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Inhibitory Effect of Sulfamido Compounds upon Development and Growth of Phage-Resistant Bacteria.

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Zaytzeff-Jern and Meleney[1] reported that, in contrast to many disinfectants, sulfanilamide and sulfapyridine do not interfere with the lytic action of bacteriophage, but may even enhance its lytic activity. The observations of these authors on the synergistic action of sulfamido compounds and staphylococcus bacteriophage could be confirmed. Furthermore, it was found that these chemotherapeutic substances inhibit or delay the development of phage-resistant staphylococcus. It was decided, therefore, to study the effect of sulfamido compounds upon development and growth of phage-resistant bacteria. The results of this investigation are presented in the following communication.

The experiments were carried out with staphylococcus, *S. dysenteriæ* (Shiga), and *E. coli* bacteriophage and the respective susceptible strains. As culture medium, brain heart infusion broth (Difco), containing infusion from calf brains and beef heart, 1% proteose-peptone, 0.2% dextrose, 0.5% sodium chloride, and 0.25% disodium phosphate, was used in all experiments.

Sulfanilamide (p-aminobenzenesulfonamide, prontylin, repurified for injection, Winthrop); sulfapyridine (2-sulfanilylaminopyridine, Merck); sulfathiazol (2-sulfanilamidothiazol, Squibb); sulfadiazine (2-sulfanilamido-pyrimidine, Lederle); and sulfanilylguanidine monohydrate were dissolved in appropriate amounts in broth by heating in a water bath. The material was then sterilized by autoclaving at 15 lb pressure for 12 minutes.

In the first experiment the effect of sulfathiazol upon the development and growth of phage-resistant staphylococci in infusion broth containing staphylococcus bacteriophage was studied. The bacteriophage was diluted in broth and in broth containing 125 mg % of sulfathiazol (volume 20 cc) ranging from 10^{-2} to 10^{-10}. Broth and sulfathiazol-broth were used as controls. Then all flasks were seeded with 0.2 cc of a 1:10,000 diluted 24 hours infusion broth culture of *Staphylococcus aureus hemolyticus*. The strain (7011)

[1] Zaytzeff-Jern, H., and Meleney, F. L., *J. Lab. Clin. Med.*, 1939, **24**, 1017.

TABLE I.

Action of Sulfathiazol (125 mg%) upon Development of Phage-Resistant Staphylococci in Infusion Broth Containing Bacteriophage.

Dilution of phage in broth	Time of Incubation: 18 Hours	48 Hours	72 Hours	96 Hours	6 Days	8 Days	9 Days	12 Days	14 Days	15 Days	17 Days	21 Days	23 Days
10^{-2}	—	—	—	—	—	—	—	—	++++	++++	++++	++++	++++
10^{-4}	—	—	+	++	++++	++++	++++	++++	++++	++++	++++	++++	++++
10^{-6}	—	+++	+++	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++
10^{-8}	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
10^{-10}	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
0	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Dilution of phage in sulfathiazol broth													
10^{-2}	—	—	—	—	—	—	—	—	—	—	—	—	+
10^{-4}	—	—	—	—	—	—	—	—	—	—	—	++	+++
10^{-6}	—	—	—	—	—	—	—	—	—	—	—	—	—
10^{-8}	—	—	—	—	—	—	—	—	—	—	—	—	—
10^{-10}	++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
0	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

— = no visible growth. + to ++++ = various degrees of visible growth.

TABLE II.

Effect Sulfanilyl-Guanidine on Development of Phage-Resistant Shiga Dysentery Bacilli in Infusion Broth Containing Bacteriophage.

Time of incubation	A—Broth containing sulfanilyl-guanidine in concentrations of (mg%): a 150	b 15	c 1.5	d 0.15	e 0	B—Bacteriophage (10^{-6}) in broth containing sulfanilyl-guanidine in concentrations of (mg%): a 150	b 15	c 1.5	d 0.15	e 0
1. 18 hr	+++	++++	++++	++++	++++	—	—	—	—	—
2. 48 "	+++	++++	++++	++++	++++	—	—	—	—	+++
3. 72 "	+++	++++	++++	++++	++++	+	—	+++	++	+++
4. 96 "	+++	++++	++++	++++	++++	—	—	+++	++	+++
5. 6 days	+++	++++	++++	++++	++++	++	—	+++	++	+++
6. 7 "	+++	++++	++++	++++	++++	++	—	+++	+++	+++
7. 8 "	+++	++++	++++	++++	++++	++	—	+++	+++	+++
8. 10 "	+++	++++	++++	++++	++++	++	+	+++	+++	+++
9. 14 "	+++	++++	++++	++++	++++	++	++	+++	+++	+++

— = no visible growth. + to ++++ = various degrees of visible growth.

was freshly isolated from a child with staphylococcal empyema. The flasks were incubated at 37°C for 23 days and the resulting growth and clearing were noted at various intervals. The results of this experiment are presented in Table I.

