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Sulfathiazole: Effect on *Staphylococcus aureus in vitro.*

CHARLES H. RAMMELKAMP AND CHESTER S. KEEFER.

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston.

Recently several thiazole analogues of sulfapyridine have been described[1] and a few reports concerning their toxicity[2] and their protective action against *Staphylococcus aureus* in experimental animals[3, 4] have been made. It appears that the thiazole compounds may prove useful clinically. However, further information concerning their action both *in vitro* and in the experimental animal is desirable. It is for this reason that the present *in vitro* study with special emphasis on the action of sulfathiazole (2-sulfanilamidothiazole) on *S. aureus* was undertaken.

Methods. Of the 4 strains of staphylococci used, 3 were isolated from the blood stream of patients with bacteremia, and the other from a case of osteomyelitis of the femur. All 4 strains produced yellow pigment on agar, 2 produced hemolysis on blood agar, and all gave a strongly positive coagulase reaction.[5] The organisms were stored in the icebox on blood-agar slants, and 16-hour peptone-broth cultures were used. All dilutions were made in saline.

To study the comparative effects of sulfanilamide, sulfapyridine, sulfathiazole, and sulfamethylthiazole on staphylococci in whole blood the method employed previously by Spink and Keefer[6] was used. Defibrinated whole blood was obtained from individuals without signs of infection. To 0.5 cc of defibrinated blood 0.1 cc of various dilutions of the 16-hour broth culture was added. The chemicals were dissolved in saline and added in 0.1 cc amounts. The final concentration in each tube was 10 mg/100 cc unless otherwise stated. Tubes with 8 different dilutions of organisms were set up in duplicate, sealed in a gas-oxygen flame, and

1 Fosbinder, R. F., and Walter, L. A., *J. Am. Chem. Soc.*, 1939, **61**, 2032.

2 van Dyke, H. B., Greep, R. O., Rake, G., and McKee, C. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 410.

3 Barlow, O. W., and Homburger, E., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 792.

4 McKee, C. M., Rake, G., Greep, R. O., and van Dyke, H. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 417.

5 Chapman, G. H., Berens, C., Peters, A., and Curcio, L., *J. Bact.*, 1934, **28**, 343.

6 Spink, W. W., and Keefer, C. S., *J. Clin. Invest.*, 1936, **15**, 21.

placed in a rotator in the incubator. The tubes were removed at 24 and 48 hours, and examined for hemolysis. Those tubes showing no hemolysis were then opened and the contents plated out and the colonies counted. The number of organisms present was calculated from the number contained in 2.5 cc of the sixth tube which was plated out at the beginning of the experiment.

Action in whole defibrinated blood. The majority of normal human defibrinated blood-samples could kill less than 10 staphylococci in 0.5 cc, although rarely as many as 1000 organisms were killed. When sulfathiazole was added to these samples the killing power was increased, and there was evidence of definite bacteriostasis in the tubes in which there was growth. To determine the concentration that produced the maximal effect, dilutions of sulfathiazole were made so that final concentrations of 1, –2.5, –5, –10, –15, and 20 mg/100 cc were obtained. Chart I demonstrates the bacteriostatic action of sulfathiazole in these various concentrations. The evidence of bacteriostasis as represented here was the absence of hemolysis at the end of 48 hours. Hemolysis in these experiments did not occur unless there were 10^8 organisms per cc. In those tubes

