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Effect of Sulfonamide Compounds upon Staphylocoagulase.*

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Coagulase is a substance elaborated by staphylococci, particularly by the invasive strains. It possesses the property of producing *in vitro* a coagulum in human, citrated plasma. The usual method of testing for the presence of coagulase is to add a loopful of organisms from a 24-hour culture on an agar slant to 0.5 cc of citrated human plasma, and then to incubate the plasma at 37°C. The presence of a coagulum can usually be detected at the end of 3 hours. In many instances, a solid clot will be formed.

While investigating the antistaphylococcal activity of sulfanilamide, sulfapyridine, sulfathiazole, and sulfadiazine, it was observed on a number of occasions that the usual effect of coagulase was reduced or absent in the presence of high concentrations of these compounds. It was assumed that the compounds interfered with the metabolism of the bacterial cells, and coagulase was not being formed as readily. However, there remained the possibility that the drugs may have acted directly upon coagulase. Since there is some evidence that the sulfonamide compounds exert their antibacterial effect by interfering with the enzyme activity of the bacterial cells, it seemed important to determine what effect the drugs had upon staphylocoagulase when the substance was separated from the cells. While the true nature of staphylocoagulase is not known, Cruickshank states it has some of the characteristics of an enzyme.[1] It is known that active staphylocoagulase may be present in sterile, broth filtrates of staphylococci.[2, 3]

Methods and Materials. Normal human plasma containing sodium citrate in a concentration of 0.3% was obtained from the blood bank of the University of Minnesota Hospitals. Three coagulase-positive strains of *S. aureus* isolated from patients with severe infections were used. In preparing a bacteria-free, broth filtrate containing staphylocoagulase, various methods were investigated and

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1 Cruickshank, R., *J. Path. and Bact.*, 1937, **45**, 294.

2 Walston, H. D., *J. Hygiene*, 1935, **35**, 549.

3 Fisher, A. M., *Bull. Johns Hopk. Hosp.*, 1936, **59**, 393.

the following method adopted. A loopful of each culture was seeded in 100 cc of veal infusion broth contained in sterile, pyrex flasks. The 3 cultures were incubated at 37°C for 7 days. At the end of this time, they were centrifuged, and the supernatant fluid sterilized with a Berkefeld N filter. Approximately 100 cc of physiologic sodium chloride solution was first permitted to run through the candle in order to remove any traces of calcium that might be present. The filtrate was then tested for sterility and stored in the refrigerator. Preliminary observations with different broth filtrates and samples of plasma were routinely made in order to determine the presence of coagulase activity. It was found that the coagulating property of coagulase in the filtrates varied considerably with different samples of plasma. In many instances, after the broth filtrate was incubated with plasma for several hours, no coagulum could be detected. Throughout the present study, a total of 52 different samples of plasma were used.

Because we had previously observed that sulfathiazole and sulfadiazine inhibited the growth of staphylococci to a greater degree than either sulfapyridine or sulfanilamide, the first mentioned drugs were first selected for study.[4] The sodium salts of sulfathiazole and sulfadiazine were used because of their greater solubility. Comparative observations were also made with solutions of sulfathiazole and sulfadiazine. When the higher concentrations of the compounds were desired in plasma, suspensions of the drugs were utilized. The first group of studies was carried out in the following way: Small glass tubes contained 0.5 cc of citrated plasma, 0.25 cc of the broth filtrate, and either 0.25 cc of physiologic, sterile sodium chloride solution, or 0.25 cc of a solution of a sulfonamide compound. The final dilution in all of the tubes was 1.0 cc. The final concentrations of the sulfonamide compounds in the plasma were 10, 100, 250, 500, and 1000 mg per 100 cc. The tubes were incubated in a water bath at 37°C. They were examined at various time intervals for the presence of a coagulum.

In another series of studies, the comparative effects of sulfanilamide, sulfapyridine, sulfathiazole and sulfadiazine were noted. The drugs were added as a saline suspension, in those instances where high plasma concentrations were desired. Otherwise, the method used was the same as described above.

Results. Table I shows the results obtained with varying concentrations of sodium sulfathiazole and sodium sulfadiazine. These results were obtained consistently with several different samples of

[4] Spink, W. W., and Jermsta, J.. PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 395.

TABLE I.
Effect of Varying Concentrations of Sodium Sulfathiazole and Sodium Sulfadiazine upon Action of Staphylocoagulase in Citrated Human Plasma.

