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In vitro Action of Urea-Sulfonamide Mixtures.

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Mixtures of urea with sulfonamides have recently been employed for topical application to infected wounds.[1] Advantages of urea are that it increases the solubility of sulfonamides, and that it has strong peptizing, lytic, and deodorant actions.[2] In addition, the value of urea would be greatly enhanced if it could be shown to actually potentiate the bacteriostatic action of the sulfonamides, or to antagonize the sulfonamide inhibitors present in pus and certain body fluids. The recent experiments of Tsuchiya and colleagues indicate that the antisulfonamide action of methionine and of *p*-aminobenzoic acid can be overcome by the addition of urea to sulfonamide solutions,[3,4] and further that urea-sulfonamide mixtures are effective against sulfonamide-resistant staphylococci.[5]

The present paper is a report of experiments performed in an attempt to confirm and extend the results of Tsuchiya *et al.*, using different technical methods.

Method. The quantitative methods evolved in this laboratory in connection with studies of sulfonamide resistance have been described elsewhere in detail.[6] Briefly, growth curves were measured turbidimetrically under rigidly controlled conditions for a period of 24 hours or more. In the present studies the organism was *E. coli*, and the synthetic medium was the same as that previously described. The addition of solutions of urea, sulfathiazole, and *p*-aminobenzoic acid did not alter the pH of the basal medium.

Three types of experiments were performed, as follows:

(1) The effect of various concentrations of urea upon the growth of the organisms was determined.

(2) Various concentrations of urea were added to *p*-aminobenzoic acid-sulfonamide mixtures to determine the effect of urea upon the antisulfonamide action of *p*-aminobenzoic acid.

(3) Various concentrations of urea were added to basal medium plus sulfathiazole plus a sulfonamide-resistant strain of *E. coli* to see if urea would potentiate the action of the sulfonamide against the resistant organisms.

Results. The effect of concentrations of urea ranging from 0.20% to 2.0% upon the growth of the test organisms is shown in Fig. 1. During the phase of active growth there was definite bacteriostasis by all the concentrations of urea employed, even as little as 0.20%, and the degree of bacteriostasis was roughly proportional to the amount of urea present. When maximal growth was reached in the control tube, growth of the partially inhibited organisms continued until the same maximal level was reached.

With the sulfathiazole-*p*-aminobenzoic acid mixtures, the addition of concentrations of urea which were not bacteriostatic (less than 0.20%) did not affect the growth of the organisms. Bacteriostatic concentrations of urea inhibited growth in the sulfathiazole-*p*-aminobenzoic acid mixtures to the same extent as they did in the control tubes, but no more; the effect was purely additive. This is illustrated in Fig. 2.

[1] Holder, H. G., and MacKay, E. M., *Military Surg.*, 1942, **90**, 509.

[2] Olson, M., Slider, E., Clark, W. G., and MacDonald, R., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 396.

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

[4] Tenenberg, D. J., Tsuchiya, H. M., Clark, W. G., and Strakosch, E. A., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 247.

[5] Tsuchiya, H. M., Tenenberg, D. J., Strakosch, E. A., and Clark, W. G., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 245.

[6] Kirby, W. M. M., and Rantz, L. A., *J. Exp. Med.*, 1943, **77**, 29.

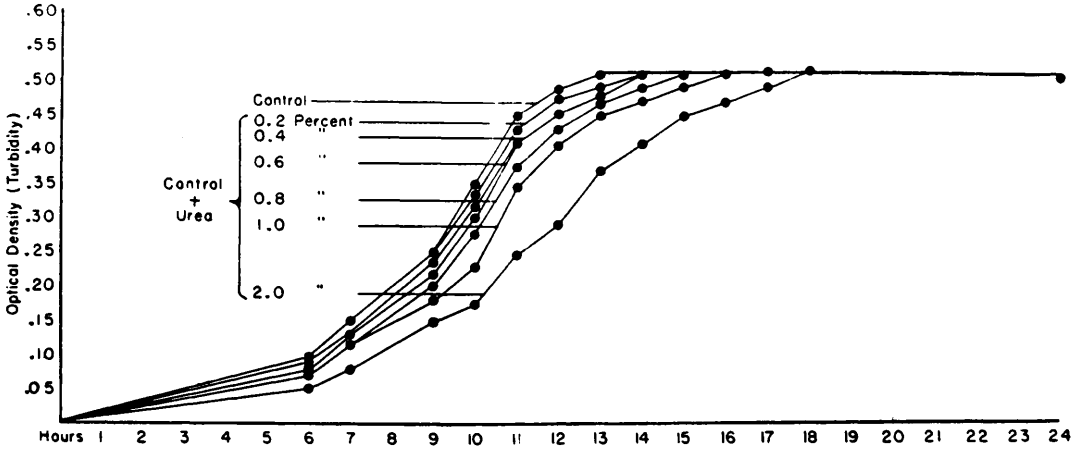

FIG. 1.
Effect of various concentrations of urea upon the growth of *E. coli*. See text for explanation.

