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Effect of Sulfonamide Compounds upon Growth of Staphylococci in Presence and Absence of p-Aminobenzoic Acid.*

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A series of observations has been made concerning the effect of sulfonamide compounds upon the growth of staphylococci. Comparative studies were made with sulfanilamide, sulfapyridine, sulfathiazole and sulfadiazine. At the same time, the inhibitory effect of p-aminobenzoic acid upon the bacteriostatic action of these compounds was investigated.

Methods. Four strains of *S. aureus* were utilized. They were isolated from patients having severe staphylococcal infections. Each strain produced coagulase and fermented mannite. To facilitate standard conditions, a synthetic medium was used throughout these studies. This chemically-defined medium was the same as described by Gladstone.[1] The advantages of using such a medium for studying the growth-inhibitory effects of the sulfonamide compounds are: it contains neither peptone nor neopeptone; it provides a basal medium of known constituency; and it supports growth of the organisms as readily as the usual broth media used for staphylococci.

Several generations of each of the 4 cultures were grown in the synthetic medium before utilizing a 24-hour culture in the experiments to be described. After several preliminary studies, it was found satisfactory for our present purposes to add 0.05 cc of a 1:10 dilution of the 24-hour culture to 11 cc of the basal medium. The approximate number of cocci added was determined by making agar pour plates of varying dilutions of the original inoculum. The following study was made with each of the strains. Control tubes contained only the basal medium and added organisms. Seven tubes containing the basal medium and organisms had p-aminobenzoic acid in varying concentrations added to them. The final concentration of p-aminobenzoic acid was respectively 2.0, 0.4, 0.08, 0.016, 0.0032, 0.00064, 0.000128 mg per 100 cc. The purpose of this was to determine whether p-aminobenzoic acid inhibited or stimulated the growth

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1 Gladstone, G. P., *Brit. J. Exp. Path.*, 1937, **18**, 322.

of staphylococci. Different concentrations of each of the sulfonamide compounds were added to tubes containing the basal medium and organisms. Freshly prepared solutions were made by dissolving the compounds in sterile distilled water. The sodium salts of sulfapyridine, sulfathiazole and sulfadiazine were used.† Under these conditions, the final concentrations of each of the sulfonamide compounds were 10, 5, 3, and 1 mg per 100 cc. Finally, a series of test tubes was set up containing the basal medium, organisms and different concentrations of p-aminobenzoic acid and each of the sulfonamide compounds as shown in Table I. All of the tubes with their contents were incubated at 37°C for 24 hours. This time interval was selected as being optimal for studying growth after many observations had been made using intervals of 12, 48, and 72 hours. The degree of bacterial growth was measured according to the turbidity of the contents in each tube at the end of 24 hours. For this purpose, readings were made with the Evelyn photoelectric colorimeter using a 540 filter. The contents of each tube were thoroughly mixed, transferred to previously standardized absorption tubes, and the amount of light transmitted through each tube was determined.

As a standard, a tube containing the water-clear basal medium was used. It was found that this method for determining bacterial growth was as satisfactory as ascertaining the number of viable organisms present by the pour-plate technic. The galvanometer readings, which are a logarithmic expression of light transmitted, were converted by means of logarithms to the per cent of light transmitted. Thus the greater the turbidity of the medium, the smaller the percent of light transmitted.

Results. Table I shows the results obtained with one of the 4 strains studied. Observations made with the other 3 strains gave essentially the same results. It is to be noted that different concentrations of p-aminobenzoic acid neither stimulated nor inhibited the growth of staphylococci. This is in agreement with the results of Strauss, Lowell and Finland, who studied the pneumococcus.[2] Woods, on the other hand, stated that p-aminobenzoic acid was an essential growth factor for *Streptococcus pyogenes*.[3] The effect of varying concentrations of each of the sulfonamide compounds upon bacterial growth is of interest. When the concentrations of each of the drugs was 10 mg per 100 cc growth of the organisms was markedly inhibited. When a concentration of 5 mg was

† The sodium sulfadiazine was supplied to us by the Lederle Laboratories, Pearl River, New York.

