which is several times the minimum requirement for growth, perhaps as much as 0.5% of the diet.[3] It was felt, therefore, that any methylating power of arsenocholine would not be observed at dietary levels lower than 0.5%. The results obtained with the combination of homocystine and arsenocholine (pen 3) did not indicate that the utilization of homocystine was appreciably increased. A minimum addition of choline (pen 4) also did not result in an appreciable increase in the utilization of homocystine. However, an addition of 0.5% choline chloride to the combination of 0.5% arsenocholine chloride and homocystine did effect a very appreciable increase in rate of gain (pen 5).

This latter result showed that a sufficient level of choline was capable of increasing the rate of gain in the presence of arsenocholine which, in itself, was comparatively ineffective. The rate of gain, 5.5%, was somewhat short of the optimal rate, which may suggest a mildly toxic action of arsenocholine. Thus, in the case of Group 3, the possibility exists that a slight methylating capacity of arsenocholine may have been almost exactly compensated for by a mild toxic action. It was also observed, however, that chicks reared from date of hatching to 14 days on the low-choline diet plus 0.5% of arsenocholine chloride made gains that were approximately normal, hence it does not seem that the arsenocholine could have been seriously detrimental to growth. Chicks fed arsenocholine possessed a strong odor of garlic, probably due to the exhalation of trimethylarsine[2, 3]

Summary. Arsenocholine as compared to choline is relatively ineffective as a methylating agent for homocysteine in the chick.

The authors are indebted to Dr. A. D. Welch for the arsenocholine, to Merck and Company, Inc., for some of the amino acids added to the diets, to Distillation Products, Inc., for the vitamin E concentrate, and to Riedel-deHaen, Inc., for the bile acids.

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In Vitro Effect of Urea-Sulfathiazole Combination on Sulfathiazole-resistant Staphylococci.*

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Several investigators have reported that under certain conditions, *Diplococcus pneumoniae, Brucella, Streptococcus, Staphylococcus, Neisseria gonorrheae, Escherichia coli,*[1-6] and possibly other organisms, develop resistance to sulfonamides ("sulfonamide-fastness"). It has been suggested that the sulfonamide-fastness is due to the production

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We wish to thank Doctor Weslie W. Spink and Mrs. Jean J. Vivino for furnishing us with the basal synthetic medium and Strain 14C used in this investigation.

† Research Fellow on above grants.

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TABLE I.
In Vitro Effect of Urea and Sulfathiazole on Sulfathiazole-fast Staphylococci.

Strain of Staph.	Organisms per ml × 1000					
	14c		S2S1		S9S2	
Experiment	1	2	1	2	1	2
Initial population	.7	.7	1.5	1.5	2.9	2.9
Plain control	500,000	500,000	128,000	197,000	450,000	630,000
SAT* control	500,000	550,000	169,000	184,000	590,000	600,000
Urea controls						
Urea 1.25%	1,380,000	1,360,000			350,000	680,000
Urea 1.75%			137,000	152,000		
SAT + urea						
SAT; urea 1.25%	13,400	1,400				
SAT; urea 1.75%			3,300	5,000	53,000	44,000

*SAT—Sodium sulfathiazole; 60 mg%.

of sulfonamide inhibitors by the organisms.[7]

In previous work[8] and in another place in this issue, we have shown that a combination of urea and sulfonamide exerts, in the presence of sulfonamide inhibitor, a greater bacteriostatic activity against *E. coli* than does sulfonamide alone in the presence of inhibitors.

These facts prompted us to investigate the effect of sulfonamide-urea combinations on sulfonamide-fast *Staphylococcus.*

Azochloramid-sulfonamide combinations,[9,3,6] and adenine or hypoxanthine-sulfonamide combinations *in vitro*[10] have been shown to exert greater bacteriostatic effect in the presence of sulfonamide-inhibitors than sulfonamides alone in the presence of inhibitors.

Experimental. Three sulfonamide-fast strains of *Staphylococcus aureus* were used. Stain 14 C was kindly given to us by Dr. W. W. Spink. This strain had been made sulfonamide-fast in his laboratory.[4] Strains S2S1 and S9S2 were isolated by one of us‡ from human abscesses infected with staphylococci, which had failed to respond to prolonged sulfonamide treatment. Strakosch and Clark[11] have discussed these clinical cases in which urea-sulfonamide mixtures had been used. The basal synthetic medium used is described by Gladstone.[12]

Preliminary experiments were carried out to establish the concentrations of urea and sodium sulfathiazole which were without inhibitory effect on growth.

At the time of inoculation the pH values of the test cultures were 7.2-7.3 as determined by the glass electrode. Terminal pH values were also determined.

Ten ml of medium containing the test substances were inoculated with 24-hour cultures of the strains of staphylococci described. After incubation at 37° C for 24 hr, these cultures were plated out in nutrient agar, 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 the conditions of the experiments, (1) the concentrations of urea used had no significant inhibitory effect on the growth of the organisms, (2) the concentration of sulfathiazole used (60 mg %) had no significant effect on the growth of the organisms, (3) urea-sulfathiazole combination caused a marked inhibition of growth in each case.

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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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