THE INHIBITION OF GROWTH OF *ESCHERICHIA COLI* BY SOME DERIVATIVES OF UREA¹

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The antibacterial action of urea and several of its derivatives has been the subject of numerous investigations (Weinstein and McDonald, 1945; Weinstein, 1948). Whereas urea itself is inhibitory to growth at concentrations of several per cent, some of its alkyl carbamate derivatives show bacteriostatic powers in quantities of the order of tenths of a per cent. In general in this series of compounds, inhibitory activity increases with chain length (Weinstein, 1948), a behavior that parallels their ability to be bound by and to denature proteins (Klotz, Triwush, and Walker, 1948). It seems likely, therefore, that the antibacterial properties of these substances are due to their general denaturing effects. In view of this method of action, it is not surprising that relatively high concentrations of agent must be present before bacteriostatic activity is observed.

There are also some compounds related to urea, such as nitrourea and S-methylisothiurea, that possess the unique property of reacting with amine groups at room temperature and at pH's near neutrality. These substances might be expected to show antibacterial properties at relatively low concentrations, since they could interfere with enzyme systems in the microorganism, which require an intact amine group. The effects of these substances on the growth of *Escherichia coli* has therefore been examined.

EXPERIMENTAL METHODS

All of the data reported here have been obtained with a culture of *Escherichia coli* that came originally from the Northwestern University Medical School. The bacteria were grown overnight on nutrient agar (Difco) and were then suspended in the synthetic medium described by MacLeod (1940). Growth was followed by turbidity measurements in a Hellige-Diller photoelectric colorimeter.

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Nitrourea, $\text{O}_2\text{N}-\text{NH}-\text{C}-\text{NH}_2$, was prepared by Mr. Frank Tietze of this laboratory from urea nitrate by the method of Davis and Blanchard (1929) and converted to the sodium salt by the procedure of Thiele and Lachman (1895).

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S-methylisothiurea, $\text{CH}_3-\text{S}-\text{C}-\text{NH}_2$, as its sulfate salt, was generously supplied by Dr. Walter Nudenberg of the University of Chicago. All other urea compounds were commercial samples of high purity. Stock solutions of

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each of the urea derivatives were adjusted to a pH between 7 and 8 before they were added to the culture medium.

RESULTS AND DISCUSSION

Nitrourea has been found by Davis and Blanchard (1929) to react readily with a wide variety of organic amines at room temperature. In addition, in this laboratory, F. Tietze has found this compound to be an inhibitor of the succinic dehydrogenase enzyme system. It was not surprising, therefore, when it was found that nitrourea has a pronounced effect on the growth of *Escherichia coli*. The pertinent data are summarized in figure 1. It is apparent that significant inhibition of growth is obtained at concentrations as low as 10 mg per cent and that complete inhibition is maintained, throughout the 8-hour period of observation, at concentrations of 50 mg per cent.

Figure 1. Effect of nitrourea on growth of *Escherichia coli* at 37 C. Counts (turbidity measurements) are given in billions per ml.

For purposes of comparison, growth curves were also obtained with urea and with urethane, and the results for a single time interval are summarized in table 1. In the case of urea, even with concentrations as high as 500 mg per cent, only slight inhibition of growth was obtained. Similarly the effect of urethane at 500 mg per cent was small, although slightly greater than that of urea, as one might expect from its increased molecular weight. Thus, each of these substances exhibits an activity in the neighborhood of that exerted by 10 mg per cent of nitrourea; in other words, nitrourea is about 50 times more active than either urea or urethane.

S-methylisothiurea is also known to react under relatively mild conditions with amine groups in polypeptides (Christensen, 1945) and in proteins (Schutte, 1943). The action of this substance as its sulfate salt was examined also, again with *E. coli*. As the results in figure 2 illustrate, only minor inhibition of growth was obtained at a concentration of 50 mg per cent, but substantial antibacterial properties were exerted in 100 mg per cent solutions.

Since thiourea, $\text{H}_2\text{N}-\text{C}-\text{NH}_2$, might be formed from the S-methylisothi-ourea, particularly in the presence of metabolizing organisms, it seemed desirable to examine the effect of this simpler compound also. As the data in table 2

TABLE 1
Inhibition of growth of E. coli by urea, urethane, and nitrourea

SUBSTANCE	CONCENTRATION (MG %)	RELATIVE GROWTH (8 HR)
Control	—	(1.0)
Urea	5	0.94
	10	0.94
	25	0.96
	50	1.09
	100	1.00
	200	0.90
	300	0.83
	400	0.82
	500	0.86
Urethane	5	1.00
	10	0.88
	25	0.87
	50	0.88
	100	0.86
	200	0.87
	300	0.77
	400	0.73
	500	0.63
Nitrourea	10*	0.83
	25*	0.54
	50*	0.027
	100*	0.03

* The nitrourea was weighed out as its sodium salt. The concentrations of active component were 0.8 of the values listed in this column.

show, no significant inhibition of growth is obtained in solutions containing as high as 100 mg per cent thiourea, a concentration that produces a very substantial effect with the S-methyl derivative. Thus there can be little doubt that the action of the latter compound is not due to any thiourea that it may produce.

The relatively weaker activity of the S-methylisothiurea, as compared to

nitrourea, is perhaps to be expected. The former substance, which structurally may be thought of as a derivative of guanidine, is a fairly strong base, as its existence normally as a salt indicates. Consequently, at pH's near 7, the substance exists primarily as a cation. Since there is strong evidence, as cited by Herriott (1947), that the guanidinating action of isourea compounds is a prop-

Figure 2. Effect of thiourea and of S-methylisothiurea on growth of *Escherichia coli* at 37°C: ▽, 50 mg per cent, and △, 100 mg per cent of S-methylisothiurea; □, 100 mg per cent of thiourea; O, control. Counts (turbidity measurements) are given in billions per ml.

TABLE 2
Comparison of effects of thiourea and S-methylisothiurea on growth of *E. coli*

SUBSTANCE	CONCENTRATION (MG %)	RELATIVE GROWTH (8 HR)
Control	—	(1.0)
Thiourea	10	0.95
	25	1.08
	50	0.95
	100	0.98
S-methylisothiurea	50*	1.0
	100*	0.15

* The S-methylisothiurea was weighed out as its sulfate salt. The concentrations of active component were 0.65 of the values listed in this column.

erty of the free base, conditions favoring formation of the cation would reduce the chemical activity of the compound.

In either case, however, it is evident that urea derivatives that are capable of reacting chemically near room temperature are more effective growth inhibitors than nonreactive derivatives of nearly equal molecular weight.

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