

## Antagonism of Sulfonamide Inhibition by Para-aminobenzoic Acid and Folic Acid in *Toxoplasma* Infected Mice.

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In feeding experiments<sup>1</sup> it has been demonstrated that sodium sulfathiazole and sodium sulfapyridine markedly reduce the fatality rate of mice infected with the protozoan parasite, *Toxoplasma*. Similar inhibitory action has been demonstrated for certain sulfonamide compounds in experimental monkey and avian malaria<sup>2,3</sup> and in experimental avian coccidiosis.<sup>4,5,6</sup>

The mode of action of sulfonamide compounds in the inhibition of multiplication of cells has been studied extensively. According to the Woods-Fildes theory sulfonamide compounds compete with the metabolite para-aminobenzoic acid and thereby interfere with cellular metabolism leading to the inhibition of multiplication of cells. Thus a high concentration of sulfonamide compound may lead to the prevention of cellular multiplication while on the other hand a high concentration of the metabolite can prevent such inhibitory action. This has been demonstrated to be true for various microorganisms both in cultures and in certain *in vivo* experiments. For example, the therapeutic action of sulfanilamide was completely nullified when administered with para-aminobenzoic acid to mice inoculated with *Streptococcus hemolyticus*<sup>7</sup> or

*Diplococcus pneumoniae* Type I.<sup>8</sup> In experimental avian malaria,<sup>9,10,11</sup> para-aminobenzoic acid has likewise been shown to have an antagonistic effect toward the therapeutic action of sulfonamide compounds.

Folic acid<sup>12</sup> and certain of its derivatives also have been shown to antagonize the bacteriostatic properties of sulfadiazine in *in vitro* experiments in which *Streptococcus faecalis* Ralston was used as a test organism. In view of this and the fact that folic acid contains a para-aminobenzoic acid moiety it might be assumed that similar results would be obtained in *in vivo* experiments with this compound.

The studies reported here were carried out to determine the effects of para-aminobenzoic acid, folic acid, sodium sulfathiazole and combinations of para-aminobenzoic acid and sodium sulfathiazole and folic acid and sodium sulfathiazole on experimental *Toxoplasma* infection in mice.

**Methods.** *The parasite and maintenance of a stock strain.* The R.H. strain of *Toxoplasma* used in all experiments was obtained through the courtesy of Dr. Joel Warren, Army Medical Center, Washington, D. C. in July, 1946. This organism has been maintained in mice by intraperitoneal inoculation of 0.2 ml of a  $1 \times 10^{-1}$  dilution of toxoplasma-containing peri-

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<sup>2</sup> Coggeshall, L. T., *Am. J. Trop. Med.*, 1938, **18**, 715.

<sup>3</sup> Levine, P. P., *Cornell Vet.*, 1939, **29**, 309.

<sup>4</sup> Wehr, E. W., and Farr, M. M., *J. Parasitol.*, 1945, **31**, 359.

<sup>5</sup> Farr, M. M., and Wehr, E. W., *J. Parasitol.*, 1945, **31**, 353.

<sup>6</sup> Horton-Smith, C., and Boyland, E., *Brit. J. Pharm.*, 1946, **1**, 139.

<sup>7</sup> Selbie, F. R., *Brit. J. Exp. Path.*, 1940, **21**, 90.

<sup>8</sup> McCarty, M. M., *Proc. Soc. Exp. Biol. and Med.*, 1941, **46**, 133.

<sup>9</sup> Maier, J., and Riley, E., *Proc. Soc. Exp. Biol. and Med.*, 1942, **50**, 152.

<sup>10</sup> Marshall, E. K., Jr., Litchfield, J. T., Jr., and White, H. J., *J. Pharm.*, 1942, **75**, 89.

<sup>11</sup> Seeler, A. O., Groessale, O., and Duesenberry, E. D., *J. Bact.*, 1943, **45**, 205.

<sup>12</sup> Lampen, J. O., and Jones, M. J., *J. Biol. Chem.*, 1946, **164**, 485.

