the folic acid was not affected by the peroxide treatment. Daily supplementation with 3000 Snell-Peterson units[20] of folic acid *precipitated marked symptoms of biotin deficiency* in groups 2, 3 and 4 (Fig. 4). The negative controls, group 1, which received neither biotin nor folic acid, showed only slight symptoms of biotin deficiency (spectacled eye). Group 5 and 6, which received 1 mg of d,l-desthiobiotin and 0.2 μg of biotin respectively, showed no manifestations of biotin deficiency. This must therefore be considered as a prophylactic experiment. The apparent biotin activity due to d,l-desthiobiotin was of the order of magnitude of 0.02%.

The striking effect of the folic acid concentrate in accentuating the biotin deficiency symptoms may arise from the fact that folic acid induces better growth and survival and thus increases the biotin requirement of the rats. Similar observations have been recorded by Daft, Ashburn and Sebrell,[21] who produced a dermatitis in rats by supplementing a diet containing succinylsulfathiazole with a liver concentrate which provided folic acid, among other things; in "the 40 rats given neither biotin nor liver extract, . . . . . any degree of biotin deficiency present in these animals was difficult to diagnose." There are indications in the recent report of Nielsen and Black[22] that the same is true for mice.

*Summary.* Synthetic d,l-desthiobiotin induces the following biological responses. (1) It is 50% as active as d-biotin in promoting the growth of *S. cerevisiae*. (2) It is approximately half as effective as d-desthiobiotin in inhibiting fermentation by *L. casei*. The inhibitory effect may diminish slightly over a period of 6 days. (3) On diets containing egg white or succinylsulfathiazole, the apparent vitamin activity in rats is of a low order of magnitude, 0.1 to 0.01% that of biotin.

18 Nielsen, E., Shull, G. M., and Peterson, W. H., *J. Nutrition*, 1942, **24**, 523.

19 Luckey, T. D., Briggs, G. M., and Elvehjem, C. A., *J. Biol. Chem.*, 1944, **152**, 157.

20 Snell, E. E., and Peterson, W. H., *J. Bact.*, 1940, **39**, 173.

21 Daft, F. S., Ashburn, L. L., and Sebrell, W. H., *Science*, 1942, **96**, 321.

22 Nielsen, E., and Black, A., *J. Nutrition*, 1944, **28**, 203.

The assistance of Miss Gertrude Engel with some of the microbiological assays is gratefully acknowledged.

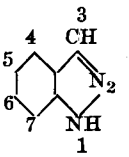
14953

## The *In vitro* Antibacterial Effects of Sulfanilamidoindazoles.

C. A. LAWRENCE AND G. R. GOETCHIUS. (Introduced by M. L. Tainter.)

*From the Research Laboratories of Winthrop Chemical Company, Inc., Rensselaer, N. Y.*

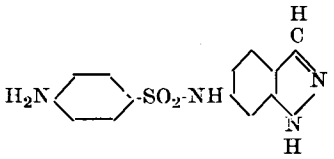
In a previous report from these laboratories, Kwartler and Lucas[1] described the preparation of several sulfanilamidoindazoles. This paper presents the results of the bacteriological investigations on 4 of the compounds, 3-, 5-, 6-, and 7-sulfanilamidoindazole. To our knowledge the only reference to the use of compounds of this nature in chemotherapy is the work of Coggeshall and Maier[2] who found that 5-sulfanilamidoindazole is ineffective in experimentally induced influenza and poliomyelitis infections in mice. The structural formula for indazoles, together with the system for numbering, is as follows:




1 Kwartler, C., and Lucas, P., *J. Am. Chem. Soc.*, 1943, **65**, 1804.

2 Coggeshall, L. T., and Maier, J., *J. Pharm. and Exp. Therap.*, 1942, **76**, 161.

The compounds which were used in these studies had a sulfanilamide radical substituted in either the 3-, 5-, 6-, or 7-position; *e. g.*, 6-sulfanilamidoindazole has the following structure.