Table I shows that in the presence of 125 mg % of sulfathiazol bacteriophage is effective in a higher dilution (10^{-8}) than bacteriophage alone (10^{-6}). Although this difference is not great, the same results were obtained in repeated experiments. The table, furthermore, reveals that the development of phage-resistant staphylococci is markedly delayed or even inhibited in the presence of sulfathiazol. Essentially the same results were obtained in several experiments with sulfathiazol, sulfapyridine (150 mg %) and sulfanilamide (500 mg %).

The experiments on the inhibitory effect of sulfathiazol on the development of phage-resistant staphylococci in broth containing bacteriophage are of particular interest, since at the present time this chemotherapeutic agent is the drug of choice in the treatment of severe staphylococcal infections. There is evidence available that bacteriophage does not cause lysis of the organisms *in vivo*, but rather promotes phagocytosis (MacNeal, McRae and Colmers[2]). This suggests experiments on the effect of sulfamido compounds on the phagocytosis of staphylococci in the presence of bacteriophage.

In the next series of experiments the effect of various sulfamido compounds upon the development of phage-resistant bacilli was investigated. *S. dysenteriæ* (Shiga) and *E. coli* and the respective bacteriophages were employed. The results of these experiments were not as striking as those obtained with staphylococci. The development of phage-resistant bacilli was usually not entirely prevented. However, in the majority of experiments sulfanilamide (1000 mg %), sulfapyridine (100-150 mg %), sulfadiazine (150 mg %) and sulfanilyl-guanidine delayed and partly inhibited the secondary growth of both *E. coli* and *S. dysenteriæ* (Shiga). A typical experiment ensues: Shiga bacteriophage was diluted one million fold (volume 5.0 cc) in broth containing sulfanilyl-guanidine in concentrations of 150 mg %, 15 mg %, 1.5 mg %, and 0.15 mg %. As controls broth containing sulfanilyl-guanidine in the same concentrations, but not bacteriophage, was used. The tubes were seeded with 0.2 cc of 1:100 diluted 24 hours brain heart infusion broth culture of Shiga dysentery bacillus. The tubes were

[2] MacNeal, W. J., McRae, M. A., and Colmers, R. A., *J. Infect. Dis.*, 1938, **63**, 25.

incubated at 37°C for 14 days. Growth was noted at various intervals. The results of this experiment are presented in Table II. It is noteworthy that both phage-resistant *E. coli* and *S. dysenteriæ* on subculture proved to be susceptible to the bacteriostatic action of sulfanilamide and sulfapyridine. It remains to be seen whether *in vivo* administration of both bacteriophage and sulfanilyl-guanidine —a drug of promise in the treatment of localized bacillary infections of the intestinal tract (Marshall[3])—or other sulfanilamide derivatives may be more efficacious than treatment with either agent alone.

Summary. (1) Sulfanilamide, sulfapyridine and sulfathiazol either completely inhibit or definitely delay the development of phage-resistant staphylococci in broth. (2) Sulfanilamide, sulfapyridine, sulfadiazine, and sulfanilyl-guanidine may delay the secondary growth of *S. dysenteriæ* (Shiga) and *E. coli* in broth containing bacteriophage. (3) The phage-resistant bacilli were found to be susceptible to the bacteriostatic action of sulfamido compounds.

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Plasma Clotting Time and Serum Calcium of Patients Recovered from Attacks of Coronary Thrombosis.

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It has been suggested that it is possible to predict among those patients who have undergone surgical operation those who are likely to have thrombosis or embolism by detecting a decreased clotting time of the blood plasma.[1] The same authors also thought that it might be possible to alter the clotting time of the blood by appropriate diet and by administering sodium thiosulfate. In view of the above considerations, it seemed worth while to investigate the

[3] Marshall, E. K., Braton, A. C., Edwards, L. B., and Walker, E., *Bull. Johns Hopkins Hosp.*, 1941, **68**, 94.

[1] Bancroft, F. W., Stanley-Brown, M., and Quick, A. J., *Am. J. Surg.*, 1935, **28**, 648.