

Summary. The volume of crude penicillin can be greatly reduced with considerable increase in potency of the active principle by a process of low temperature-partial vacuum

concentration. After an initial drop the titer of the concentrated material kept in a dry-ice cabinet is maintained for as long as 6 months.

14323

Synergistic Action of Thiourea and Guanidine with Sulfathiazole.

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An advantageous co-action between sulfonamides and urea has been found to exist, both in wounds,¹ and *in vitro*.² The conclusion

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

² Tsuchiya, H. M., Tenenberg, D. J., Clark, W. G., and Strakosch, E. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1942, **51**, 247. See also previous papers.

reached by Tsuchiya *et al.* in the latter case have been contested by Kirby.³ In this paper we have confirmed the finding of Tsuchiya *et al.*, and have extended the observations to include a study of thiourea and iminourea (guanidine). The structural similarity of these two compounds to urea is obvious from the formulæ:

³ Kirby, W. M. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1943, **53**, 109.

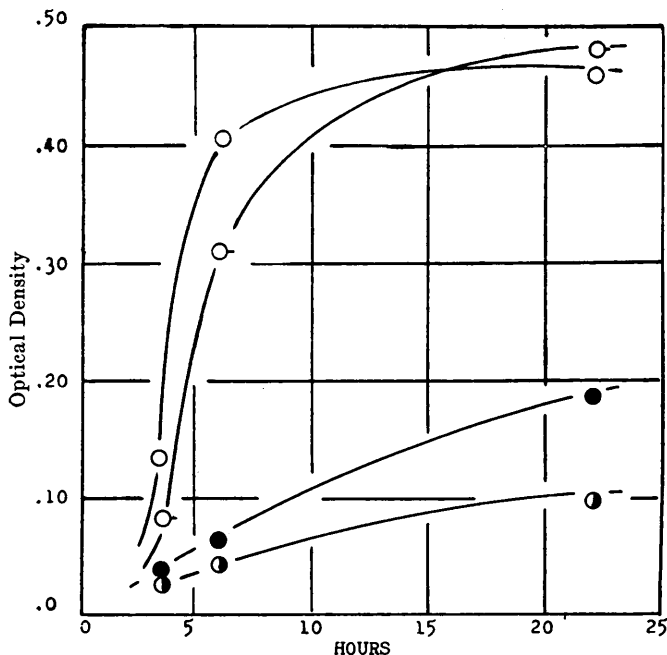


FIG. 1. TIME-GROWTH CURVES OF *E. COLI*, AS INFLUENCED BY UREA, SODIUM SULFADIAZINE AND THEIR COMBINATION.

○ = Control.

○ = Urea, 1.6% or 0.28 M.

● = Sodium Sulfadiazine, 1 mg. %.

● = Urea, 1.6% or 0.28 M, and sodium sulfadiazine, 1 mg. %.

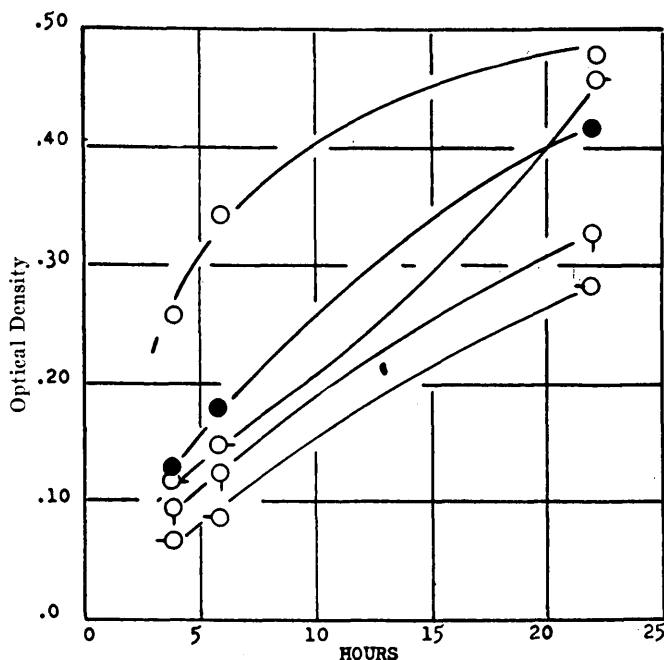
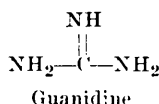
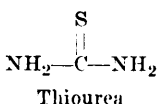
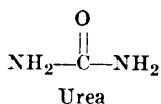


FIG. 2. TIME-GROWTH CURVES OF *E. COLI*, AS INFLUENCED BY SULFATHIAZOLE ALONE AND COMBINED WITH UREA, THIOUREA OR GUANIDINE.

- = Control.
- = Sulfathiazole, 10^{-3} M.
- = Sulfathiazole, 10^{-3} M, and urea, 0.066 M.
- = Sulfathiazole, 10^{-3} M, and thiourea, 0.066 M.
- = Sulfathiazole, 10^{-3} M, and guanidine, 0.066 M.