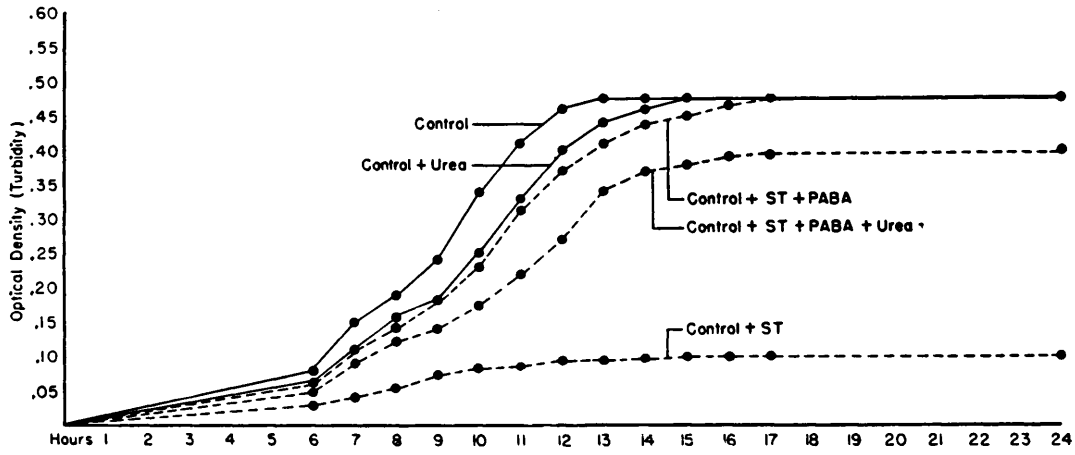

FIG. 2.
Effect upon a non-resistant strain of *E. coli* of adding urea 1% to a mixture of *p*-amino-benzoic acid (PABA) 1 mg% and sulfathiazole (ST) 10 mg%. See text for explanation.

Growth of the sulfonamide resistant organisms plus sulfathiazole was not inhibited by concentrations of urea which were not bacteriostatic. The degree of inhibition of growth produced by bacteriostatic concentrations of urea plus sulfathiazole was identical with that observed in the control tubes; here again the effect was purely additive.

Discussion. The observation of Tsuchiya and co-workers that concentrations of urea which are not bacteriostatic have an anti-sulfonamide inhibitor action has not been confirmed. The present experiments indicate that high concentrations of urea are in themselves bacteriostatic, but that concentrations of urea which are not bacteriostatic do not counteract sulfonamide inhibitors, nor do they potentiate the bacteriostatic action of the sulfonamides against resistant organisms. The conclusions of Tsuchiya's group were based upon plate counts made only at the end of 24 hours, and inspection of Figs. 2 and 3 will show why readings made at this time are not valid. Due to the limiting effect of the medium itself the partially inhibited organisms gradually catch up with the controls following the phase of logarithmic growth, so that at the end of 24 hours the bacteriostatic action of urea alone is no longer apparent, while the combined inhibition of urea plus sulfathiazole is great enough to prevent maximal growth in this tube. There is no actual potentiation of sulfonamide action;

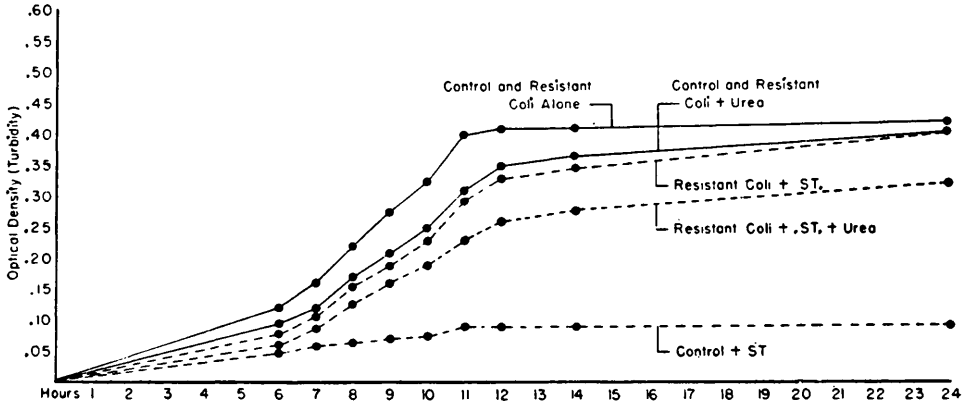

FIG. 3.

Effect upon a non-resistant and a sulfonamide-resistant strain of *E. coli* of adding urea 1% in the presence of sulfathiazole (ST) 10 mg%. See text for explanation.

the effect of urea, a bacteriostatic agent itself in high concentrations, is purely additive.

It is not to be inferred that urea-sulfonamide mixtures are of no value. On the contrary, the fact that urea is in itself a bacteriostatic agent, in addition to its solvent, peptizing, lytic, and deodorant actions, would seem to make the combination highly desirable for topical application.

Summary. Contrary to previous reports the present experiments indicate that concentrations of urea which are not bacteriostatic do not counteract sulfonamide inhibitors, nor do they potentiate the bacteriostatic action of the sulfonamides against resistant organisms. High concentrations of urea have a bacteriostatic action of their own which may be of value when they are used in combination with the sulfonamides.

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Presence of Hippuric Acid in Milk.

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The non-protein nitrogen, or residual nitrogen of cow's milk is known to consist in part of urea, uric acid, creatinine, amino acids, and purine bases. The remainder is composed to a large extent of compounds of unknown structure. We wish to report that one of these heretofore unidentified substances of the residual nitrogen fraction of cow's milk is hippuric acid.

In connection with the isolation of crystalline biotin from milk in this laboratory,[1] we had occasion to fractionate a biotin concentrate from milk, generously supplied by the S.M.A. Corporation of Chagrin Falls, Ohio. This material had been prepared by charcoal adsorption from a commercial milk residue which remained after the removal of protein and sugar from milk. One gram of the biotin concentrate represented approximately 15 kg of milk.

The first steps in the fractionation of this concentrate for the isolation of biotin included esterification of the concentrate with alcoholic HCl and then extraction with ethyl acetate of a slightly alkaline solution of the esterified material. The ethyl acetate extracts were concentrated to remove solvent and the residue

[1] Melville, D. B., Hofmann, K., Hague, E., and du Vigneaud, V., *J. Biol. Chem.*, 1942, **142**, 615.