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Combined Bacteriostatic Activity of Sulfanilamide and Azochloramid upon Group A Hemolytic Streptococcus and Enterococcus.

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The therapeutic effectiveness of sulfanilamide may be markedly enhanced in the presence of other antibacterial agents, notably immune serums. The question presents itself as to whether or not a similar synergistic action may be obtained by the combined use of two different chemotherapeutic substances. *In vitro* studies on the combined bacteriostatic activity upon pneumococci of sulfapyridine or sulfathiazole and optochin hydrochloride (ethylhydrocupreine hydrochloride) or beta hydroxyethylapocupreine dihydrochloride (Parke, Davis & Co.) failed to reveal such a synergistic effect.[1] On the other hand, it could be shown that with *E. coli* as test organism, the combined use of pyridium (phenylazo-alpha-alpha-diaminopyridine mono-hydrochloride) and sulfamido compounds resulted in bacteriostatic effects beyond that exerted by either compound alone.[2] The activity of sulfamido compounds and azochloramid used in combination against hemolytic streptococcus Group A and enterococcus is reported in the following communication. Azochloramid (N, N'-Dichloroazodicarboxamidine) (Wallace and Tiernan products) was chosen as a representative of chlorine compounds. It is a bacteriostatic and bactericidal agent which is relatively stable and whose effectiveness in the presence of organic matter is inhibited to a slight degree only.

As test microörganisms a strain of beta hemolytic streptococcus (Group A Lancefield) which was isolated from the chest fluid of a patient with empyema and a strain of hemolytic enterococcus that was isolated from the urine of a patient with infection of the urinary tract, were used. Brain heart infusion and ¼% maltose phenol red broth (Difco) were employed as culture media. Sulfanilamide [p-aminobenzenesulfonamide, prontylin, repurified for injection (Winthrop)] and sulfathiazole [2-sulfanilamidothiazole (Squibb)] were dissolved in appropriate amounts in broth by heating in a

[1] Neter, E., *J. Bact.*, 1941, **41**, 273.

[2] Neter, E., and Loomis, T. A., *The Urol. and Cutan. Review*, 1941, **45**, 295.

water bath. These solutions as well as the broth media were sterilized by autoclaving at 15 lb pressure for 12 minutes. Azochloramid was dissolved in sterile broth and kept in the dark; the azochloramid-broths were not heated. Controls for sterility were carried out.

The number of viable organisms was determined by means of poured blood agar plates. The specimens were diluted in broth containing p-aminobenzoic acid and/or sodium sulfite in order to counteract the activity of sulfanilamide and azochloramid.

A typical experiment on the combined bacteriostatic activity of sulfanilamide and azochloramid upon Group A hemolytic streptococcus ensues. Maltose phenol red broths containing sulfanilamide or azochloramid or both (volume 4.8 cc) were inoculated with 0.2 cc of 1 to 10 diluted 24-hour infusion broth culture of hemolytic streptococcus. The number of organisms present were approximately 50,000 per cubic centimeter. The tubes were shaken well and incubated in the dark at 37°C. The resulting growth was noted at various intervals. Contaminations were excluded by preparations of films and subcultures. The results of this experiment are presented in Table I.

TABLE I.
Combined Bacteriostatic Activity of Sulfanilamide and Azochloramid upon Group A Hemolytic Streptococcus.

	Mediums	Hr of incubation				
		8	18	24	48	72
1.	Control broth	++	++++	++++	++++	++++
2.	Sulfan. (25 mg%) broth	++	+++	++++	++++	++++
3.	" (100 ") "	++	+++	++++	++++	++++
4.	Azochl. (1/800,000) broth	—	++	+++	++++	++++
5.	" (1/400,000) "	—	++	+++	++++	++++
6.	" (1/200,000) "	—	—	—	+++	++++
7.	Azochl. (1/800,000) Sulfan. (25 mg%) broth	—	++	+++	++++	++++
8.	Azochl. (1/800,000) Sulfan. (100 mg%) broth	—	—	—	++	+++
9.	Azochl. (1/400,000) Sulfan. (25 mg%) broth	—	—	—	—	—
10.	Azochl. (1/400,000) Sulfan. (100 mg%) broth	—	—	—	—	—
11.	Azochl. (1/200,000) Sulfan. (25 mg%) broth	—	—	—	—	++
12.	Azochl. (1/200,000) Sulfan. (100 mg%) broth	—	—	—	—	—

— = No visible growth.
+ to ++++ = Various degrees of visible growth.

Table I reveals that with a large inoculum of hemolytic streptococcus the bacteriostatic action of sulfanilamide (25 and 100 mg %) was very slight. Azochloramid in concentrations of 1/200,000 to 1/800,000 exerted some bacteriostatic activity although it failed to prevent entirely the growth of the organisms. The combination of sulfanilamide and azochloramid exerted greater bacteriostatic activity than either compound alone. Used together these two agents in suitable dilutions are more effective than four times the concentration of sulfanilamide alone and twice the concentration of azochloramid alone (Table I). Essentially the same results were obtained in several experiments.

A quantitative study confirmed these results. In one representative experiment the number of viable streptococci per 0.2 cc after 24 hours' incubation at 37° C were as follows: (1) Control broth: 4,000,000; (2) 100 mg % sulfanilamide broth: 2,000,000; (3) 1/800,000 azochloramid broth: 3,000,000; (4) 1/200,000 azochloramid broth: 600; (5) broth containing 25 mg % of sulfanilamide and azochloramid in a dilution of 1/800,000: 2,100. In the same experiment, after 48 hours of incubation, no viable streptococci were demonstrable in broth containing 25 mg % of sulfanilamide and azochloramid in a dilution of 1/400,000, whereas broth containing azochloramid in a dilution of 1/200,000 had 8,000,000 viable streptococci.

Experiments with hemolytic enterococcus revealed that sulfanilamide (1,000 mg %) used in combination with azochloramid (1/800,000) may completely prevent the growth of enterococcus and exert greater bacteriostatic activity than azochloramid alone even in a concentration twice as great.

It remains to be seen whether synergistic effects also may be obtained by the combined use of azochloramid and various sulfamido compounds upon pneumococci and other organisms; furthermore, whether these *in vitro* results may be duplicated *in vivo*.*

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