## Reversal of the Antibacterial Activity of Simple and Complex Sulfonamides by p-Aminobenzoic Acid

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Previous studies by Woods (1) clearly demonstrated that p-aminobenzoic acid (PABA) competitively antagonized the antibacterial activity of sulfanilamide (Fig. 1). There appears to be little information as to

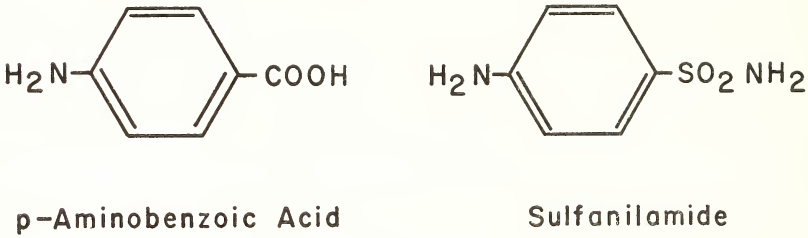


Fig. 1. Structure of p-aminobenzoic acid and sulfanilamide.

whether the more complex sulfonamide derivatives currently used in chemotherapy act like the simple sulfonamides in inhibiting susceptible bacteria by interfering with the utilization of PABA. Studies on the reversal of the *in vitro* antibacterial activity of sulfanilamide, N-(6-meth-

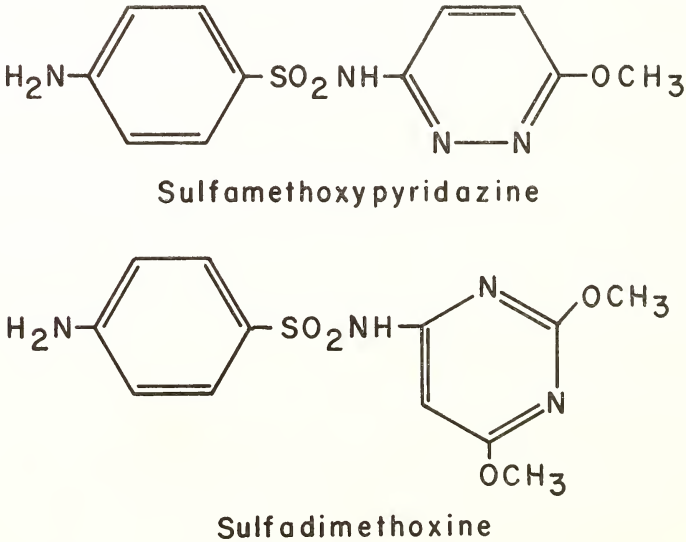


Fig. 2. Structures of sulfamethoxypridazine and sulfadimethoxine.

oxy-3-pyridazinyl)-sulfanilamide (sulfamethoxypyridazine) and N-(2,6-dimethoxy-4-pyrimidinyl)sulfanilamide (sulfadimethoxine) in *Lactobacillus plantarum*, a PABA dependent culture, are described in this report (Fig. 2). Studies on the PABA reversal of growth inhibition caused by sulfadimethoxine in *E. coli* are also included.

## Materials and Methods

The medium of Sarett (2) was used with *L. plantarum*, strain 17-5. Since some of the compounds were not readily water soluble, five to ten mg. quantities were dissolved in five ml. portions of 0.1N NaOH, diluted promptly to 400 ml. with distilled water, adjusted to pH 8.0 with HCl and diluted to 500 ml. to give $6X10^{-5}$ M solutions. Solutions of the sulfonamide analogues (pH 8.0) and PABA (pH 7.0) were sterilized by Seitz filtration and added to previously autoclaved culture tubes containing five ml. portions of basal medium and graded levels of sterile, distilled water to give eight to nine ml. volumes. Final assay volumes were 10 ml. per 20X150 mm. culture tube. The sulfonamide analogues were tested at a final concentration of $6X10^{-6}$ M. In the preparation of the inoculum, precautions were taken to minimize the carry-over of PABA by appropriate washing of the culture and by using a dilute cell suspension. Growth was measured turbidimetrically as optical density using a Coleman Junior spectrophotometer at a wave length of 620 $\mu$, following a 24 hr. incubation period at 35°C.

