# SECTION J, BIOCHEMISTRY

## Growth Stimulation of Escherichia coli by 2-Thiouracil

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2-Thiouracil has been extensively tested as a feed additive to rations prepared for domestic animals (Committee on Animal Nutrition, 1959). In some cases, it was found to promote growth and produce animals possessing superior market quality. Growth promotion by 2-thiouracil is usually attributed to a potent antithyroid activity (Pearson, et al., 1966). We have found that 2-thiouracil in the presence of uracil promotes the growth of *E. coli*. Since 2-thiouracil has been found in tRNA (transfer RNA) of *E. coli*, it seemed possible that growth stimulation resulted from a "cytokinin" effect (Skook, et al., 1966) produced by the incorporation of 2-thiouracil into tRNA. Our report offers evidence that most of the growth stimulation by 2-thiouracil in *E. coli* results from a donation of sulfur for the synthesis of sulfur-containing amino acids.

### MATERIALS AND METHODS

*E. coli* was grown at 37 C without aeration in minimal salt medium containing 14.0 g $K_2HPO_4$, 6.0 g $KH_2PO_4$, 2.0 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4$, and 10 g glucose in 1000 ml. U-*E. coli* (uracil-requiring *E. coli*) was grown in the minimal medium containing uracil ($4 \times 10^{-4}M$). Growth was followed by turbidity measurements using a Klett-Summerson photoelectric colorimeter. The exponential growth curve was approximated by freehand curve fitting and by the method of least squares. Lag period was defined as the time the approximated exponential growth curve would have a turbidity value of 20. Confidence intervals and the least-squares approximation of the growth curve were computed with an IBM 7040 computer. Biochemicals were purchased from Sigma Chemical Company, St. Louis, Mo.

### RESULTS

2-Thiouracil mixed with uracil stimulated the growth of U-*E. coli* (Fig. 1). Growth stimulation consisted of a decreased lag period and a longer period of growth which resulted in increased growth density. Growth rate in the exponential phase was not affected. The decreased lag phase produced by 2-thiouracil was a consistent finding. Lag phase was virtually eliminated in medium containing 2-thiouracil and uracil while it was 1 to 2 hr in duration for organisms grown in medium containing only uracil. 2-Thiouracil stimulated growth density by producing a stationary phase turbidity averaging about 15% higher than for organisms grown in medium containing uracil alone. Increasing the uracil concentration of the medium from $4.5 \times 10^{-4}M$ to $10 \times 10^{-4}M$ in these experiments did not change the results, nor did increasing 2-thiouracil concentration from $0.7 \times 10^{-4}M$ to $39 \times 10^{-4}M$. Uracil at $10^{-4}M$ and 2-thiouracil at $0.7 \times 10^{-4}M$ were approximately the lowest concentrations which would regularly produce a maximum stimulation.

Methionine, cystine and cysteine were tested for their ability to stimulate the growth of U-*E. coli* in minimal medium containing $4.5 \times 10^{-4}M$ uracil (Fig. 1). All of the amino acids stimulated growth but in a manner different from the stimulation produced by 2-thiouracil. Effects produced by cystine and cysteine were essentially the same and distinctly different from the effects produced by methionine. Mixtures of cystine and cysteine containing methionine or 2-thiouracil produced effects similar to those produced respectively by methionine or 2-thiouracil alone. Cystine or cysteine ($3 \times 10^{-4}M$) added to minimal medium containing

uracil ($4 \times 10^{-4}M$) reduced the lag period by about 50%, and stimulated growth density by approximately 15%, but did not increase growth rate. Methionine was almost as effective as 2-thiouracil in reducing lag phase, increased growth density by approximately 15% and increased the growth rate by approximately 7%.

Growth stimulation produced by mixtures of methionine and 2-thiouracil is shown in Fig. 2. Low concentrations ($0.7 \times 10^{-4}M$) of each component produced an additive effect in growth stimulation. The lag period was reduced as had been found with 2-thiouracil alone, and growth rate was stimulated approximately 7% as had been found with methionine alone. 2-Thiouracil ($6.3 \times 10^{-4}M$) and methionine ($5.4 \times 10^{-4}M$) mixed at higher concentrations inhibited growth by increasing the lag period but did not affect growth rate or growth density.

2-Thiouracil was compared with 6-methyl-2-thiouracil, 6-propyl-2-thiouracil and 1-methyl-2-mercaptoimidazole, which are also antithyroid compounds, for the ability to stimulate growth in wild *E. coli* (Fig. 3). 2-Thiouracil at high concentrations ($15.6 \times 10^{-4}M$) in the absence of uracil inhibited the growth of wild *E. coli*, while the other antithyroid compounds did not show this effect. Each of the antithyroid compounds (including 2-thiouracil when uracil was present) increased the growth density and reduced the lag period of *E. coli*. All of the compounds produced approximately the same increase in growth density but 2-thiouracil

