Comparison of Antibacterial Activity of Single and Combined Sulfonamides.* (19677)

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Schweinburg and Rutenberg(1) reported that combinations of some of the sulfonamides were less bactericidal, in some instances, than the single agents which composed them. Although decreased antibacterial activity was most notable against gram negative organisms, it was also observed, to a lesser degree, with gram positive ones. Additive and even potentiating activity was produced by mixing some of the drugs. Differences in effect of the sulfonamide mixtures were related to variation in species and strain susceptibility. The purpose of the present report is to present confirmation of the work of Schweinburg and his coworker and to indicate the percentile incidence of the various types of reactions (additive, potentiating or depressing) which occur when sulfonamide drugs are combined.

Methods. All of the bacterial inocula were grown and the tests for inhibition by the sulfonamides carried out in peptone broth. Seventy-one strains of organisms were used: *E. coli* (7), *A. aerogenes* (5), *Salmonella* of various types (5), *Shigella* of different types (6), *B. subtilis* (3), alpha *Streptococcus* (8), beta-hemolytic *Streptococcus* (10), and *Staphylococcus aureus* (7).

The drugs studied were sulfadiazine (SD), sulfathiazole (ST), sulfamerazine (SM), and sulfamethazine (SMT). They were tested singly and in the following combinations: SMT+SD, SMT+ST, SD+ST, SD+SM, SMT+SD+ST, and SM+SD+ST. The technic of determining antibacterial activity was the same as that employed by Schweinburg and Rutenberg(1): "1000 mg % stock solutions of each sulfonamide in broth were prepared and brought to a pH of 8.2. Stock solutions of the sulfonamide combinations were prepared by mixing equal volumes of stock solutions of the individual sulfonamides. Serial dilutions of each of these stock solutions were prepared in concentrations of 750, 500, 333, 250, 100, 50, 25, and 10 mg % respectively. One cc of each of the 8 dilutions was placed in sterile Wassermann tubes. To each tube was added 0.1 cc of a bacterial suspension prepared by diluting a 24-hour culture so that 0.1 cc contained 500-5000 bacteria. A control tube containing broth without sulfonamide was also inoculated with 0.1 cc of the same bacterial suspension. All tubes were incubated at 37°C for 24 hours (or longer for streptococci)." The bactericidal titer was determined by making a subculture from each tube showing no growth onto a peptone-agar plate containing no drug; the amount of sulfonamide in the first clear tube yielding no growth on subculture was taken to indicate the bactericidal quantity of sulfonamide, either single or in combination.

Results. The reactions noted with each of the combinations of sulfonamides were classified as additive effect (A), potentiation (P), and decreased antibacterial activity (D). The following was considered to represent an additive effect: The bactericidal titer for a strain of *E. coli* was 100 mg % of sulfadiazine and 50 mg % of sulfathiazole. A mixture composed of equal parts of the 2 drugs inhibited growth of the organism at a level of 75 mg %. Also interpreted as an additive response was the following: A strain of *Salmonella* was killed by 100 mg % of sulfadiazine and by 100 mg % of sulfamerazine; a combination of the two, 50 mg % of each, showed a bactericidal level of 100 mg %. This type of reaction might be assumed to indicate an absence of any change in antibacterial activity. We prefer, however, to include it in the category of an additive effect because the mixture was bactericidal at the same level as each of the drugs when tested alone despite the fact that sub-effective quantities of each (50 mg %) made up the combination. A potentiative

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effect was defined as one in which a mixture of 2 or 3 of the sulfonamides was lethal at a concentration lower than that of the drugs used singly or that could be expected from mere addition of antibacterial activity. Thus, one strain of *Proteus vulgaris* was inhibited by 100 mg % of sulfadiazine and sulfathiazole, respectively. When the 2 agents were combined, the bactericidal titer was 25 mg %. The following was considered as demonstrating a decrease in antibacterial effect: A strain of *B. subtilis* was killed by 250 mg % of sulfamethazine, 100 mg % of sulfadiazine, and 50 mg % of sulfathiazole, respectively. When the 3 drugs were mixed in equal proportions, the organism grew well at a concentration of 750 mg % of the combination.

