due to synthesis of inadequate amounts of pyridoxamine. This is suggested by the fact that *L. casei* and *L. delbrückii* require ten times as much pyridoxamine for growth without the above three amino acids as with them.

An explanation is now available for the disagreement between investigators as to the essentiality of lysine and threonine for growth of *L. arabinosus*.[9] During heat sterilization of the medium sufficient pyridoxamine may be formed from the interaction of the pyridoxine and amino acids[2] to permit good growth of *L. arabinosus* in the absence of lysine or threonine. Autoclaving a medium deficient in lysine for 30 minutes instead of the customary 15 or 20 minutes permitted maximum growth, whereas the same medium autoclaved without pyridoxine and to which Seitz filtered pyridoxine was added, failed to support growth unless lysine was present.

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THE EFFECT OF UREA, URETHANE AND OTHER CARBAMATES ON BACTERIAL GROWTH[1,2]

UREA has been shown to have anti-bacterial properties by Peju and Rajat[3] and Foulger and Foshay[4] and to be effective in the treatment of infected wounds and diphtheria carriers by Symmers and Kirk.[5] Holder and MacKay[6,7] and Ilefeld[8] have reported favorable response of infected wounds to treatment with mixtures of urea and sulfonamides. Tsuchiya, Tenenberg, Clark and Strakosch[9,10,11] have recently shown that urea inhibits para-aminobenzoic acid and methionine, substances which antagonize the action of the sulfonamides. These findings could not be confirmed by Kirby[12] but have been confirmed by Lee, Epstein and Foley.[13]

Urethane has received very little attention with respect to its action on bacterial growth but has been found to depress the respiratory rate of yeasts and, in low concentrations, to stimulate, and in larger amounts to depress the luminescence and respiration of the luminescent bacteria.[17, 18, 19] Johnson[14] has claimed that urethane exerts an anti-sulfonamide effect on luminous bacteria but McIlwain[15] and Martin and Fisher[16] using streptococci in *in vitro* and *in vivo* studies could not confirm this work.

The investigations reported here indicate that urea and urethane are both bacteriostatic and bactericidal for many organisms, that they antagonize slightly the sulfonamide inhibitors and that they increase the solubility and bacteriostatic activity of the sulfonamides. Urethane appears to be greatly superior to urea in all respects.

Six per cent. urea and 3 per cent. urethane were found to be bacteriostatic for *E. coli* in veal infusion broth containing 50 per cent. horse serum. *Staphylococcus aureus* required higher concentrations of either drug in the same medium to produce a similar effect; 4 per cent. urethane and 10–12 per cent. urea inhibited growth of this organism. The bacteriostatic levels of both drugs were found to be lower in synthetic media. The growth of *Pneumococcus,* hemolytic *Streptococcus, Proteus vulgaris, E. typhi, Pseudomonas pyocyaneus, S. schotmulleri* and *S. paradysenteriae* (Flexner) in serum-veal infusion medium was found to be inhibited by 2 per cent. urethane and 6 per cent. urea, these being the final concentrations of the drugs in the medium. Several strains of some of these bacteria were examined and all were found to react in the same manner.

Studies of the effect of other urea derivatives such as propyl and butyl carbamate on the same group of organisms showed that bacteriostasis is produced by lower concentrations than those necessary with either urea or urethane. One to 2 per cent. propyl carbamate and 0.5 to 0.75 per cent. butyl carbamate were found to inhibit the growth of all the organisms listed above.

In a large number of bactericidal tests in which the bacteria enumerated above were exposed to various concentrations of either urea or urethane at a temperature of 37° C. and subcultures taken at periodic intervals, it was found that killing occurred after 5 to 15 minutes of contact with 10 per cent. urethane in

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[2] Aided by a grant from the Johnson Research Foundation, New Brunswick, New Jersey.

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veal-serum broth even when inocula as large as 50,000 to 75,000 bacteria were used except with *Staphylococcus aureus* which, in some of the tests, was killed only after 3 to 4 hours of exposure; with small inocula of this organism bactericidal action was often noted in 30 minutes. With a concentration of 5 per cent. urethane the time required to produce bacterial death was appreciably longer than with the larger amount. The action of urea was found to be much weaker than that of urethane and 2 hours of contact were often required before 20 per cent. urea in broth produced the same degree of effect as did 10 per cent. urethane in 5 minutes. Twenty per cent. urea was found not to be bactericidal for *Staphylococcus aureus* even after exposure for 24 hours. *Pseudomonas pyocyaneus* and *Proteus vulgaris* appeared to be the most susceptible to the action of the carbamates.

