

## Bacteriostatic and Bactericidal Action of Sulfadiazine *in Vitro* on Gram-Negative Bacteria.

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Animal studies<sup>1</sup> and clinical results in patients<sup>2, 3</sup> have indicated that sulfadiazine may be a chemotherapeutic agent of low toxicity and wide therapeutic applicability. *In vitro* studies of this drug on the pneumococcus are reported separately.<sup>4</sup> In this paper a comparison is made of the bactericidal and bacteriostatic activity *in vitro* of sulfadiazine and sulfathiazole on a number of common pathogenic gram-negative bacteria in a simple medium.

**Materials and Methods.** Stock laboratory strains of *Escherichia coli*, *Salmonella typhimurium* (aertrycke), *S. enteritidis*, *S. paratyphi*, *S. schottmüller*i, *S. choleraesuis* (supestifer), *Klebsiella pneumoniae* (type A) and *Shigella paradysenteriae* (Flexner) were used. The identity of the organisms was verified by their typical cultural, fermentation and serological reactions.\* The medium used was that of Sahyun as modified by MacLeod<sup>5</sup> by the addition of 0.2% of an acid hydrolysate of casein. Growth curves were performed by the method previously described.<sup>6</sup> Incubation was at 37°C. The stock cultures were stored at 4°C. on plain agar slants and were transferred 2 or 3 times in the semi-synthetic medium before testing.

**Results.** The comparative activity of sulfadiazine and sulfathiazole on the organisms tested is shown in Fig. 1. With original inocula of from 100 to 1000 organisms per milliliter, 1 or 2 mg

<sup>1</sup> Feinstone, W. H., Williams, R. D., Wolff, R. T., Huntington, E., and Crossley, M. L., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 427.

<sup>2</sup> Finland, M., Strauss, E., and Peterson, O. L., *J. Am. Med. Assn.*, 1941, **116**, 2641.

<sup>3</sup> Flippin, H. F., Rose, S. B., Schwartz, L., and Domm, A. H., *Am. J. Med. Sci.*, 1941, **201**, 585.

<sup>4</sup> Strauss, E., and Finland, M., *Proc. Soc. Exp. Biol. and Med.*, 1941, **47**, 428.

\* The strains were obtained from Miss Marian Lamb of the Bacteriological Laboratory, Boston City Hospital. The medium used was unsatisfactory for studies on the stock strain of *Eberthella typhi*.

<sup>5</sup> MacLeod, C. M., *J. Exp. Med.*, 1940, **72**, 217.

<sup>6</sup> Spring, W. C., Jr., Lowell, F. C., and Finland, M., *J. Clin. Invest.*, 1940, **19**, 163.

% of either drug is bactericidal in 24 hours against *E. coli*, *Shigella paradysenteriae* (Flexner) and *S. choleraesuis* (*suipestifer*). For *S. enteritidis*, *S. schottmülleri* and *S. typhimurium* (*aertrycke*), 5 mg % is bactericidal; for *S. paratyphi* this concentration is bacteriostatic. In the case of *Klebsiella pneumoniae* (type A) both drugs are bactericidal in concentrations of 10 mg %.

*Comment.* The marked bacteriostatic and bactericidal activity of both drugs in concentrations as low as 1 mg % is in contrast to their only moderate bacteriostatic effect in broth in concentrations from 10 to 50 times as great.<sup>7</sup> In studies to be reported separately, we have tested various sulfonamide drugs on *E. coli* in a completely synthetic medium in which inorganic salts are the only source of nitrogen. The activity of sulfadiazine and sulfathiazole is essentially as reported here, while sulfapyridine, sulfaguanidine and sulfanilamide are progressively less active in the order given. In the case of *E. coli*, therefore, there is no evidence that there is any significant degree of inhibition of sulfadiazine and sulfathiazole by the medium used in these studies. The two drugs probably have about the same order of activity for this organism. Whether or not the medium used here had any inhibitory effect on the drugs in the case of the other organisms studied cannot be stated.

