

SUSCEPTIBILITY OF SHIGELLA PARADYSENTERIAE TO SODIUM SULFATHIAZOLE AND SULFAGUANIDINE IN VITRO

WITH SOME INFORMATION REGARDING EBERTHELLA TYPHOSA, SALMONELLA PARATYPHOSA, ESCHERICHIA COLI, SALMONELLA MORGANI AND PROTEUS

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THE purpose of this study was to compare the bacteriostatic and bactericidal concentrations in vitro of sodium sulfathiazole* and sulfaguanidine* for cultures of *Shigella paradysenteriae* Flexner, *Shigella paradysenteriae* Sonne, *Shigella alkalescens*, *Escherichia coli*, *Eberthella typhosa*, *Salmonella paratyphi*, *S. morgani*, and *Proteus*. All of these cultures had been isolated from the stools of children acutely ill.

This study differs from other in vitro studies in two respects: first, the observations were made over a period of twenty-one to twenty-eight days instead of one, two, or three days as is the case in most reports; and second, each culture was studied in a series of at least nineteen different concentrations of the drugs while most reports are based on one, two, or three concentrations of the drugs. Lawrence¹ used only one short period of observation, twenty-three to twenty-five hours, and only one concentration of sulfonamide, 10 mg. per cent. His data indicated that sulfathiazole and sulfamethylthiazole were somewhat more bacteriostatic over this short incubation period than were sulfapyridine and sulfanilamide on cultures of *E. typhosa*, *Esch. coli*, *S. dysenteriae*, *S. paratyphi*, *S. suispestifer*, *S. enteritidis*, *A. aerogenes*, and *Proteus vulgaris*. Remmelkamp and Jewell² made their observations at the end of a twenty-four-hour incubation period and used only one concentration of sulfonamide, 10 mg. per cent. They used cultures of *E. typhosa*, *S. paratyphi*, and *Shigella paradysenteriae* Flexner and Sonne in broth medium containing sulfathiazole, sulfanilamide, sulfapyridine, and sulfamethylthiazole. Their results showed that there was some bacteriostatic effect on *S. paratyphi* and *Shigella paradysenteriae* Flexner when the inoculum was about ten microorganisms per cubic centimeter. Bornstein and Straus³ observed at the end of twenty-four hours that sulfanilylguanidine was bacteriostatic in less than 10 mg. per cent for *Shigella paradysenteriae* Flexner and *S. cholerae suis*, in 10 mg. per cent concentration for *S. paratyphi* A, and in 25 mg. per cent for *E. coli*. Long and Bliss⁴ made their observations at the end of from seven-

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teen to twenty-four hours on the bacteriostatic effect of 10 mg. per cent of sulfathiazole, sulfapyridine, and sulfanilamide on cultures of *Esch. coli* and *B. proteus*. They found sulfathiazole as effective as sulfapyridine. Lawrence and Sprague⁵ used concentrations of sulfanilamide, sulfapyridine, sulfathiazole, and sulfanilylguanidine varying from 100 to 200 mg. per cent. They observed that sulfathiazole was more effective in vitro as to bacteriostatic and bactericidal activity upon bacteria of the colon-typhoid-dysentery group. Straus and Finland⁶ obtained viable bacterial counts after six, twelve, and twenty-four hours and observed that with small inocula sulfadiazine and sulfathiazole were bactericidal in concentrations as low as 1 or 2 mg. per cent on *Esch. coli* and *Shigella paradysenteriae* Flexner, and in 5 mg. per cent on *S. schottmülleri*. Both drugs were bacteriostatic in 5 mg. per cent for *S. paratyphi*.