TABLE I.  
Effect of Different Concentrations of Sodium Sulfathiazole and Para-aminobenzoic Acid on *Toxoplasma* Infection in Mice. Results in the table are based on the last day in the experiment on which an accurate LD<sub>50</sub> titer could be calculated for the control animals.

| Compound and conc. in diet   | Proportion of inoculated mice dying at dilution of |                    |                    |                    | LD <sub>50</sub> titer on last day of exp. | Last day of exp. |
|------------------------------|----------------------------------------------------|--------------------|--------------------|--------------------|--------------------------------------------|------------------|
|                              | 1x10 <sup>-1</sup>                                 | 1x10 <sup>-2</sup> | 1x10 <sup>-3</sup> | 1x10 <sup>-4</sup> |                                            |                  |
| Sodium sulfathiazole 1.0%    | 0/7                                                | 1/7                | 1/6                | 0/4                | —0.5                                       | 10               |
| Control                      | 7/7                                                | 6/7                | 7/7                | 3/7                | 3.93                                       | 10               |
| Sodium sulfathiazole 0.15%   | 0/7                                                | 0/7                | 0/7                | 0/7                | —0.5                                       | 9                |
| Control                      | 7/7                                                | 7/7                | 6/7                | 3/7                | 3.75                                       | 9                |
| Sodium sulfathiazole 0.03%   | 6/6                                                | 2/6                | 0/6                | 0/6                | 1.74                                       | 7                |
| Control                      | 6/6                                                | 6/6                | 6/6                | 0/6                | 3.50                                       | 7                |
| Para-aminobenzoic acid 3.5%  | 7/7                                                | 6/6                | 5/6                | 3/7                | 3.74                                       | 10               |
| Control                      | 7/7                                                | 6/7                | 7/7                | 3/7                | 3.93                                       | 10               |
| Para-aminobenzoic acid 0.15% | 5/6                                                | 6/6                | 6/6                | 3/6                | 3.83                                       | 8                |
| Control                      | 6/6                                                | 5/6                | 5/6                | 2/6                | 3.51                                       | 8                |

toneal fluid obtained from previously infected mice. Transfers of the strain have been made every 5 to 6 days.

*Titration of the organism and inoculation of experimental animals.* Toxoplasma-containing peritoneal fluid obtained from 4 stock mice on the fifth or sixth day of infection was pooled. Serial dilutions of 1x10<sup>-1</sup>, 1x10<sup>-2</sup>, 1x10<sup>-3</sup> and 1x10<sup>-4</sup> were prepared in Locke's solution. Six to 7 mice, weighing 15 to 20 g each, were inoculated with 0.1 ml of the first dilution and this procedure was repeated for each of the succeeding dilutions using a similar number of mice in each case. The animals were observed for a period of 15 days. Deaths which occurred within 3 days following inoculation are not included in the results. Mice which died between the fourth and fifteenth days were examined for the parasite and included in the data only if the parasite was observed in large numbers in the peritoneal fluid. The LD<sub>50</sub> titer calculations were made according to the methods of Reed and Muench.<sup>13</sup>

*Maintenance of mice during the test period.* During the experimental period the mice were fed a standard diet which consisted of sucrose 3650 g, casein (vitamin free) 900 g, Crisco 250 g, salt mixture 150 g,<sup>14</sup> cellu flour Type

B 50 g, cod liver oil 75 drops, potassium iodide 75.6 mg, thiamin hydrochloride 25 mg, riboflavin 35 mg, calcium pantothenate 75 mg, niacin 70 mg, pyridoxine hydrochloride 25 mg, and choline chloride 5 g. Test compounds were administered to the animals in this diet. Approximately 4 g of diet or diet-drug mixture was provided for each mouse per day. For each drug tested one group of uninoculated mice was fed the diet-drug mixture thereby serving as a drug control. Water was provided at all times.

*Results.* Sodium sulfathiazole in a concentration of 1.0% in the diet protected 22 out of 24 mice inoculated with *Toxoplasma* for 15 days. One death occurred in the 1x10<sup>-2</sup> dilution group and one in the 1x10<sup>-3</sup> dilution group. None of the inoculated mice treated with 0.15% sodium sulfathiazole succumbed to the experimental disease. At a concentration of 0.03% protection was slight although deaths occurred somewhat later than in the untreated controls. (Table I)

Toxic effects of sodium sulfathiazole were observed in mice fed a concentration of 1.0% of the drug. These were manifested as lethargy, ruffling of the coat and loss of weight. No toxic effects were noted in animals fed 0.15% or 0.03% sodium sulfathiazole.

Para-aminobenzoic acid at concentrations of 3.5% and 0.15% in the diet had no effect upon the experimental disease in mice. There

<sup>13</sup> Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

<sup>14</sup> Wesson, L. G., *Sci.*, 1932, **75**, 339.

TABLE II.