*Methods and Results.* Inasmuch as these compounds are comparatively insoluble in water, they were used in the form of their sodium salts. A series of dilutions of each compound, varying from 1:100 to 1:10,000, was prepared in distilled water. To tubes containing 4.4 cc of nutrient medium was added 0.5 cc of the aqueous drug solution. The tubes were autoclaved at 10 lb for 10 min, and, upon cooling, each tube was inoculated with 0.1 cc of a 1:200 broth dilution of a 24-hr culture of test organism. The final drug concentrations were, therefore, 1/10 the original concentrations of the aqueous solutions. Veal infusion broth containing 0.15% dextrose and 0.1% horse serum was used in testing the compounds against the pneumococcus and streptococcus. The Brucella organisms were tested in tryptose phosphate broth containing 0.1% agar. Beef extract broth was used for *Staphylococcus aureus* and several gram-negative enteric organisms. Studies on the anaerobic Clostridia were carried out in Brewer's fluid thioglycollate medium.

The inoculated test media, with controls, were incubated at 37°C and observed for visible growth after 24, 48, and 72 hr. Due to their slower rate of growth, the Brucella were observed at 48, 72, and 96 hr. The test compounds were considered to show bacteriostatic action when the tubes containing them showed less than one-half the growth noted in the broth controls at the end of the initial incubation period. In order to differentiate between bacteriostatic and possible bactericidal effects, lack of visible growth in the drug-broth tubes at the end of 72 hr (or 96 hr for the Brucella) was checked for the presence of viable organisms by transferring three 4-mm loopfuls of the test medium to a tube containing the same medium without the drug. Absence of growth in the subculture tubes indicated that the organisms were destroyed in the original medication tubes.

The results of tests against the several bacteria are presented in Table I. Although not indicated in the table, the sulfanilamidoindazoles compare with sulfathiazole and sulfadia-

TABLE I.
Highest Dilution of Four Sulfanilamidoindazoles Exhibiting Antibacterial Action.

| | Sulfanilamidoindazole | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3- | | 5- | | 6- | | 7- | |
| Organism | Bs × 1000 | Bc × 1000 | Bs × 1000 | Bc × 1000 | Bs × 1000 | Bc × 1000 | Bs × 1000 | Bc × 1000 |
| Pneumococcus Type I | 5 | 5 | 10 | 10 | 10 | 10 | 25 | 25 |
| " " II | 10 | 10 | 10 | 5 | 10 | 5 | 20 | 15 |
| " " III | 5 | 5 | 5 | 5 | 10 | 5 | 20 | 15 |
| *Strep. hemolyticus* | <5 | <5 | 5 | <5 | 5 | <5 | <5 | <5 |
| *Staph. aureus* | 2.5 | <1 | 5 | <1 | 40 | <1 | 50 | <1 |
| *E. typhi* | 2.5 | <1 | 7.5 | 1 | 5 | <1 | 5 | <1 |
| *S. paratyphi A* | 2.5 | <1 | 5 | <1 | 2.5 | <1 | <1 | <1 |
| *S. dysenteriae* | 2.5 | <1 | 5 | <1 | 2.5 | <1 | 1 | <1 |
| *Prot. vulgaris* | 5 | <1 | 7.5 | <1 | 2.5 | <1 | 50 | <1 |
| *V. cholerae* | 50 | 1 | 50 | <1 | 50 | 1 | 50 | <1 |
| *Br. abortus* | 100 | 10 | 25 | 5 | 100 | 10 | 100 | 10 |
| " *melitensis* | 100 | 10 | 25 | 7.5 | 100 | 10 | 100 | 10 |
| " *suis* | 100 | 2.5 | 75 | 2.5 | 100 | 2.5 | 100 | 5 |
| *Cl. welchii* | <1 | <1 | <1 | <1 | <1 | <1 | <1 | <1 |
| " *tetani* | <1 | <1 | <1 | <1 | <1 | <1 | <1 | <1 |
| " *histolyticus* | <1 | <1 | <1 | <1 | <1 | <1 | <1 | <1 |
| " *oedematiens* | <1 | <1 | <1 | <1 | <1 | <1 | <1 | <1 |

Bs = Bacteriostatic
Bc = Bactericidal
< = Greater concentrations not tested.