Bacto nutrient broth plus sulfonamide was the medium used in this study, and the inoculum was usually between 50 and 150 microorganisms per cubic centimeter. Our cultures are stored in a semisolid medium composed of bacto brain heart infusion plus 0.3 per cent nutrient agar, in which heavy growth occurs. Two serial subcultures were made from the semisolid culture into synthetic medium which afforded moderate growth. The second culture was diluted 1:100,000 with nutrient broth, and 0.2 c.c. of this was inoculated into each of a series of tubes containing 10 c.c. of nutrient broth with various concentrations of sulfonamide. The different concentrations of sulfonamide were obtained by first preparing the highest concentration in nutrient broth. This was then sterilized in the autoclave and all other concentrations were prepared by making dilutions in sterile nutrient broth. The inoculated tubes of sulfonamide broth were incubated at 37° C. for at least three weeks, observed frequently, and subcultures made at regular intervals to determine the bactericidal concentration.

Daily observations of broth cultures containing increasing concentrations of sulfonamide show increasing bacteriostatic levels. If such observations are made over a sufficient number of days, the final bacteriostatic concentration is the same as the final bactericidal concentration. Bacteriostatic and bactericidal sulfonamide in vitro data are influenced greatly by certain factors which we have pointed out recently⁷ such as the number of bacteria in the inoculum, the quality of the medium and the length of the period of observation. Any period of observation less than that necessary to give data regarding the final bacteriostatic and final bactericidal concentrations provides data which is incomplete and dependent on the time of observation. We feel that our final results justify carrying the experiment through to completion. It is our experience that the comparison of such final data for a number of strains of bacteria is significant, and we have found that fairly constant results may be obtained by repetition under similar controlled conditions. A similar method could be developed which would be ap-

TAB
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SHIGELLA PARADYS
BACTERIOSTATIC AND BACTERICIDAL CONCENTRATIONS OF SODIUM SULFATHIAZOLE

STRAINS	INOCULUM BACTERIA PER C.C.	SODIUM SULFATHIAZOLE BACTERIOSTATIC CONCENTRATIONS (MG. PER CENT)										SULFAGUANIDINE BACTERIOSTATIC CONCENTRATIONS (MG. PER CENT)				FINAL BACTERICIDAL CONCENTRATIONS (21 DAYS)		
		DAYS					DAYS					DAYS				SODIUM SULFATHIAZOLE	SULFAGUANIDINE	
		1	2	4	7	14	21	1	2	4	7	14	1	2	4			7
B. (R.) SS	51	<1	<1	<1	<1	30	30	30	30	50	50	5	20	30	50	50	80	130
B. R. I	46	0.1	0.1	1	1	30	30	30	30	50	50	5	20	20	150	200*	100	>200*
J. K. I	88	0.1	0.1	1	1	40	40	40	40	70	70	5	5	>200*	>200*	>200*	40	>200*
J. K. 5 SS	85	0.1	0.1	1	1	50	50	50	50	70	70	5	5	170	170	>200*	70	>200*
D. B. I	78	0.1	0.1	1	1	50	50	50	50	50	50	5	80	90	200	200	50	200
D. B. 5 SS	96	0.1	0.1	1	1	10	10	10	10	10	10	5	80	80	>200*	>200*	10	>200*
V. O. I	140	0.1	0.1	0.1	10	20	20	20	20	20	20	5	10	70	70	200	20	>200*
V. O. 5 SS	150	0.1	1	1	10	30	30	30	30	30	30	5	10	100	>200*	>200*	30	>200*
P. W. I	110	0.1	0.1	1	50	60	70	70	70	90	140	5	5	10	170	200	120	>200*
P. W. 23	106	0.1	0.1	1	70	90	140	140	140	140	140	5	5	30	170	200	140	>200*
P. S. 21	119	<0.1	<0.1	<0.1	50	200	200	200	200	200	200	5	5	10	>200*	>200*	200	>200*
P. S. 10	67	1	20	40	120	160	160	160	160	160	160	30	200	>200*	>200*	>200*	160	>200*

*Higher concentrations necessary for bacteriostatic and bactericidal effects.

plicable as a standard technique for testing the final bactericidal and changing bacteriostatic concentrations of sulfonamides for various bacteria. As conditions are now, each investigator in this field uses his own choice of media, number of bacteria inoculated, quantity of drug, and time of observation, and he usually observes only for bacteriostasis. More recently we have determined by bacterial plate counts that gross evidence of growth in our sulfonamide broth media does not become apparent until the bacterial count reaches 15 million *Shigella paradysenteriae* per cubic centimeter. Thus it would seem that evidence of growth in sulfonamide broth media is not a delicate measurement of the bacteriostatic influence of a drug on a microorganism. Profuse multiplication must occur before bacteriostasis is ruled out by this method.