The Effects of Sodium Sulfathiazole and Para-aminobenzoic Acid, Separately and Combined, on *Toxoplasma* Infection in Mice. Results in the table are based on the last day in the experiment on which an accurate LD<sub>50</sub> titer could be calculated for control animals.

| Compound and conc. in diet                               | Proportion of inoculated mice dying at dilution of |                    |                    |                    | LD <sub>50</sub> titer on last day of exp. | Last day of exp. |
|----------------------------------------------------------|----------------------------------------------------|--------------------|--------------------|--------------------|--------------------------------------------|------------------|
|                                                          | 1x10 <sup>-1</sup>                                 | 1x10 <sup>-2</sup> | 1x10 <sup>-3</sup> | 1x10 <sup>-4</sup> |                                            |                  |
| Sodium sulfathiazole 0.15%                               | 0/6                                                | 0/6                | 0/6                | 0/6                | —0.5                                       | 8                |
| Para-aminobenzoic acid 0.15%                             | 5/6                                                | 6/6                | 6/6                | 3/6                | 3.83                                       | 8                |
| Para-aminobenzoic acid + sodium sulfathiazole 0.15% each | 6/6                                                | 6/6                | 5/6                | 0/6                | 3.39                                       | 8                |
| Control                                                  | 6/6                                                | 5/6                | 5/6                | 2/6                | 3.51                                       | 8                |

TABLE III.

The Effect of Sodium Sulfathiazole and Folic Acid Alone and in Combination on *Toxoplasma* Infection in Mice. Results in the table are based on the last day in the experiment on which the accurate LD<sub>50</sub> titer could be calculated for the control animals.

| Drug and conc. in diet                           | Proportion of inoculated mice dying<br>at dilution of |        |        |        | LD <sub>50</sub> titer<br>on last day<br>of exp. | Last day<br>of exp. |
|--------------------------------------------------|-------------------------------------------------------|--------|--------|--------|--------------------------------------------------|---------------------|
|                                                  | 1x10-1                                                | 1x10-2 | 1x10-3 | 1x10-4 |                                                  |                     |
| Exp. I                                           |                                                       |        |        |        |                                                  |                     |
| Folic acid 0.08%                                 | 6/6                                                   | 6/6    | 6/6    | 3/6    | 4.00                                             | 9                   |
| Sodium sulfathiazole 0.08%                       | 0/6                                                   | 0/6    | 0/6    | 0/6    | —0.5                                             | 9                   |
| Folic acid + sodium<br>sulfathiazole 0.08% each  | 6/6                                                   | 6/6    | 4/6    | 0/6    | 3.24                                             | 9                   |
| Control                                          | 6/6                                                   | 6/6    | 6/6    | 2/6    | 3.74                                             | 9                   |
| Exp. II                                          |                                                       |        |        |        |                                                  |                     |
| Folic acid 0.08%                                 | 6/6                                                   | 5/6    | 5/5    | 1/6    | 3.29                                             | 8                   |
| Sodium sulfathiazole 0.08%                       | 2/6                                                   | 2/6    | 0/6    | 0/5    | 1.20                                             | 8                   |
| Folic acid + sodium<br>sulfathiazole 0.08 % each | 4/5                                                   | 6/6    | 4/6    | 2/6    | 2.29                                             | 8                   |
| Control                                          | 6/6                                                   | 6/6    | 6/6    | 3/6    | 4.00                                             | 8                   |

was no evidence of toxicity resulting from the administration of this compound. (Table I)

When fed a diet containing both sodium sulfathiazole and para-aminobenzoic acid at 0.15% concentration each, the experimental disease in mice was not altered, *i.e.* these animals died at the same rate as untreated inoculated control animals. In this reversal experiment sodium sulfathiazole alone at 0.15% concentration afforded complete protection of all mice at all dilutions of the inoculum while 0.15% para-aminobenzoic acid failed to protect any of the mice. (Table II)

Two experiments were conducted to determine the effects of folic acid and sodium sulfathiazole singly and in combination on *Toxoplasma* infection in mice. A diet containing 0.08% of sodium sulfathiazole prevented the death of inoculated mice while a diet containing 0.08% of folic acid failed to protect any of the mice. When fed a diet

containing both drugs at 0.08% concentration each, mice were likewise not protected, *i.e.* animals so treated died at approximately the same rate as inoculated control animals. This experiment was repeated and similar results were obtained. (Table III)

*Summary.* Mice inoculated with fatal dosages of *Toxoplasma* (R.H. strain) were protected by the administration of sodium sulfathiazole in the diet in concentrations of 1.0%, 0.15%, and 0.08% but not by a concentration of 0.03%.

Neither para-aminobenzoic acid nor folic acid were observed to exert a significant effect on experimental toxoplasmosis in mice.

The protection afforded toxoplasma-infected mice by treatment with sodium sulfathiazole was nullified by the simultaneous administration of para-aminobenzoic acid or folic acid.