Some of the difficulties involved in attempting to predict thera-

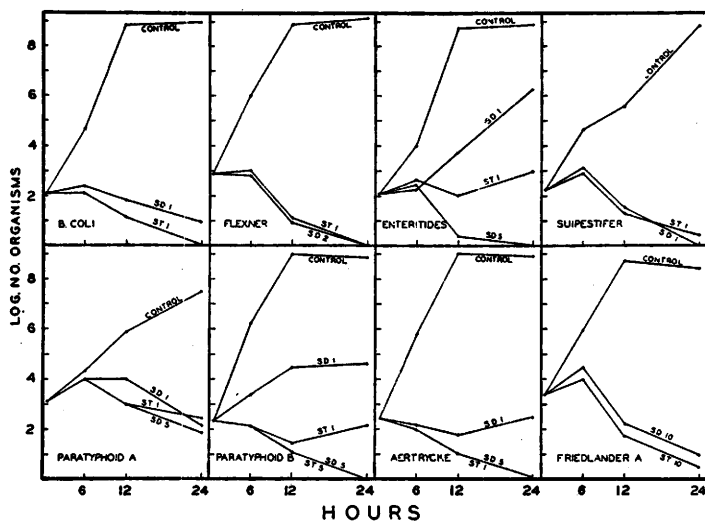


FIG. 1.

Growth curves of 8 strains of gram-negative bacteria in a simple medium. Control contains no drug. SD = sulfadiazine, ST = sulfathiazole, and the numbers indicate the concentration of these drugs in mg per 100 ml.

<sup>7</sup> Klinefelter, H. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1941, **46**, 591.

peutic efficacy on the basis of *in vitro* results of sulfonamide action have been commented upon elsewhere.<sup>4</sup> The activity of a drug *in vitro* does not necessarily parallel its activity in experimental animals or in human subjects. *In vitro* tests may be useful as preliminary guides to *in vivo* studies, but cannot substitute for them.

*Summary.* When small inocula are planted in a semi-synthetic medium, sulfadiazine and sulfathiazole are bactericidal in concentrations as low as 1 or 2 mg % on *E. coli*, *Shigella paradysenteriae* (Flexner) and *S. choleraesuis* (*suipestifer*); and in 5 mg % on *S. enteritides*, *S. schottmülleri* and *S. typhimurium* (*aertrycke*). In the latter concentration they are bacteriostatic for *S. paratyphi* and in 10 mg % they are bactericidal for *Klebsiella pneumoniae*, type A.

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#### Radiosensitivity of Skin of New-Born Rats. III. Sensitivity at Different Temperatures.\*

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In a previous paper<sup>1</sup> it has been reported that temperatures of 0-5°C during roentgen irradiation (1,300 r) decreased the amount of injury produced in the skin of new-born rats. In a second series of experiments<sup>2</sup> this increased resistance of animals irradiated at 0-5°C was studied quantitatively for dosages ranging from 300 to 3000 roentgens. The purpose of this investigation was to determine whether the resistance would change quantitatively with increasing temperature.

The animals were of the same strain used in the previous studies.<sup>†</sup> The temperature was controlled during the irradiation by placing the animals in a double walled box, constructed of transparent sheet plastic, containing water at the desired temperature. The

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\* Aided by grants from the Committee on Radiations of the National Research Council, and the Rockefeller Fund for Research on Physiology of the Cell.

The writers are indebted to Professors J. H. Bodine and H. D. Kerr for their interest and helpful criticisms.

<sup>1</sup> Evans, T. C., *J. Roentgenol. and Rad. Ther.*, in press.

<sup>2</sup> Evans, T. C., Robbie, W. A., Goodrich, J. P., and Slaughter, J. C., *Proc. Soc. Exp. Biol. and Med.*, 1941, **46**, 662.

<sup>†</sup> These animals were kindly supplied by Dr. Emil Witschi from his rat colony.