TABLE II.
Comparison of the Effects of Sulfathiazole and 6-Sulfanilamidoindazole Upon *Brucella melitensis* in the Presence of Paba.

| | 6-Sulfanilamidoindazole | | | | | | Sulfathiazole | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1:16,000 | | 1:32,000 | | 1:64,000 | | 1:1000 | | 1:2000 | |
| Paba | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 48 hr |
| 1:1000 | 0 | 0 | 0 | 1 | 1 | 4 | 1 | 2 | 2 | 4 |
| 1:5000 | 0 | 0 | 2 | 2 | 2 | 4 | 4 | 4 | 4 | 4 |
| 1:10,000 | 0 | 0 | 1 | 3 | 3 | 4 | 4 | 4 | 4 | 4 |
| 1:50,000 | 0 | 0 | 1 | 3 | 3 | 4 | 4 | 4 | 4 | 4 |
| 1:100,000 | 0 | 0 | 1 | 3 | 1 | 4 | 4 | 4 | 4 | 4 |
| 1:500,000 | 0 | 0 | 1 | 3 | 1 | 4 | 4 | 4 | 4 | 4 |
| None | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 |

0 = No growth (bacteriostatic only)
1-3 = Slight to heavy growth
4 = Maximum growth

zine in degree of activity against most of the bacteria. The Brucella organisms, however, are more sensitive to the sulfanilamidoindazoles than to sulfathiazole or sulfadiazine. Under corresponding experimental conditions, the latter compounds were bacteriostatic in dilutions of 1:16,000 to 1:32,000 but failed to exhibit a bactericidal action against the Brucella even when used in concentrations of 0.1%

*Tests with p-Aminobenzoic Acid.* While studies on *p*-aminobenzoic acid-sulfonamide relationships were being conducted, it was noted that the sulfanilamidoindazoles are relatively resistant to antagonism by *p*-aminobenzoic acid. A series of tests was consequently carried out to measure the degree of antagonism displayed by *p*-aminobenzoic acid (PABA) against the antibacterial activity of the sulfanilamidoindazoles and to compare it to the extent of antagonism shown by PABA against sulfathiazole. Dilutions of 1:100, 1:500, 1:1000, 1:5000, 1:10,000, and 1:50,000 of PABA were prepared in water. Of each dilution of the acid 0.5 cc was added to 4.5 cc of tryptose-phosphate-0.1% agar broth containing either a 1:16,000, 1:32,000, or 1:64,000 dilution of the sodium salt of one of the sulfanilamidoindazoles. Sodium sulfathiazole was used in dilutions of 1:1000 and 1:2000. After autoclaving, each tube was inoculated with a loopful of a 24-hr broth culture of *Brucella melitensis*. The amount of visible growth developing in the tubes was observed after 24 and 48 hr of incubation at 37°C. Table II presents the results of a typical test using 6-sulfanilamidoindazole. In general, results comparable to those obtained with this compound were also noted when 3-, 5-, and 7-sulfanilamidoindazoles were used.

It may be readily observed (Table II) that 6-sulfanilamidoindazole is only slightly affected by the presence of PABA. This effect is noticeable only in the zone where the endpoint of the bacteriostatic activity of the compound occurs (1:32,000-1:64,000). Unlike 6-sulfanilamidoindazole, sulfathiazole in high concentration loses its bacteriostatic activity in the presence of corresponding amounts of PABA.

*Summary.* The *in vitro* antibacterial effects of 3-, 5-, 6-, and 7-sulfanilamidoindazole against a variety of bacteria are presented. Data are also given to show that there is a several-thousand-fold decrease in the antagonism displayed by *p*-aminobenzoic acid against the sulfanilamidoindazoles as compared with its antagonism against sulfathiazole.

The authors wish to acknowledge the technical assistance of Virginia L. Wilson.