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Sulfonamide Resistant *Shigella paradysenteriae* Flexner and *Shigella sonnei*.

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In a previous publication[1] we reported on 2 sulfathiazole resistant strains of Shigella, one a *Shigella sonnei*, the other a *Shigella paradysenteriae* Flexner type. Data are presented at this time regarding bacteriostatic and bactericidal concentrations of 6 sulfonamides for 3 parent strains of Shigella, 1 Flexner and 2 Sonnei, together with a sulfonamide resistant substrain of each. The 6 sulfonamide* compounds studied were sodium sulfathiazole, sodium sulfapyridine, sulfanilamide, sodium sulfadiazine, sulfacetamide, and sulfapyrazine. Two additional sulfonamides, succinyl sulfathiazole and sul-

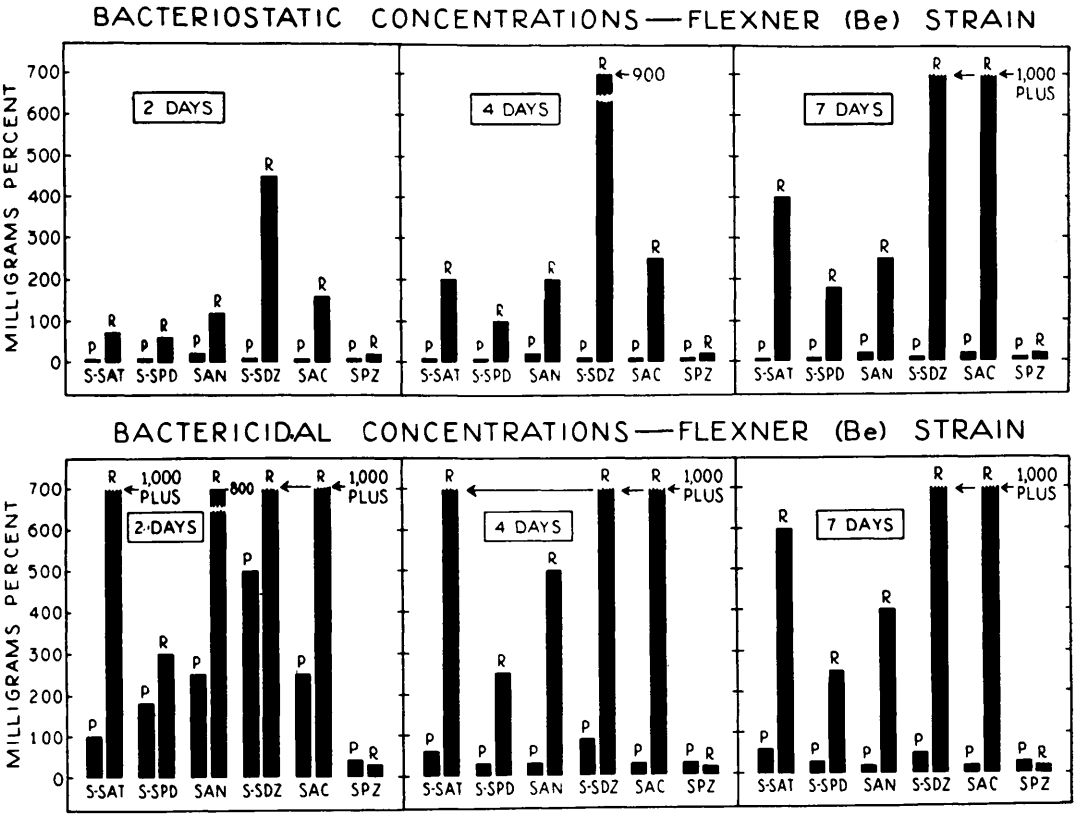

FIG. 1.

Bacteriostatic and bactericidal concentrations of 6 sulfonamides for a parent strain of *Shigella paradysenteriæ* Flexner and its sulfonamide resistant substrain.

1 Cooper, Merlin L., and Keller, Helen M., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 238.

* We are indebted to the following for certain of the sulfonamides: Sulfapyrazine, Mead Johnson and Co.; sodium sulfathiazole and sulfanilylguanidine, E. R. Squibb & Sons; sulfacetamide, Schering Corp.; succinyl sulfathiazole, Sharp and Dohme, Inc.