Time of Incubation	Control	Na Sulfathiazole mg per 100 cc					Na Sulfadiazine mg per 100 cc				
		1000	500	250	100	10	1000	500	250	100	10
15 min.	0	0	0	0	0	0	0	0	0	0	0
30 "	0	0	0	0	0	0	0	0	0	0	0
45 "	0	+	+	+	+	0	0	0	0	0	0
60 "	0	+	+	+	+	+	0	0	0	0	0
90 "	+	+	+	+	+	+	0	0	0	0	0
2 hr	+	+	+	+	+	+	0	0	0	+	+
3 "	+	+	+	+	+	+	0	0	0	+	+
6 "	+	+	+	+	+	+	0	0	0	+	+
18 "	+	+	+	+	+	+	0	0	0	+	+

+ = Coagulum.
0 = No coagulum.

plasma and broth filtrates. The outstanding features are that sulfathiazole in concentrations between 100 and 1000 mg per 100 cc accelerated the coagulating activity of staphylocoagulase, while sulfadiazine in the same concentrations inhibited this activity. Control tubes containing only plasma and the sulfonamides did not reveal any clotting effect. No coagulum was present in tubes with a mixture of plasma, veal infusion broth and either sulfathiazole or sulfadiazine. The amounts of the sodium salts of the two compounds which were employed did not result in any significant change in the hydrogen-ion concentration of the plasma.

The comparative effects of crystalline sulfanilamide, sulfapyridine, sulfathiazole, and sulfadiazine in varying concentrations are shown in one of the experimental results in Table II. Again, it is to be noted that sulfathiazole accelerated the coagulating activity of staphylocoagulase, whereas, sulfadiazine acted in an inhibitory manner. In this particular experiment, it would appear that a concentration of 100 mg per 100 cc of sulfadiazine accelerated coagulation, but this observation is inconsistent with other experiments. Sulfanilamide operated in a manner similar to sulfathiazole, while the effect of sulfapyridine approximated the results in the control tubes and in some instances inhibited the coagulating effect.

A series of observations were made to determine if p-aminobenzoic acid would inhibit the action of the sulfonamides upon staphylocoagulase. Concentrations of p-aminobenzoic acid up to 20 mg per 100 cc did not show any consistent results. When concentrations of 100 mg per 100 cc were used, it was found that all of the compounds had an accelerating effect in the presence of p-aminobenzoic acid. This compound, when studied alone in the presence of a broth

TABLE II.

Effect of Various Concentrations of 4 Sulfonamide Compounds upon Action of Staphylocoagulase in Citrated Human Plasma.

Hrs of Incubation	Control		Sulfanilamide mg per 100 cc				Sulfapyridine mg per 100 cc				Sulfathiazole mg per 100 cc				Sulfadiazine mg per 100 cc			
	A	B	1000	500	250	100	1000	500	250	100	1000	500	250	100	1000	500	250	100
1	0	0	2+	0	0	0	0	0	0	0	3+	3+	3+	0	0	0	0	0
1½	0	0	3+	2+	2+	1+	0	0	0	0	3+	3+	3+	1+	0	0	0	3+
2	0	0	3+	2+	3+	1+	0	0	0	0	3+	4+	4+	1+	0	0	0	2+
2½	0	0	4+	4+	4+	2+	0	0	0	1+	3+	4+	4+	1+	0	0	0	2+
3	1+	0	4+	4+	4+	3+	2+	1+	1+	1+	3+	4+	4+	1+	0	0	0	2+
3½	1+	1+	4+	4+	4+	3+	2+	1+	1+	1+	3+	4+	4+	1+	0	0	0	2+
4	1+	1+	4+	4+	4+	3+	2+	1+	2+	1+	3+	4+	4+	1+	0	0	0	2+
5	1+	1+	4+	4+	4+	3+	3+	3+	3+	3+	3+	4+	4+	1+	0	0	0	1+
6½	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	3+	4+	4+	2+	3+	0	3+	4+
24	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	2+	4+	1+	4+	4+

1+ to 4+ = Minimum and maximum clotting of plasma.

filtrate and plasma, had a markedly accelerating coagulating effect, similar to sulfanilamide.

No explanation is put forward to account for the influence which the sulfonamides have upon staphylocoagulase activity. It should be emphasized that the presence of significant traces of calcium in the sulfonamide solution was ruled out.

Summary. 1. Sulfathiazole and, to a slightly lesser extent, sulfanilamide accelerated the clotting action in citrated plasma of staphylocoagulase contained in a sterile broth filtrate. Sulfadiazine markedly inhibited the coagulating effect, while sulfapyridine had only slight inhibitory activity. 2. Para-aminobenzoic acid operated like sulfanilamide and accelerated the coagulating action. 3. Broth filtrates with staphylocoagulase did not cause coagulation of every sample of normal, citrated human plasma. The degree of coagulation also varied.

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Influence of Tissue on Bacteriostatic Effect of Paranitrobenzoic Acid.*

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King and Henschel[1] reported that paranitrobenzoic acid exerted a bacteriostatic action against Type II pneumococcus in tissue culture clots which compared favorably with that of sulfathiazol in the same medium. Earlier the same authors[2] reported that " . . . . the characteristic effects of sulfanilamide on beta hemolytic streptococci are markedly inhibited by the products of tissue-breakdown."

In this paper we report the results of a study on the inhibition of the bacteriostatic effect of the sodium salt of paranitrobenzoic acid by the products of tissue-breakdown. The details of the tissue culture technic used have been described in the above papers.

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1 King, J. T., and Henschel, A. F., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 400.

2 *Ibid.*, 1939, **41**, 208.