In Table I are presented the summarized data of the entire study in terms of the effect of combining several sulfonamides on their antibacterial activity. The results obtained using the drugs singly are not included in this report in order to conserve space. However, most of the strains of both the gram negative and positive organisms were inhibited by each of the agents approximately in the same range of concentrations as reported by Schweinburg and Rutenberg(1). The data in Table I are stated in relation to the activity of the single sulfonamides which composed the mixtures studied. For example, the number 3 under *E. coli* and SMT+SD in the column labeled D indicates that 3 out of 6 strains of this organism were less susceptible to a combination of sulfamethazine and sulfadiazine than they were to either used alone. Very striking was the marked variation in the reaction of different strains of a single organism to the same combination of drugs. Notable also were the differences in susceptibility of different species of bacteria to some mixtures of the drugs. These observations illustrate the unpredictability of the bactericidal potency of a sulfonamide combination. Certain of the mixtures depressed the antibacterial effect against most organisms. Thus, SD+SM and SD+SM+SMT were least active against the gram negative bacteria while these same combinations, in addition to several others, showed the greatest decrease in effectiveness when tested against gram positive cocci.

TABLE I. Effect of Combining Various Sulfonamides on Their Antibacterial Activity.

Sulfonamide combination	Organism — No. of strains — Effect of sulfonamide mixture																											
	E. coli(7)			*Ps. aeruginosa*(5)			*K. pneumonia*(6)			*Proteus vulgaris*(9)			*Aerobacter aerogenes*(5)			Salmonella(5)			Shigella(6)			*Bacillus subtilis*(3)			α-Strep.(8)			β-Strep.(10)			*Staph. aureus*(7)		
	P	A	D	P	A	D	P	A	D	P	A	D	P	A	D	P	A	D	P	A	D	P	A	D	P	A	D	P	A	D	P	A	D
SMT+SD	0	4	3	0	1	4	0	1	5	4	3	2	0	2	3	0	3	2	1	4	1	0	0	3	0	8	0	0	0	10	0	1	6
SMT+ST	3	3	1	0	4	1	4	1	1	4	5	0	0	4	1	3	1	1	4	1	1	0	1	2	0	0	8	0	2	8	0	4	3
SD+ST	1	4	2	0	3	2	3	1	2	2	7	0	1	2	2	0	2	3	0	4	2	0	3	0	0	0	8	0	2	8	0	5	2
SD+SM	0	2	5	0	0	5	1	5	0	6	3	0	0	0	5	0	0	5	0	2	4	0	0	3	0	0	8	1	8	1	0	4	3
SMT+SD+ST	0	5	2	1	0	4	4	1	1	0	7	2	0	0	5	1	3	1	0	4	2	0	1	2	0	0	8	0	9	1	0	1	6
SM+SD+ST	0	0	7	1	0	4	1	1	4	0	7	2	0	2	3	0	1	4	0	1	5	0	0	3	0	0	8	0	5	5	0	0	7

P = Potentiating effect; A = Additive effect; D = Decrease in bactericidal potency.

TABLE II. Effect of Various Combinations of Sulfonamides on Gram Negative and Positive Organisms.

Drug combinations	Organisms	Effect of drug combination, No., and % of strains: Additive effect No.	Additive effect %	Potentiation No.	Potentiation %	Decreased activity No.	Decreased activity %
SMT+SD	Gram negative, 43 strains	18	41.7	5	11.6	20	46.7
SMT+ST		19	44.5	18	41.7	6	13.8
SD+ST		23	53.3	7	16.2	13	30.5
SD+SM		12	27.8	7	16.2	24	56
SMT+SD+ST		20	46.7	6	13.8	17	39.5
SM+SD+ST		12	27.9	2	4.6	29	67.5
SMT+SD	Gram positive, 28 strains	9	32.1	0	0	19	67.9
SMT+ST		7	25	0	0	21	75
SD+ST		10	35.7	0	0	18	64.3
SD+SM		12	42.8	1	3.5	15	53.7
SMT+SD+ST		11	39.2	0	0	17	60.8
SM+SD+ST		5	17.9	0	0	23	82.1

These data confirm the work of Schweinburg and his coworker for the gram negative bacteria. They indicate, however, that there may also be an even more marked reduction in bactericidal power for the gram positive cocci.

In Table II are presented the data, without relation to particular bacterial species, in terms of the percentage of strains of gram negative and positive organisms showing various types of reaction to the sulfonamide combinations studied. These results indicate that SD+ST was the combination producing an additive and SMT+ST a potentiative effect most frequently against the gram negative bacteria. The poorest mixtures were SD+SM and SM+SD+ST. It appeared, therefore, that the addition of sulfamerazine to any other single or pair of sulfonamides produced a marked depression of antibacterial activity. The presence of sulfathiazole, on the other hand, especially in the double combinations, increased the bactericidal potency.

