Discussion. The strains of staphylococci used in these experiments are resistant to the bacteriostatic action of sodium sulfathiazole. It might be noted that strain 14 C was made sulfonamide-fast *in vitro,* while the other 2 strains were sulfonamide-fast when isolated from sulfonamide-treated patients.

The parent strain of 14 C and 2 additional strains from our laboratory museum all proved to be susceptible to 5 mg % of sodium sulfathiazole, under conditions identical to those used in these experiments.

The terminal hydrogen ion concentrations (pH 7.3 to 6.9) varied in direct proportion to the amount of growth and since the initial pH values of the various test cultures were identical (pH 7.2-7.3), it is indicated that the results observed may not be due to the effect of hydrogen ion concentration upon sulfonamide ionization. It has been claimed by several workers[13-15] that the bacteriostatic efficiency of sulfonamides is influenced by the degree of their ionization.

The mechanism of sulfonamide-fastness is not completely understood. Some workers have claimed that sulfonamide-fastness is due to the production of sulfonamide-inhibitors such as p-amino benzoic acid by the organisms.[7] Whatever the cause of sulfonamide-fastness may be, the results noted here indicate that in the concentrations used, sodium sulfathiazole-urea combinations exert a bacteriostatic effect whereas sodium sulfathiazole alone does not.

Summary. Three strains of sulfathiazole-resistant staphylococci in a synthetic medium, were found to be susceptible to urea-sodium sulfathiazole combinations whereas they were unaffected by these two agents used separately, under the conditions of the experiments described.

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In vitro Effect of Sulfonamides plus Urea on *Escherichia coli* in Presence of Para-aminobenzoic acid.*

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At the time that studies on sulfonamides were being initiated, it was noticed by the earlier workers that certain substances inhibited the bacteriostatic action of these drugs. Methionine and p-aminobenzoic acid are among these inhibitors.[1] In a previous paper[2] it was shown that in the presence of methionine, urea and sodium sulfadiazine will inhibit the growth of *Escherichia coli,* whereas in the presence of inhibitor, sodium sulfadiazine alone had little or no bacteriostatic action. To see if urea and sodium sulfadiazine, and urea and other sulfonamides, would

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† Research Fellow on above grants.

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2 Tsuchiya, H. M., Tenenberg, D. J., Clark, W. G., and Strakosch, E. A., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 262.

exert a bacteriostatic effect on *E. coli* in the presence of another sulfonamide inhibitor, p-aminobenzoic acid, the present experiments were carried out.

Experimental. A freshly isolated strain of *E. coli* was used in these experiments. Before the tests were run, the organism was first serially transferred several times in the synthetic medium, the composition of which is given in our previous paper.[2]

Preliminary experiments were performed to establish the tolerance range of our organism in the synthetic medium, to sodium sulfadiazine, sodium sulfathiazole, sulfanilamide, urea, and p-aminobenzoic acid. Various ratios of concentrations of the inhibitor and the sulfonamides were tested to determine the least amount of p-aminobenzoic acid which would be necessary to exhibit anti-sulfonamide activity.

The initial pH of the synthetic medium plus the various substances was 6.8 ± 0.1, as measured by the glass electrode just prior to inoculation. The terminal pH values were also determined.

Twenty-five ml amounts of the synthetic medium containing the various test substances were inoculated with 0.1 ml of a 24 hr synthetic medium culture. These cultures were incubated at 37° C for 24 hr, and the populations were determined by plating out in nutrient agar medium, using 5 replicate plates per test culture. The plates were counted after incubation at 37° C for 48 hr.

Results. The means of the 5 replicate plates for the various test cultures are given in Table I.

Table I shows that, under our experimental conditions, (1) Urea alone, in the concentrations used, had no significant effect on growth, (2) sulfanilamide, and the sodium salts of sulfathiazole and sulfadiazine alone, in the concentrations used, markedly inhibited growth, (3) p-aminobenzoic acid, in the concentrations used, significantly inhibited sulfonamide bacteriostasis, (4) combinations of urea and sulfonamides were bacteriostatically effective, in the presence of p-aminobenzoic acid.

Discussion. These experiments extend the

TABLE I.

In Vitro Effect of Sulfonamides and Urea on *E. coli* in Presence of Para-aminobenzoic Acid.

Experiments with	Populations expressed in millions per ml					
	SAN		SAT		SAD	
	1	2	1	2	1	2
Control	372	334	211	200	135	140
Urea controls						
Urea 4%			225	241	165	155
" 6%	299	272				
Sulfonamide controls						
SAN 15.0 mg%	67	55				
SAT 1.0 "			12.9	11.9		
SAD 1.0 "					19.4	23.1
Sulfonamide-p-aminobenzoic acid controls						
SAN 15 mg%; PAB .001 mg%	279	248				
SAT 1 " ; PAB .02 "			165	145		
SAD 1 " ; PAB .02 "					55	86
Sulfonamide-p-aminobenzoic acid-urea						
SAN 15 mg%; PAB .001 mg%; urea 6%	38	34				
SAT 1 " ; PAB .02 " " 4%			30.1	33.3		
SAD 1 " ; PAB .02 " " 4%					26	32

SAN—sulfanilamide.
SAT—Na-sulfathiazole.
SAD—Na-sulfadiazine.
PAB—p-aminobenzoic acid.

observations made in our previous work,[2] in which methionine was used as the inhibitor, to p-aminobenzoic acid as the inhibitor. Furthermore, using this inhibitor, 2 additional sulfonamides, sulfanilamide and sodium sulfathiazole, showed the same effect, whereas sodium sulfadiazine was the sulfonamide selected in the previous work.

The hydrogen ion concentration values determined at the time of plating, in general were found to increase with the amount of growth. This indicates that the urea effect may not be due to sulfonamide ionization. This point is being studied further. Fox and Rose,[3] Schmelkes, Wyss, *et al.*,[4] Cowles,[5] and others,[6, 7] claim that the bacteriostatic efficiency of sulfonamides is influenced by their degree of ionization.

The mode of action of urea is not as yet elucidated. It is not known whether or not urea acts similarly to azochloramid and certain purines such as adenine and hypoxanthine, which when used in conjunction with sulfonamides, were bacteriostatically effective in the presence of sulfonamide inhibitors.[8-11]

Summary. The concentrations of sulfanilamide, sodium sulfathiazole and sodium sulfadiazine which were required to exert bacteriostatic effect on *Escherichia coli* in a synthetic medium, were determined. Concentrations of para-aminobenzoic acid which caused a significant anti-sulfonamide activity were determined. Concentrations of urea which had no effect on bacterial growth were also determined.

Combinations of these concentrations of sulfonamides and urea, proved to possess bacteriostatic action even in the presence of the sulfonamide inhibitor.

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On the Adenosine Triphosphate-Myosin System.

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Recent data indicate that the chemical energy resulting from the breakdown of carbohydrate in muscle is converted to the mechanical energy required for muscle contraction by means of a reaction between adenosine triphosphate and myosin.[1] Engelhardt and Ljubimova have shown that this is an enzymatic reaction during which adenosine triphosphate is hydrolyzed to adenosine diphosphate and inorganic phosphate.[2] Needham *et al.* suggest that this reaction is associated with the stretching of the myosin fiber from the contracted state, to which it returns on nerve stimulation.[3] Since the adenosine triphosphate-myosin system is so intimately related to the contraction of the myosin fibrils,

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