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Action of Urea and Some of Its Derivatives on Bacteria. V. Antibacterial Activity of Methyl- and Thiourea.*

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It has been reported[1-3] that urea and urethane are highly bacteriostatic and bactericidal for many Gram-negative and several Gram-positive organisms, potentiate moderately the activity of sulfonamides, inhibit *p*-aminobenzoic acid moderately, and increase the solubility of sulfanilamide and sulfathiazole. To determine whether increased bacteriostatic potency results from substitution of one of the NH_2 groups in urea by $—CH_3$ radicals, as was suggested by the superiority of urethane over urea, other derivatives of carbamide were investigated. The present paper deals with a study of antibacterial and anti-*p*-aminobenzoic acid activity of methyl and thio derivatives of urea, the $—CH_3$ group replacing one $—NH_2$ in the first compound and —S being substituted for the =O in urea in the second.

A review of the literature reveals no information concerning the activity of methyl urea against microorganisms. Thiourea is reported[4] to be antibacterial and to increase the effectiveness of sulfathiazole. This action was thought to be synergistic.

Methods. The methods used in the studies described in this paper are similar to those detailed in the previous ones of this series and, therefore, will not be described here. Veal-infusion broth with added horse serum is designated as VIB-S. The pH of 5% methyl- and thiourea solutions in VIB-S was found to be 7.2 to 7.25.

Results. I. *The Bacteriostatic Effects of Methyl Urea.* A study of the bacteriostatic effect of methyl urea in amounts varying from 2 to 8% in VIB-S revealed that a concentration of approximately 6% produced suppression of growth of several Gram-negative organisms. *Ps. aeruginosa* was completely inhibited by a 4% solution of the drug. Moderate bacteriostasis of *E. coli, S. schottmulleri, P. vulgaris,* and *E. typhi* resulted from exposure to 5% methyl urea. *P. vulgaris* was partially inhibited by 6% and an 8% solution completely arrested growth for 96 hours.

In a synthetic medium,[5] the concentration

TABLE I.

Effect of Varying Concentrations of Methyl Urea on Growth of Various Organisms in Different Media.

			Concentration of methyl carbamate Growth at various periods											
			6%		5%		4%		3%		2%		No drug	
Medium	Organisms inoculated	hrs	48	96	48	96	48	96	48	96	48	96	48	96
VIB-S	*E. coli*—6500		0	0	0	3+	2+	4+	4+	4+	4+	4+	4+	4+
	S. schottmulleri—1050		0	0	0	2+	1+	3+	4+	4+	4+	4+	4+	4+
	Ps. aeruginosa—29,000		0	0	0	0	0	0	1+	3+	4+	4+	4+	4+
	*P. vulgaris**—34,000		0	2+	1+	4+	4+	4+	4+	4+	4+	4+	4+	4+
	E. typhi—525		0	0	0	0	0	0	1+	3+	3+	4+	4+	4+
Synthetic medium	*E. coli*—22,000,000		0	0	0	0	0	0	0	3+	4+	4+	4+	4+

* 8% completely inhibited for 96 hours.

* This study was aided by a grant from the Johnson Research Foundation, New Brunswick, N.J.

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2 Weinstein, L., and McDonald, A., *J. Immunol.,* 1946, **54**, 117.

3 Weinstein, L., and McDonald, A., *J. Immunol.,* 1946, **54**, 131.

4 Lee, S. W., Epstein, J. A., and Foley, E. J., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 105.

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

TABLE II.
Effect of Two Concentrations of Methyl Urea on Varying Amounts of Para-Aminobenzoic Acid in VIB-S.
Inoculum of *E. coli*—810,000 organisms.

Conc. of methyl urea, %	Sulfanilamide mg/100 ml	*p*-aminobenzoic acid mg/100 ml	Growth (hr) 24	48	72	96
2	—	—	4+	4+	4+	4+
4	—	—	4+	4+	4+	4+
—	15	0.001	0	0	0	3+
—	15	0.0025	0	0	3+	4+
—	15	0.005	0	3+	4+	4+
—	15	0.0075	0	4+	4+	4+
—	15	0.01	2+	4+	4+	4+
—	15	0.025	4+	4+	4+	4+
—	15	0.05	4+	4+	4+	4+
—	15	1.00	4+	4+	4+	4+
—	15	2.50	4+	4+	4+	4+
2	15	0.001	0	0	0	0
2	15	0.0025	0	1+	4+	4+
2	15	0.005	0	3+	4+	4+
2	15	0.0075	1+	4+	4+	4+
2	15	0.01	2+	4+	4+	4+
2	15	0.025	3+	4+	4+	4+
2	15	0.05	4+	4+	4+	4+
2	15	2.50	4+	4+	4+	4+
4	15	0.001	0	0	0	1+
4	15	0.0025	0	0	0	2+
4	15	0.005	0	0	3+	4+
4	15	0.0075	0	1+	4+	4+
4	15	0.01	0	2+	4+	4+
4	15	0.025	0	2+	4+	4+
4	15	0.05	0	2+	4+	4+
4	15	0.75	0	2+	4+	4+
4	15	0.10	0	3+	4+	4+
4	15	2.50	0	3+	4+	4+
—	—	2.50	4+	4+	4+	4+
2	15	—	0	0	0	0
4	15	—	0	0	0	0
—	15	—	0	0	0	0
—	—	—	4+	4+	4+	4+