2 Strauss, E., Lowell, F. C., and Finland, M., *J. Clin. Invest.*, 1941, **20**, 189.

3 Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

TABLE I.

Bacteriostatic Effect of Sulfonamide Compounds on *Staphylococcus aureus* in the Presence and Absence of p-Aminobenzoic Acid.

Growth expressed in % of light transmitted through culture											
			Basal medium + sulfonamide compound + p-aminobenzoic acid Mg per 100 cc p-aminobenzoic acid								
Basal medium	Basal medium plus p-amino-benzoic acid	Basal medium plus sulfonamide compound	2.0	.4	.08	.016	.0032	.00064	.000128	Mg per 100 cc sulfonamide compound	Sulfonamide compound
28	28	81			28	28	35	72	83	10	Sulfanilamide
28	28	86			28	32	73	86	87		Sulfapyridine
28	28	92	28	28	31	65	92				Sulfadiazine
28	28	93	28	29	81	91	93				Sulfathiazole
28	28	50			28	28	29	39	51	5	Sulfanilamide
28	28	82			28	29	47	79	85		Sulfapyridine
28	28	91	28	28	31	39	87				Sulfadiazine
28	28	93	28	28	39	93	92				Sulfathiazole
28	28	32			28	28	29	35	36	3	Sulfanilamide
28	28	72			28	30	31	56	72		Sulfapyridine
28	28	91	28	28	29	32	76				Sulfadiazine
28	28	92	28	28	32	85	93				Sulfathiazole
28	28	29			28	28	28	29	29	1	Sulfanilamide
28	28	31			28	28	31	30	32		Sulfapyridine
28	28	53	28	28	28	29	33				Sulfadiazine
28	28	91	28	29	29	33	85				Sulfathiazole

Inoculum = 700,000 organisms per cubic centimeter.

present, the inhibitory action of sulfanilamide and sulfapyridine was considerably less than that obtained with sulfadiazine and sulfathiazole. This was also true with concentrations of 3 mg per 100 cc. The results were quite striking when a concentration of 1 mg per 100 cc was employed. Sulfathiazole was definitely more inhibitory than sulfadiazine, while this concentration of sulfanilamide and sulfapyridine had very little effect.

The inhibitory effect of p-aminobenzoic acid upon the bacteriostatic action of the compounds revealed that the larger quantities of the acid produced the greater inhibitory effect. Sulfathiazole inhibited bacterial growth more consistently than the other compounds, and p-aminobenzoic acid was the least effective in inhibiting this bacteriostatic action of sulfathiazole. In other words, these results show that the greater the inhibitory effect of a compound upon bacterial growth, the less the effect of p-aminobenzoic acid upon this action of the compound, which confirms the observations of others for the pneumococcus.[2] One observation that we have made several times was that the smaller concentrations of p-aminobenzoic acid in combination with a sulfonamide compound often appeared to inhibit growth more than was obtained with the sulfonamide compound alone. Control tubes showed that these smaller concentrations of p-aminobenzoic acid did not stimulate growth. These observations have been too few and inconsistent from which to draw final conclusions, but this paradoxical finding merits further investigation.

Conclusions. A synthetic, chemically-defined medium has proved satisfactory for the study of the bacteriostatic effect of the sulfonamide compounds upon staphylococci. Sulfathiazole and sulfadiazine have a definitely greater inhibitory effect upon growth compared to sulfapyridine and sulfanilamide, with sulfathiazole superior to sulfadiazine. While p-aminobenzoic acid inhibits this bacteriostatic action of all the compounds studied, the greater the effect of a compound upon growth of Staphylococci, the less inhibitory is p-aminobenzoic acid against this action of the compound.