoic acid neutralized the action of sulfanilamide against lymphogranuloma venereum. The failure of para-aminobenzoic acid to inhibit the protective action of sulfanilamide and sulfamethyldiazine on mice infected with lymphogranuloma venereum suggests that the mode of action of these drugs on lymphogranuloma venereum differs from that which the drugs exert on other agents of disease. In this connection it is of interest that while sulfanilamide and sulfamethyldiazine do protect mice infected with lymphogranuloma venereum they do not eradicate the virus. Jones, Rake and McKee (1941) recently reported that they were able to recover lymphogranuloma virus from mice which had been treated with sulfonamides. Similarly we have been able to recover the virus from mice which had received 6 months intensive sulfanilamide treatment and clinically appeared normal. Thus, the sulfonamides differ in their action on lymphogranuloma venereum from their behavior in bacterial infection in that they are not inhibited by para-aminobenzoic acid and also in that they, apparently, do not make it possible for the mouse to eliminate the infecting agent although they do prevent the animal's death from the infection.

SUMMARY

Para-aminobenzoic acid inhibits the action of sulfamethyldiazine on *Plasmodium lophurae* infections in Pekin ducklings, but does not affect the action of sulfanilamide and sulfamethyldiazine on lymphogranuloma venereum in mice.

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