TABLE III.
Bacteriostatic Effect of Thiourea in Serum-Veal-Infusion Medium.

Organisms. No. in inoculum	hrs	Concentration of thiourea. Growth at various periods: None 48	None 96	2% 48	2% 96	1% 48	1% 96	0.5% 48	0.5% 96	0.25% 48	0.25% 96
E. coli—3250		4+	4+	0	0	0	0	1+	1+	3+	4+
S. aureus—2400		4+	4+	0	0	0	1+	1+	3+	4+	4+

of methyl urea required to suppress growth of *E. coli* was found to be less than that necessary in VIB-S.

A study of the potentiating effect of methyl urea on sulfanilamide in a synthetic medium, using *E. coli* as the test organism, revealed that a 2% concentration of the carbamate produced no apparent increase in activity of

the sulfonamide. Four per cent methyl urea was bacteriostatic when used alone and any stimulation of the activity of sulfanilamide by it could, therefore, not be determined.

II. *The Effect of Methyl Urea on Para-aminobenzoic Acid.* A 2% concentration of methyl carbamate did not inactivate *p*-aminobenzoic acid. Four per cent was moderately effective, the sulfonamide-inhibiting substance being inactivated in amounts as large as 0.1 mg per 100 ml for 24 hours. As with urea and urethane, the anti-PABA activity of methyl urea appeared to be of short duration.

III. *The Bacteriostatic Effects of Thiourea.* A 2% concentration of thiourea inhibited growth of *S. aureus* for 96 hours, 1% for only 48 hours. Quantities less than 1% were ineffective. One per cent thiourea suppressed completely the multiplication of *E. coli.*

Summary and Discussion. Methyl urea was found to be bacteriostatic for several Gram-negative bacteria in both VIB-S and in a synthetic medium. The concentration of drug required to suppress growth varied somewhat with each organism, but it ranged generally between 6 and 8% except for *Ps. aeruginosa,* which was inhibited by a 4% solution. The activity of the drug in a synthetic medium was greater than in VIB-S.

The substitution of one $—NH_2$ in carbamide by a single $—CH_3$ group increased the activity of urea very little since approximately the same concentrations of the 2 compounds were required to inhibit the growth of bacteria. Urethane, as previously reported, was more highly bacteriostatic than either urea or methyl urea. It would appear, therefore, that the insertion of $—CH_2CH_3$ in place of one $—NH_2$ in urea results in greater activity than that following the addition of only a single carbon atom. As shown by the few data presented, the replacement of $=O$ by $—S$ in the urea molecule is apparently very effective in increasing its antibacterial properties since a 1% concentration of thiourea produced approximately the same results with *E. coli* as 3% urethane or 6% urea.

Conclusions. 1. Methyl- and thiourea are bacteriostatic for Gram-negative bacteria. 2. The substitution of an $—NH_2$ group by $—CH_3$ in urea results in no demonstrable increase in antibacterial activity. 3. The replacement of the $=O$ group in urea by $—S$ increases markedly the antibacterial activity. 4. Methyl urea, in the concentrations studied, does not potentiate the sulfonamides. It does have a moderate antagonistic effect on *p*-aminobenzoic acid.

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Interrelation Between α-Tocopherol and Protein Metabolism: Body Weight and Tooth Pigmentation of Rats.*

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A relation between vitamin E and protein metabolism has been suggested in 3 reports in the literature. Cerecedo and Vinson[1] observed that a 58% litter incidence of weanling paralysis in mice was reduced to 28% by increasing the casein in the diet of the mothers.

Dam[2] noted that when rats were placed upon a low-protein diet, the group receiving α-tocopherol survived somewhat longer than the E-free controls. However, the *rate* of body weight loss was the same in the 2 groups, and the E-fed group had a lower

* Communication No. 106.

1 Cerecedo, L. R., and Vinson, L. J., *Fed. Proc.*, 1944, **3**, 55.

2 Dam, H., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 55.