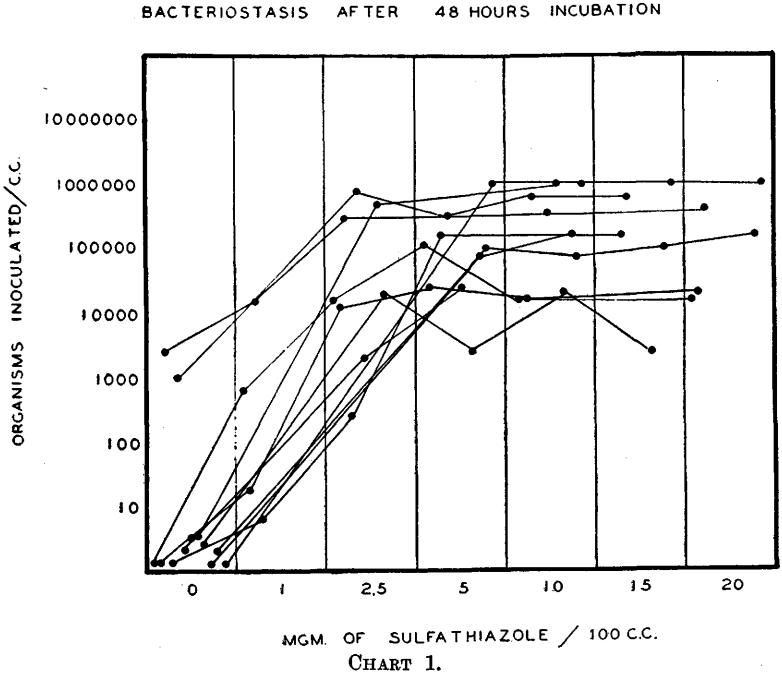

CHART 1.

Each dot represents the largest inoculum of *S. aureus* that failed to produce hemolysis after 48 hours' incubation. The lines connect individual experiments on the same sample of blood.

showing no hemolysis there were 10^4 organisms or less per cc. Similar results were obtained when the tubes were examined at the end of 24 hours, but the color change was not so marked. In the tubes containing the drugs, the hemolysis was greatly delayed as compared to the controls. From the chart it can be seen that the effective bacteriostatic level of sulfathiazole was between 2.5 and 5 mg/100 cc and that higher concentrations of the drug produced little increase in this action.

The increase in the bactericidal effect upon the addition of sulfathiazole was not as striking, but it was definite. Chart 2 represents a typical experiment on one sample of defibrinated blood. In all, 12 such experiments were done. Again, the results shown in this chart are taken after 48 hours' incubation in the rotator; however, the results at 24 hours were similar. Indeed, there was little change in the number of organisms between the 24th and the 48th hour. The

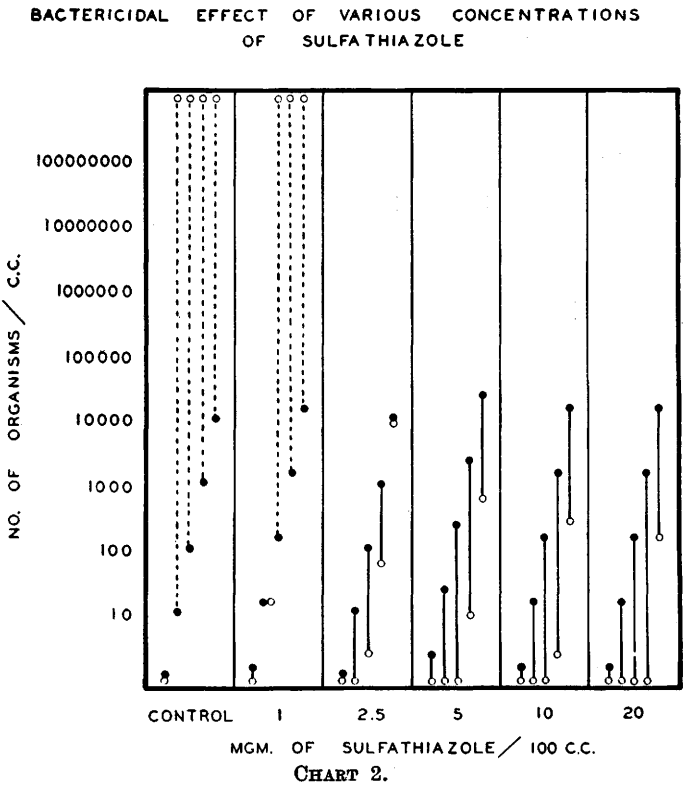

CHART 2.

Solid dots represent original inoculum per cc of *S. aureus*. Circles represent final number of organisms per cc at the end of 48 hours' incubation. Solid lines indicate killing; broken lines growth.

addition of sulfathiazole to defibrinated whole blood which kills less than 10 staphylococci increased the bactericidal power at least 100 times. In 2 experiments in which the blood killed 50 to 100 organisms the addition of sulfathiazole enhanced the bactericidal effect 10 to 100 times. Again it is evident from this chart that concentrations of between 2.5 and 5 mg/100 cc gave effective results, and that increasing the level above this concentration did not enhance the bactericidal effect appreciably.