Thiourea and guanidine have been found to be much more active than urea, in the above sense.

The early work of Tsuchiya *et al.*² has been repeated in a quantitative manner. Fig. 1 clearly shows these results. The conditions for these experiments were identical to those of Tsuchiya *et al.*, except for the initial population of *E. coli*, which was about 6 million organisms per ml. The stimulatory effect of urea (after 24 hours) at the concentration used (1.6%, 0.28 M), as well as its synergistic action with sodium sulfadiazine, is shown. The bacterial growth was determined turbidimetrically, and the density readings plotted in Fig. 1 are taken directly from the drum of a Coleman Universal Spectrophotometer. With this organism, the population is roughly lin-

early related to the optical density. As determined by plating, the bacterial count per ml corresponding to a density of 0.46 is 6.5×10^8 . Using the exact plating method of Tsuchiya *et al.*, the counts obtained were well in line with the related density readings.

It is seen from Fig. 1 that urea alone exerts a slight bacteriostatic effect in the early part of the growth curve, in essential agreement with the work of Kirby. However, the magnitude and even the presence of this growth diminution is dependent on the inoculum size. This dependence is discussed in the subsequent paper. Otherwise, the results are similar to those of Tsuchiya *et al.*, but are here given quantitatively.

As was the case with urea, preliminary experiments were carried out to determine the lowest concentration of thiourea and guanidine which did not adversely affect the growth rate of *E. coli*, in a synthetic medium consisting of glucose and various salts (SG).⁴ A concen-

tration of 0.066 M was chosen, and equimolar solutions of urea, thiourea, and guanidine (in the form of its sulfate) were made up in sterile SG medium. One ml of sulfathiazole solution (10^{-3} M) was added to 99 ml portions of the above solutions for the mixed drug solutions. These, together with SG medium (control) and 10^{-5} M sulfathiazole in SG medium, were added in 10 ml amounts to sterile tubes. One-half ml of a 1:2.5 dilution of an overnight culture of *E. coli* was added to each tube, giving an initial population of about 1.1×10^6 organisms per ml. The suspensions were incubated at 37° , and their densities determined at various times. The result of this experiment is shown in Fig. 2, each point representing an average of 4 or 5 determinations.

It can be seen from Fig. 2 that, over the entire period of 24 hours, the order of increasing synergistic action is urea, thiourea, and guanidine. At 24 hours, the time chosen by

Tsuchiya *et al.*,² the picture is more favorable for the latter two compounds. The urea, in the presence of the greater number of organisms (which is essentially a dilution from the standpoint of a single enzyme molecule) shows a growth stimulating effect. The presence of thiourea (0.066 M) brings about a 22%, and guanidine a 34% growth decrease, in addition to that caused by the sulfathiazole (10^{-5} M), which was about 13%. The figures on thiourea and guanidine are on the basis of the growth of the organism in the presence of sulfathiazole (10^{-5} M), which is the related control.

Summary. Guanidine and thiourea, in this order, have been found to be much more active than urea (all at the same molar concentration), when used jointly with sulfathiazole in inhibiting the growth of *E. coli* on a simple medium. The action is a synergistic one.

The synergistic action of urea with sodium sulfadiazine on *E. coli* has been confirmed, using the conditions defined by Strakosch and associates.

⁴ Kohn, H. I., and Harris, J. S., *J. Pharm. and Exp. Therap.*, 1943, **77**, 1.

14324

Synergism of Urea and Sulfathiazole on *E. coli*. Effect of Inoculum Size.

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In a previous paper (see companion article) the varying opinions of Tsuchiya *et al.*¹ and Kirby² concerning the nature of the action of urea with the sulfonamides upon *E. coli* has been mentioned. In conjunction with other problems, a few experiments have been carried out in this laboratory which are considered to bear on this problem.

It should first be pointed out that the experiments of Kirby² were not carried out exactly as were those of Tsuchiya *et al.*, and

because of these variations the results should probably not have been contrasted. The obvious points of difference in the two series of experiments were media and inoculum size. The medium of Kirby contained sugar, salts and casamino acids, Difco, whereas that of Tsuchiya *et al.* consisted only of salts and glucose, similar in nature to that which has been used in experiments reported here (medium SG³).

The much greater departure in conditions, it is felt, concerns the inoculum size. Tsuchiya *et al.* worked with the large initial population of about 800,000 organisms per ml. Kirby

¹ Tsuchiya, H. M., Tenenberg, P. J., Strakosch, E. A., and Clark, W. G., *PROC. SOC. EXP. BIOL. AND MED.*, 1942, **51**, 245. (See also previous papers.)

² Kirby, W. M. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1943, **53**, 109.

³ Kohn, H. I., and Harris, J. S., *J. Pharm. and Exp. Therap.*, 1943, **77**, 1.