In Table I is seen data regarding the changing bacteriostatic and the final bactericidal concentrations of sodium sulfathiazole and sulfaguanidine for the twelve Flexner strains. The bacteriostatic effect was pronounced until the seventh day for all strains except one. During subsequent days growth appeared in the tubes of broth containing increasing concentrations of the sulfonamide approaching the bactericidal concentration. The Flexner strains showed a wide variation in susceptibility to sodium sulfathiazole. Bactericidal concentrations as low as 10 mg. per cent and as high as 200 mg. per cent were observed for different strains. Eight of the twelve strains were killed by 100 mg. per cent or less of the sulfonamide in medium. The other four strains were killed by the sulfonamide in concentrations between 100 and 200 mg. per cent. All 12 strains of *Shigella paradysenteriae* Flexner were killed by concentrations of sodium sulfathiazole varying between 10 and 200 mg. per cent. Such results are in keeping with clinical therapeutic results published previously⁸ in which it was shown that children with bacillary dysentery derived benefit from the use of sulfathiazole and those with nonspecific diarrhea did not derive benefit from its use. All of the Flexner strains showed growth earlier and more rapidly in medium containing sulfaguanidine than in similar medium containing sodium sulfathiazole. Only two of the twelve strains were killed by 200 mg. per cent of sulfaguanidine in medium. The solubility of sulfaguanidine is 200 mg. per cent.

The seven strains of *Shigella paradysenteriae* Sonne, Table II, were much less susceptible to both sulfonamides than were the Flexner strains. The Sonne strains showed growth earlier in medium containing higher concentrations of both sulfonamides than did the Flexner strains. It was necessary to repeat studies on three strains with higher concentrations of sodium sulfathiazole in the medium in order to obtain an end point for the bacteriostatic and bactericidal concentrations, since in the first studies with these particular strains growth occurred in medium containing all concentrations of the sulfonamide. The two sets of data for each of

TABLE II
SHIGELLA PARADYSENTERIAE SONNE BACTERIOSTATIC AND BACTERICIDAL CONCENTRATIONS OF SODIUM SULFATHIAZOLE AND SULFAGUANIDINE IN VITRO

STRAINS	INOCULUM BACTERIA PER C.C.	SODIUM SULFATHIAZOLE BACTERIOSTATIC CONCENTRATIONS (MG. PER CENT)										SULFAGUANIDINE BACTERIOSTATIC CONCENTRATIONS (MG. PER CENT)							FINAL BACTERICIDAL CONCENTRATIONS (21 DAYS)							
		DAYS					DAYS					DAYS							SODIUM SULFATHIAZOLE	SULFAGUANIDINE						
		1	2	4	7	14	21	1	2	4	7	1	2	4	7											
G. B. I }	86	30	120	170	>200*	>200*	>200*	170	120	270	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	
G. B. I }	89	5	50	120	270	>200*	120	170	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*
G. B. 3 SS }	112	1	40	90	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300
G. B. 3 SS }	100	5	30	60	120	250	350	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400
R. W. I }	82	0.1	50	60	170	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*
R. W. 18 M }	78	0.1	50	60	170	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*	>200*
Ch. }	36	2	10	60	350	350	350	350	350	350	350	350	350	350	350	350	350	350	350	350	350	350	350	350	350	350
Ch. }	105	1	30	60	250	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400
P. S. I }	97	5	5	70	250	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400
M3. I }	60	1	5	40	80	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160

*Higher concentrations necessary for bacteriostatic and bactericidal effects.