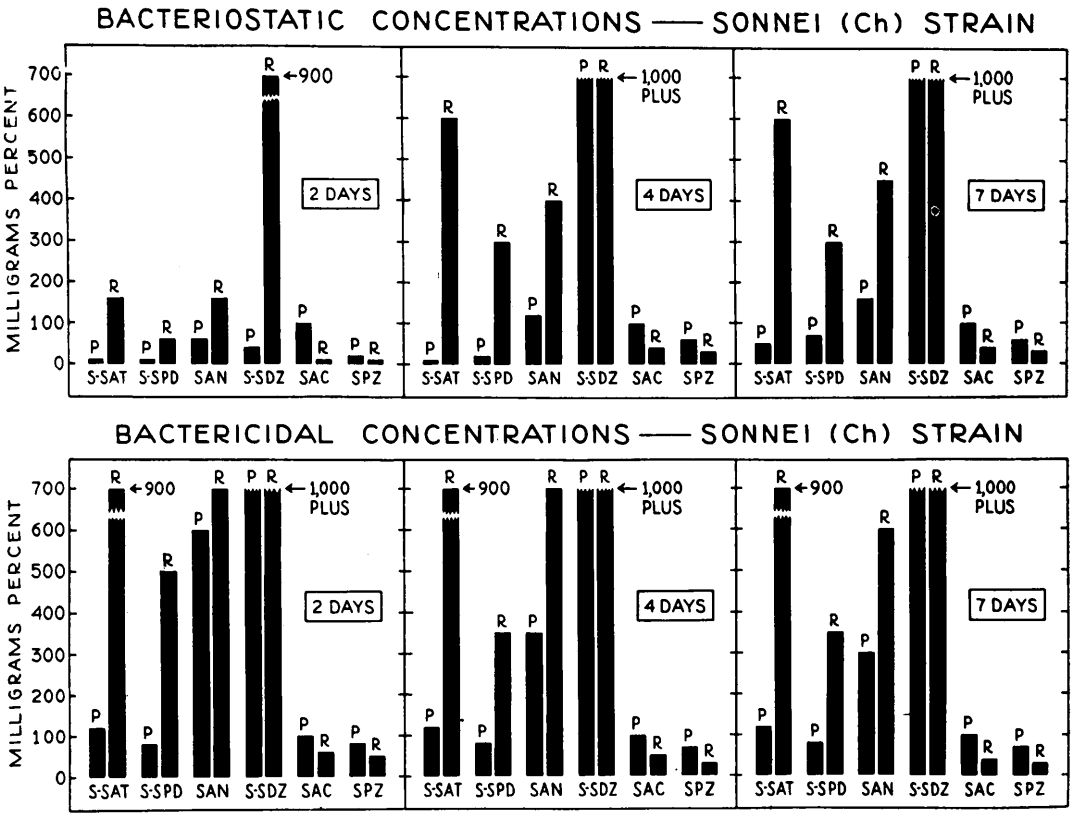

FIG. 2.
Bacteriostatic and bactericidal concentrations of 6 sulfonamides for a parent strain of *Shigella sonnei* (Ch) and its sulfonamide resistant substrain.

fanilylguanidine, were tested, but their relative insolubility did not permit obtaining positive data, except for the Flexner parent strain. These 2 drugs are, therefore, omitted from the accompanying figures.

The sulfonamide resistant strains were developed *in vitro* by adaptation to increasing concentrations of sodium sulfathiazole. The parent strains had been isolated originally from the stools of children acutely ill with dysentery.

Each strain of Shigella was inoculated into several different concentrations of each sulfonamide from 1 mg % to several hundred mg %. The various concentrations of sulfonamides were obtained by first preparing a high concentration of each sulfonamide in Bacto nutrient broth, taking into consideration the solubility of each compound, and sterilizing in the autoclave. Lower concentrations were prepared by making appropriate dilutions in similar sterile medium. Drug concentrations from 1 to 100 mg % differed by 10 mg %, from 100 to 200 mg % they differed by 20 mg %, and above 200 mg % by 50 mg %. Bactericidal concentrations were determined by making subcultures after 2, 4 and 7 days from tubes not showing visible growth. The inoculum for each tube of 10 cc of sulfonamide broth was 30 to 45 microörganisms per cc.

The data are presented graphically in 3 figures.