The action of urea and urethane on para-aminobenzoic acid was examined a number of times using *E. coli* and *Staphylococcus aureus* in synthetic and horse serum-veal infusion media. The amounts of sulfanilamide used were 15 mgms per 100 cc in the synthetic and 75 mgms per 100 cc in the serum-veal infusion medium for *E. coli* and 50 mgms per 100 cc in synthetic and 100 mgms per 100 cc in infusion broth for the *Staphylococcus*. In one series of experiments two non-bacteriostatic amounts of urea (2 and 4 per cent.) and urethane (1 and 2 per cent.) were titrated against fourteen concentrations of para-aminobenzoic acid ranging from 0.001 to 2.5 mgms per 100 cc; in another, seven different quantities of para-aminobenzoic acid were tested against six varying amounts of urea. Both urea and urethane produced inhibition of the sulfonamide antagonist, the effect being evident, however, only with very small quantities of para-aminobenzoic acid. Inhibition of PABA by urea occurred for 96 hours only when 0.001 mgms per 100 cc of the acid were used. The carbamates antagonized slightly higher concentrations of PABA only for short periods of time, while larger amounts of this substance showed complete lack of inhibition by either urea or urethane. Urethane was more active than urea since about one half the concentration produced the same effect on PABA.

The combination of non-bacteriostatic amounts of urea and urethane with non-bacteriostatic quantities of sulfanilamide produced pronounced inhibition of bacterial growth. The bactericidal potency of urethane was also increased moderately by the addition of non-bactericidal concentrations of sulfathiazole. Non-bacteriostatic amounts of urea and urethane in combination with sulfanilamide produced marked flattening of the growth curves of *E. coli* and *Staphylococcus aureus*, each substance alone yielding a normal curve of bacterial multiplication.

It has been shown that urea increases the solubility of the sulfonamides. A similar effect was noted with urethane; thus, the solubility of sulfanilamide at 20° C. was 400 mgms per 100 cc in water and 1,000 mgms per 100 cc in 10 per cent. urethane solution in water. Two hundred mgms of sulfathiazole could be dissolved in 100 cc of 10 per cent. urethane in water, but only 69 mgms went into solution in water alone at 20° C.

The mode of action of urethane and urea on bacterial growth is not clear, but certain factors appear to have been ruled out. The osmotic pressure of the solutions probably plays no rôle since it has been found that concentrations of urea and urethane which inhibit bacterial growth have no effect on the human erythrocyte even after exposure for 24 hours at 37° C. Furthermore, while 10 per cent. urethane invariably kills all the organisms studied, 10 per cent. sodium chloride, 20 per cent. sucrose and 20 per cent. glucose solutions show no bactericidal action even after 24 hours at 37° C. All the solutions of the carbamates in the media in which the tests were carried out had a pH of from 7.2 to 7.4. Harvey[17] and others[18, 19] have shown that narcotics, among them urethane, have a depressant effect on the enzyme systems of luminescent bacteria in certain concentrations; it is possible that the same type of action is responsible for the effects noted in the studies reported here.

A small number of various types of infections in man have been treated with a mixture containing ten grams of urethane and 1 gram of sulfanilamide in 100 cc of water, and those in which Gram negative bacteria were the predominant organisms responded very rapidly with sterilization and healing of the wounds. Infections with Gram positive bacteria were much more refractory to treatment. Some evidence was also obtained which indicates that urethane does not impede wound healing in man.

The studies reported here indicate that urethane and urea exert both a bacteriostatic and bactericidal action (depending on the concentration used) mainly against Gram negative bacteria but also to a lesser degree against Gram positive organisms, that they antagonize sulfonamide inhibitors, increase the solubility of sulfonamide compounds, and are of value in the treatment of Gram negative infections in man. The activity of the four carbamate compounds tested against bacteria appears to be in the following order; most active is butyl carbamate and least effective urea, with urethane and propyl urea occupying a middle ground.

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