TABLE III
 EBERTHELLA TYPHOSA, SALMONELLA PARATYPHI, SALMONELLA MORGANI, SHIGELLA ALKALESCENS, ESCHERICHIA COLI, AND PROTEUS
 BACTERIOSTATIC AND BACTERICIDAL CONCENTRATIONS OF SODIUM SULFATHIAZOLE AND SULFAGUANIDINE IN VITRO

STRAINS	INOCULUM BACTERIA PER C.C.	SODIUM SULFATHIAZOLE BACTERIOSTATIC CONCENTRATIONS (MG. PER CENT)							SULFAGUANIDINE BACTERIOSTATIC CONCENTRATIONS (MG. PER CENT)							FINAL BACTERICIDAL CONCENTRATIONS (21 DAYS)	
		DAYS							DAYS							SODIUM SULFA- THIAZOLE	SULFA- GUANI- DINE
		1	2	4	7	14	21		1	2	4	7	14				
Esch. coli B}	153	>400*	>400*	>400*	>400*	>400*	>400*	200	>200*	>200*	>200*	>200*	>200*	>400*	>200*		
Esch. coli B}	40	>2000*	140	180	250	300	350	100	>200*	>200*	>200*	>200*	>200*	350	>200*		
Esch. coli M	74	1	5	60	180	350	350	40	100	>200*	>200*	>200*	>200*	350	>200*		
Esch. coli N	130	1	5	450	550	600	600	40	140	>200*	>200*	>200*	>200*	600	>200*		
Esch. typhosa P.	60	1	5	180	250	300	300	40	180	>200*	>200*	>200*	>200*	300	>200*		
Esch. typhosa O	90	1	5	400	450	550	550	100	>200*	>200*	>200*	>200*	>200*	600	>200*		
S. paratyphi	105	20	250	400	450	550	550	100	>200*	>200*	>200*	>200*	>200*	600	>200*		
B. L-1																	
S. 10-SS-1	76	20	30	80	120	200	200	30	>200*	>200*	>200*	>200*	>200*	250	>200*		
S. paratyphi A	87	1	5	5	250	250	350	30	50	90	180	>200*	>200*	350	>200*		
S. paratyphi B}	68	5	250	600	800	800	>800*	70	>200*	>200*	>200*	>200*	>800*	>200*			
S. paratyphi B}	80	1	250	750	850	850	900	180	>200*	>200*	>200*	>200*	900	>200*			
S. paratyphi B}	162	20	20	150	300	>400*	>400*	180	>200*	>200*	>200*	>200*	>400*	>200*			
S. morgani B}	100	5	30	250	400	500	500	60	90	>200*	>200*	>200*	500	>200*			
S. morgani B}	290	1	5	5	40	60	60	60	90	>200*	>200*	>200*	60	>200*			
S. morgani N	82	1	40	80	120	120	120	100	180	>200*	>200*	>200*	120	>200*			
Shigella alkalescens																	
Br.																	
Shigella alkalescens	87	1	10	90	100	100	120	40	90	>200*	>200*	>200*	>200*	120	>200*		
Be.																	
Proteus J.	118	0.1	0.1	5	40	70	70	5	10	>200*	>200*	>200*	>200*	70	>200*		
Proteus N.	276	0.1	1	10	20	70	70	5	10	>200*	>200*	>200*	>200*	70	>200*		

*Higher concentrations necessary for bacteriostatic and bactericidal effects.

these three strains are included in Table II to show that these data are characteristic of strains of the particular bacteria and not merely culture differences of the same strain. We have had occasion to repeat our sulfonamide studies on other strains, and the results obtained on repetition have always been similar to those obtained previously. The bactericidal concentrations for the Sonne strains varied between 250 and 400 mg. per cent in contrast to the Flexner strains, where all but one were killed by concentrations of the drug less than 200 mg. per cent. Sulfaguanidine in the medium had only a slight and brief bacteriostatic effect on the Sonne cultures. All but one of the seven Sonne strains tested in sulfaguanidine showed growth on the second day in medium containing 200 mg. per cent of the drug, the maximum concentration used. This one strain showed growth in medium containing 200 mg. per cent of the drug on the fourth day. None of these strains were killed by 200 mg. per cent of sulfaguanidine in medium.