Comparison, in Fig. 1, 2 and 3 of the bacteriostatic concentrations for the 3 parent non-resistant strains, shows that the 6 sulfonamides were all bacteriostatic in low concentrations (10 to 20 mg %) for the parent Flexner strain, while for the 2 Sonnei strains, the bacteriostatic concentrations varied widely, being 10 mg % for sodium sulfathiazole and more than 1000 mg % for sodium sulfadiazine.

Comparison of the bacteriostatic concen-

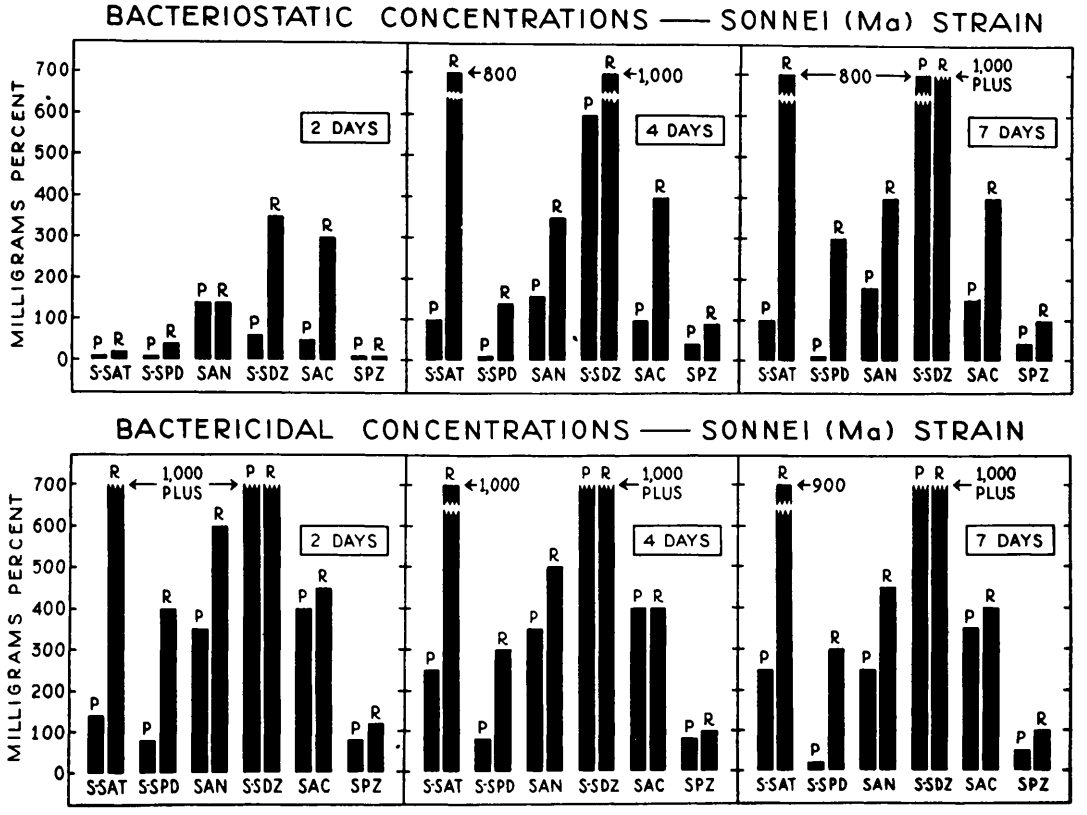

FIG. 3.

Bacteriostatic and bactericidal concentrations of 6 sulfonamides for a parent strain of *Shigella sonnei* (Ma) and its sulfonamide resistant substrain.

trations for the 3 resistant strains shows that: All 3 strains were resistant to sodium sulfathiazole, sodium sulfapyridine, sulfanilamide and sodium sulfadiazine; all 3 strains were not resistant to sulfapyrazine; the Sonnei (Ch) strain was, in addition, not resistant to sulfacetamide; the Flexner strain was most resistant to sodium sulfadiazine and sulfacetamide; and the 2 Sonnei strains were most resistant to sodium sulfadiazine and sodium sulfathiazole.

The above findings are impressive. We are not aware of any instance in which a microörganism resistant to one sulfonamide has not also been found resistant to all other sulfonamides tested. Studies by Schmidt[2,3] and Lowell[4] with sulfonamide resistant pneumococci have shown that such pneumococci resistant to one sulfonamide were also resistant to others.