With none of sulfonamide combinations was a remarkable degree of additive or potentiating effect observed when gram positive bacteria were used as the test organisms. SD+SM was the mixture which heightened the degree of bactericidal activity most often. Next in order of increased effect were SD+ST and SD+SMT. It is apparent, therefore, that for the gram positive organisms, the addition of sulfadiazine to a combination produced the greatest rise in antibacterial potency. Combining SMT+SD+ST resulted in the greatest reduction in growth-inhibiting effect against both gram positive and negative bacteria.

Summary and discussion. The data obtained in this study indicate that combining several of the sulfonamides in mixtures containing either 2 or 3 of these agents may produce 3 types of change in their antibacterial activity as compared to the effect of the drugs used alone; an additive, potentiating or depressing effect. The results point out clearly, as did the studies of Schweinburg and Rutenberg, that the effect of mixing sulfonamides on the bactericidal potency against gram negative and positive bacteria is almost totally unpredictable. This raises considerable question as to the usefulness of combined sulfonamide therapy in human infections. In order to be certain of the clinical value of any mixture, determination of the actual sensitivity of the infecting organism to it would have to be carried out. This makes effective application of sulfonamide combinations highly impractical.

In contrast to the observations of Schweinburg and his coworker, there was a high incidence, in the present study, of depression of antibacterial effect when sulfonamides were combined and the tests carried out with gram positive organisms. The discrepancy is probably related to the fact that different strains of bacteria were used in the 2 studies. This possibility is supported by the data indicating marked variation in the susceptibility of different strains of the same bacterial species to a single drug mixture. These studies suggest

that the presence of sulfathiazole tends to increase and of sulfamerazine to decrease the lethal effect of combined sulfonamides on gram negative bacteria. On the other hand, the addition of sulfadiazine increases the bactericidal potency for gram positive ones. The combination producing the greatest depression of antibacterial activity for all organisms, regardless of their tinctorial reaction, appears to be one containing sulfamerazine, sulfadiazine, and sulfathiazole.

Conclusions. 1. The antibactericidal activity of various mixtures of sulfonamides is unpredictable. 2. The observed effect varies markedly with different species and with various strains of the same species of organism. 3. Depression of bactericidal potency against both gram negative and positive bacteria frequently results from combining sulfonamides. 4. The greatest and most constant decrease in antibacterial effect is produced by mixing sulfamerazine, sulfathiazole, and sulfadiazine. 5. These results suggest that combinations of sulfonamides may have little clinical usefulness in many instances, since they are often not as effective as the single agents which compose them.

1. Schweinburg, F. B., and Rutenberg, A. M., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 480.

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Studies on Passive Immunity in Poliomyelitis. V. Lansing Antibody Levels in Humans after Gamma Globulin Administration.* (19678)

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Gamma globulin has been used successfully in man not only for the prevention or attenuation of measles, but also mumps(1) and infectious hepatitis(2,3). There is thus some justification for an investigation of gamma globulin in the prophylaxis of poliomyelitis; this possibility has been discussed by Hammon(4), who concludes that the available evidence warrants a large scale trial. The present paper reports a study that has been carried out to determine poliomyelitis antibody levels in serum after inoculation of the doses of gamma globulin likely to be used in such a study. Advantage has been taken of the fact that gamma globulin contains antibody to the Lansing as well as the other types of poliomyelitis virus(5-7), so that tests for antibody can be carried out in mice.

Materials and methods. Persons inoculated. It was necessary to examine the sera of a considerable number of persons in order to find those whose serum contained no Lansing antibody. Fifty-one children were bled at the Home of the Imperial Order of the Daughters of the Empire, Toronto. These children were from one to 11 years of age, and were hospitalized for tuberculosis, some being convalescent. The use of such patients offered the advantage that temperatures were regularly recorded, and any reactions would be quickly noted. Sera were tested for Lansing antibody by a screening test, the details of which are given below. The sera of 19 children were found to be free of demonstrable Lansing antibody. The sera of over 300 adults attending the Blood Bank at The Hospital for Sick Children, Toronto, were tested by a similar method, and the sera of 30 were found to be free of demonstrable Lansing antibody. It may be stated that in no case did any patient complain of more than mild local discomfort after the inoculation; there were no febrile reactions. *Gamma globulin.* Gamma globulin prepared from pools of human plasma was supplied through the courtesy

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