The above observations were repeated after giving sulfathiazole by mouth to normal persons. Blood was withdrawn both before and after the drug was administered, and the organisms were added. The second sample showed an increase in both the bactericidal and bacteriostatic action against the staphylococcus as compared with the sample of blood before the drug was given.

Comparative effects of sulfanilamide derivatives. Since sulfanilamide and sulfapyridine have both been disappointing in their effects

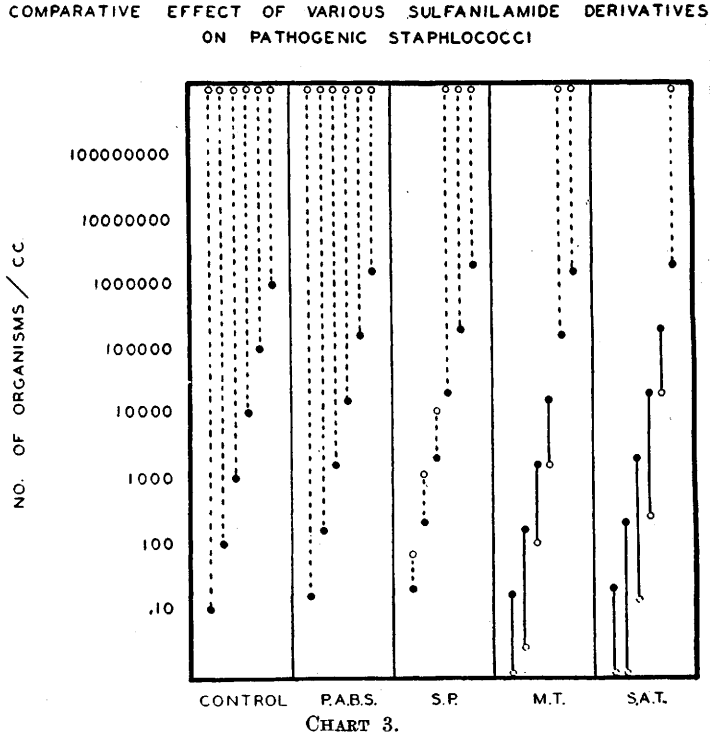

CHART 3.

Solid dots represent original inoculum. Circles represent number of organisms per cc at the end of 48 hours' incubation. Broken lines indicate growth; solid lines indicate killing. P.A.B.S. = sulfanilamide; S.P. = sulfapyridine; M.T. = sulfamethylthiazole; S.A.T. = sulfathiazole.

on the clinical course of cases with *S. aureus* bacteremia, it seemed desirable to compare the action of these drugs with the thiazole compounds (sulfathiazole and sulfamethylthiazole) *in vitro.*

The drugs were added to the blood so that the final concentrations were 10 mg/100 cc. In each of 8 experiments, the various drugs were added to the same sample of blood; 8 dilutions of organisms were made, and set up in duplicate tubes and incubated for 24 and 48 hours. The tubes were then opened and the contents cultured. Chart 3 illustrates the results of a typical experiment. When 10 or more organisms were added to the blood without any drug, and incubated for 48 hours, there were at least 10^8 organisms present at the end of 24 hours. With sulfanilamide the same was true. It was noted, however, that at the end of 24 hours there was evidence of bacteriostasis in that hemolysis of the blood was delayed. This, however, was never very striking. The bacteriostatic effect of sulfapyridine was shown when small numbers of organisms were used, that is less than 10,000 per cc of blood. Both sulfathiazole and sulfamethylthiazole showed bacteriostatic and bactericidal properties. The numbers of organisms actually decreased during incubation and this was striking even when as many as 100,000 organisms per cc were used. From our experiments, using whole blood, sulfathiazole was slightly superior to sulfamethylthiazole although the difference was not great. It seems justifiable to say that in whole defibrinated blood, sulfathiazole is more effective against staphylococci than sulfamethylthiazole, sulfapyridine, or sulfanilamide.

Conclusions. Sulfathiazole is an effective bacteriostatic and bactericidal agent against *S. aureus* when added to defibrinated whole blood *in vitro.* Concentrations between 2.5 and 5 mg/100 cc are necessary to obtain the maximal effect. Comparison of this drug with sulfamethylthiazole, sulfanilamide, and sulfapyridine show it to be somewhat superior in the experiments reported.

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