Comparison of the data regarding the bactericidal concentrations for the 3 parent non-resistant strains shows that: The 6 sulfonamides were bactericidal in lower concentrations for the Flexner strain than for the 2 Sonnei strains; for the Flexner strain, in 2 days, sulfapyrazine was bactericidal in the lowest concentration (40 mg %) and sodium sulfadiazine required the highest concentration (500 % mg) for bactericidal effects; for the Flexner strain after 4 and 7 days the bactericidal concentrations of all 6 sulfonamides were low, 20 to 90 mg %. For the 2 parent non-resistant Sonnei strains the

[2] Sesler, Clara L., and Schmidt, L. H., *J. Bact.*, 1942, **43**, 73.

[3] Schmidt, L. H., Claugus, C. E., and Starks, E., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 256.

[4] Lowell, F. C., Straus, Elias, and Finland, Maxwell, *Ann. Int. Med.*, 1940, **14**, 1001.

bactericidal concentrations varied widely, from 20 mg % for sodium sulfapyridine after 7 days to more than 1000 mg % for sodium sulfadiazine; the bactericidal concentrations of sulfapyrazine and sulfapyridine were consistently lower for the two strains than were the other sulfonamides; sulfacetamide was bactericidal in low concentrations for one of the Sonnei strains (Ch); sodium sulfadiazine was not bactericidal for either Sonnei strain in a concentration of 1000 mg %.

Comparison of the bactericidal concentrations of the 6 sulfonamides for the 3 resistant strains shows that: None of these 3 strains were resistant to sulfapyrazine; that both Sonnei resistant strains were, in addition, not more resistant than the parent strains to sulfacetamide; that the Flexner resistant strain was most resistant to sulfacetamide, sodium sulfadiazine and sodium sulfathiazole; that the 2 resistant Sonnei strains were most resistant to sodium sulfadiazine and sodium sulfathiazole.

Bacteriostatic concentrations increased with time of observation while bactericidal concentrations either decreased or remained the same. Bactericidal concentrations became stabilized much earlier than did the bacteriostatic concentrations.

Summary. Bacteriostatic and bactericidal concentrations of 6 sulfonamides *in vitro* after 2, 4 and 7 days for a resistant strain of *Shigella paradysenteriae* Flexner and 2 resistant strains of *Shigella sonnei* show that: The 3 resistant strains were not resistant to sulfapyrazine; the Sonnei (Ch) strain was in addition, not resistant to sulfacetamide; the Sonnei (Ma) strain was also not resistant to sulfacetamide according to the bactericidal concentrations, but as judged by the bacteriostatic concentrations was more resistant to sulfacetamide than was its parent strain; these 3 strains were quite resistant to the other sulfonamides.

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Choleretic Effect of Chloroacetylcholine.

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A choleretic response to monochloroacetic acid was first noted by Chabrol *et al.*[1] and has been confirmed by one of us with J. L. Morrison. Data concerning this agent will be reported elsewhere. Of interest here is the singular latency of choleresis following intravenous injection of chloroacetate; the maximum response is usually delayed for 45-90 min. This latency indicates that perhaps chloroacetic acid does not act as a direct chloretic but through the slow formation of some more potent metabolite. Chloroacetate may be converted to glycolate in the body.[2] However, glycolate is not an active choleretic even when given in large doses intravenously. Similarly, mercaptoacetate and aminoacetate do not significantly stimulate formation of bile. An opportunity to test another "physiologic" derivative of chloroacetate, chloroacetylcholine, was afforded through the kindness of Dr. Morrison and Dr. David Fielding Marsh, who supplied us with a small amount of the chloride of this ester. Other pharmacologic actions of chloroacetylcholine have been recently described.[3]

The technic used was similar to that employed previously in noting the response to chloroacetate in some 20 dogs. A large male dog was lightly narcotized with pentobarbital and received supplemental amounts of

1 Chabrol, E., Charonnat, R., Maximin, M., and Waitz, R., *Compt. Rend. Soc. Biol.*, 1931, **106**, 17.

2 Morrison, J. L., *Comparative Biological Effects of Halogenated Fatty Acids*, Ph.D. diss., 1941, Univ. of California.

3 Morrison, J. L., to be published.