For the studies with *E. coli*, strain W, the minimal medium of Davis and Mingioli (3) was used. Except for the concentrations of sulfadimethoxine and PABA, the procedures used with *E. coli* were similar to those described for *L. plantarum*.

## Results and Discussion

PABA reversed the growth inhibition of both a simple and two complex sulfonamides in *Lactobacillus plantarum*, a PABA requiring culture

TABLE I

Reversal of the Antibacterial Activity of Sulfonamides by PABA in *L. plantarum*

| PABA Molarity X $10^{10}$ | Sulfanilamide | Sulfamethoxypyridazine | Sulfadimethoxine |
|---|---|---|---|
| | % Reversal of Growth Inhibition | | |
| 0.02 | 3 | 3 | 4 |
| 0.04 | 2 | 3 | 3 |
| 0.08 | 3 | 4 | 4 |
| 0.20 | 14 | 16 | 4 |
| 0.40 | 57 | 54 | 6 |
| 0.75 | 91 | 92 | 40 |
| 1.50 | 100 | 100 | 84 |
| 3.00 | 100 | 100 | 100 |

(Table I). The ability of PABA to reverse the antibacterial activities of sulfanilamide and sulfamethoxypyridazine was equivalent. In contrast, the inhibition of bacterial growth caused by sulfadimethoxine was much more resistant to PABA reversal.

In the PABA reversal studies with *L. plantarum,* a 150 fold range in PABA concentrations was employed starting with a level which gave a maximal growth response in control tubes. At this base level, one will note that inhibition of growth was about 95% with all three sulfonamides at equimolar concentrations.

It was also of interest to determine whether the antibacterial activity of sulfadimethoxine could be reversed in a micro-organism not requiring exogenous PABA, such as *E. coli.* This organism is not as sensitive to the antagonistic effect of the sulfonamides as is *L. plantarum;* hence higher concentrations of the inhibitor were employed with this culture. A level of sulfadimethoxine, $1.6 \times 10^{-5}$M, which caused an 85% inhibition of *E. coli* growth was completely reversed by the addition of $1.5 \times 10^{-9}$M PABA. A 19 fold increase in the concentration of sulfadimethoxine produced a 95% inhibition of growth and a 70% reversal of this inhibition occurred

TABLE II

PABA Reversal of Sulfadimethoxine Growth Inhibition in *E. coli*

| PABA Molarity X $10^9$ | Sulfadimethoxine $1.6 \times 10^{-5}$M | Sulfadimethoxine $30.3 \times 10^{-5}$M |
|---|---|---|
| | % Reversal of Growth Inhibition | |
| 0.0 | 14 | 6 |
| 0.1 | 20 | 5 |
| 0.2 | 70 | 4 |
| 0.4 | 74 | 4 |
| 0.8 | 84 | 4 |
| 1.5 | 100 | 7 |
| 3.0 | 100 | 7 |
| 6.0 | 100 | 12 |
| 12.0 | 100 | 48 |
| 24.0 | 100 | 70 |

when $24 \times 10^{-9}$M PABA was added (Table II). These data clearly show that the antibacterial activity of sulfadimethoxine also can be readily reversed by PABA in a culture not requiring the addition of PABA for growth.

These data confirm the expected conclusion that with both simple and complex sulfonamides the inhibition of susceptible bacteria occurred through an interference in the utilization of PABA.

## Summary

The *in vitro* antibacterial activity of both simple and complex sulfonamide compounds was readily reversed by the addition of PABA in *L. plantarum,* a PABA requiring organism. The growth inhibition observed with sulfadimethoxine was more refractory to PABA reversal than was the inhibition with sulfanilamide or sulfamethoxypyridazine. The growth inhibitory activity of sulfadimethoxine was also reversed by PABA in *E. coli,* a bacterium capable of PABA synthesis.

As expected, these studies have shown that the antibacterial activities of sulfamethoxypyridazine and sulfadimethoxine are due primarily to antagonism of PABA utilization.

## Literature Cited

1. WOODS, D. D. 1940. Brit. J. Expt. Path. **21** : 74.
2. SARETT, H. P. 1951. Arch. Biochem. Biophys. **34** : 378.
3. DAVIS, B. D., and E. S. MINGIOLI. 1950. J. Bacteriol. **60** : 17.