The miscellaneous group of fifteen strains, Table III, composed of *Esch. coli*, *E. typhosa*, *S. paratyphi*, *S. morgani*, *Shigella alkalescens*, and *Proteus* varied in their susceptibility to the bacteriostatic effect of sodium sulfathiazole. Twelve of these fifteen strains showed heavy growth within seven days in medium containing high concentrations of this drug. Eleven of these twelve strains were killed by concentrations of sodium sulfathiazole varying between 120 and 900 mg. per cent in medium. One strain, *Esch. coli*, was not killed by 2,000 mg. per cent. The other three strains, one of *Proteus* and two of *S. morgani*, were quite susceptible to the bacteriostatic effect of sodium sulfathiazole and were killed by 60 and 70 mg. per cent of the drug in medium. Again sulfaguanidine in the medium had only slight and brief bacteriostatic effect on all these cultures. All but two strains showed heavy growth within four days in 200 mg. per cent of sulfaguanidine in broth. Sulfaguanidine in 200 mg. per cent concentration was not bactericidal for any of the cultures in this group. Our strains of *E. typhosa* and *S. paratyphi* had been isolated from children acutely ill with these infections who were subsequently treated with sulfathiazole or sulfaguanidine or both separately without any favorable response. One strain of *Shigella alkalescens* (Br.) had been isolated from a patient with recurring severe bloody diarrhea. This patient was treated with large doses of sulfaguanidine over a number of days without any improvement clinically and without the disappearance of the microorganism from the stool.

SUMMARY

Studies are reported on the bacteriostatic and bactericidal effects of sodium sulfathiazole and sulfaguanidine in vitro on twelve strains of *Shigella paradysenteriae* Flexner, seven strains of *Shigella paradysenteriae* Sonne, and fifteen strains of a miscellaneous group of intestinal bacteria, most of them pathogenic.

These studies differ from preceding studies in that the observations were made over a period of twenty-one days rather than one or two days, as is the usual custom, and each strain was studied in a series of at least nineteen different concentrations of the drugs instead of one, two, or three concentrations as is usual.

Sulfaguanidine was bactericidal for only two of the thirty-four different strains tested, and these were both strains of *Shigella paradysenteriae* Flexner.

Sodium sulfathiazole was bactericidal for all strains tested except one strain of *Esch. coli* which survived in media containing 2,000 mg. per cent of the drug.

For the Flexner strains, sodium sulfathiazole was bactericidal for all in concentrations between 10 and 200 mg. per cent inclusive.

For the Sonne strains, sodium sulfathiazole was bactericidal for all in concentrations between 250 and 400 mg. per cent inclusive.

For the miscellaneous group, sodium sulfathiazole was least bactericidal for strains of *Esch. coli*, *E. typhosa*, *S. paratyphi*, and two of the *S. morgani*. The bactericidal concentrations for these varied between 250 and more than 2,000 mg. per cent. For one strain of *S. morgani*, two of *Shigella alkalescens*, and two of *Proteus*, the bactericidal levels were between 60 and 120 mg. per cent, which are comparable to those observed for the Flexner cultures.

CONCLUSION

These results indicate that sodium sulfathiazole was more bacteriostatic and bactericidal for Flexner strains of *Shigella paradysenteriae* than for Sonne strains and still less bactericidal for *E. typhosa* and *S. paratyphi*.

These results also indicate that sodium sulfathiazole was much more bactericidal than sulfaguanidine for the strains tested.

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