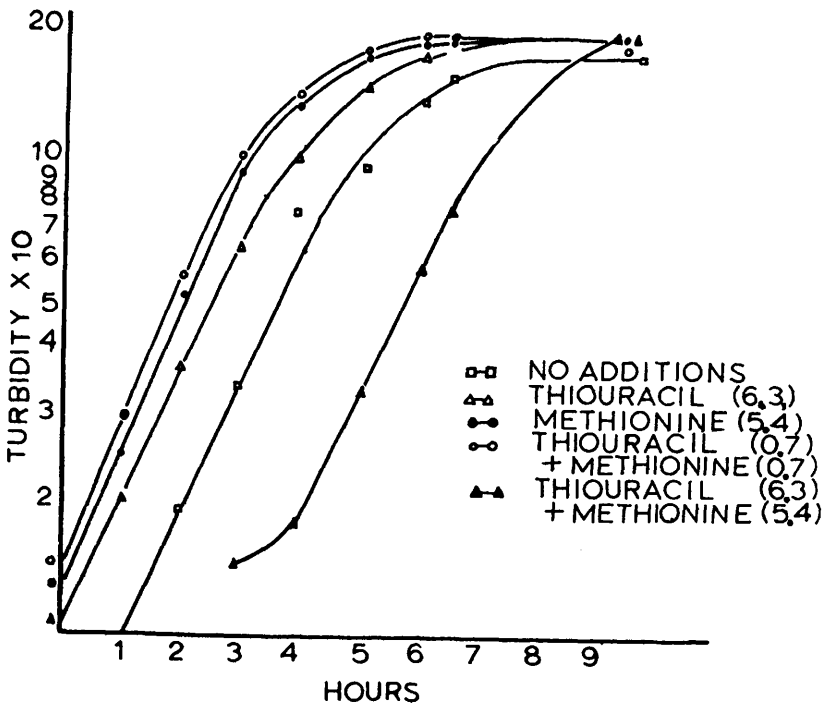


**Figure 1.** Growth stimulation of a uracil-requiring strain of *E. coli* by 2-thiouracil, uracil, uracil mixed with 2-thiouracil, methionine, cysteine and cystine in minimal salt medium. Concentrations are $M \times 10^{-4}$. The cultures were incubated at 37 C without aeration.

plus uracil consistently produced a slightly greater reduction in the lag period.

## Discussion

The detailed mechanism of growth stimulation by 2-thiouracil is not known although it has been generally assumed to result from antithyroid activity. Different results have been obtained when different animal species were used and when different antithyroid drugs were used. This variability of results suggests that the mechanism of growth stimulation is complicated. This report presents evidence for a growth stimulation by 2-thiouracil which is unrelated to thyroid hormone.

It is known that 2-thiouracil can be metabolized unchanged to nucleotides and incorporated into RNA (Brockman and Anderson, 1963), and it is known that 2-thiouracil can donate sulfur to sulfur-containing amino acids (Bauernfeind and Ledoux, 1963). Because the physiological effect of these metabolic events is not known, our experiments were designed to relate them to growth stimulation. Growth stimulation is an easily measurable physiological response. Cell size and cell viability were not changed to a detectable extent when *E. coli* was grown in minimal medium containing uracil in the presence of high concentrations of 2-thiouracil (Cardeilhac, 1967). High concentrations of 2-thiouracil in the absence of uracil inhibited growth in *E. coli* by inhibiting the production of L-ureidosuccinic acid. Production of L-ureidosuccinic acid appeared

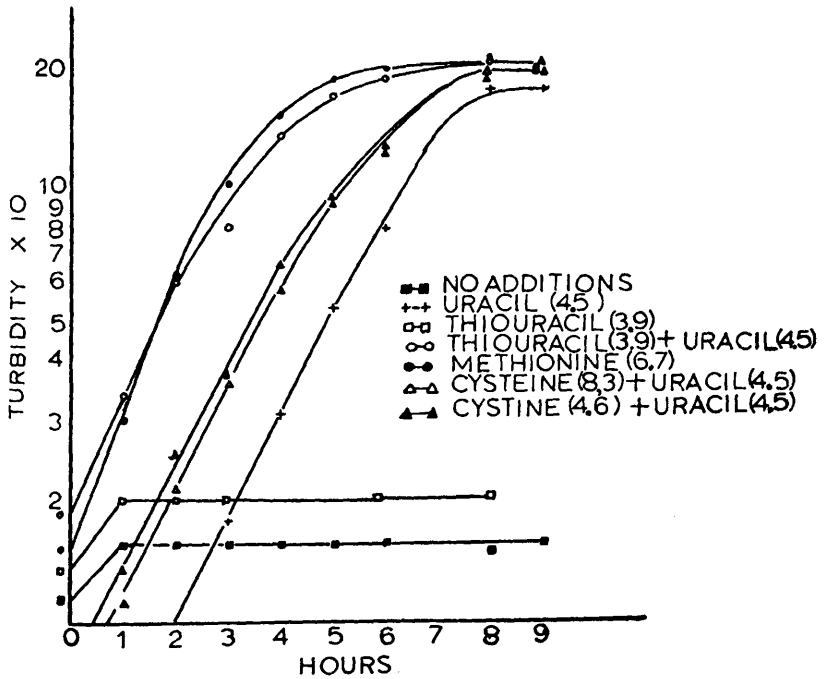


Figure 2. Growth stimulation of a uracil-requiring strain of *E. coli* by thiouracil, methionine and mixtures of thiouracil and methionine in minimal salt medium containing uracil. Concentrations are $M \times 10^{-4}$. The cultures were incubated at 37 C without aeration.

to result from an inhibition of aspartate transcarbamylase. The inhibition could be completely reversed by uracil, and it was shown that 2-thiouracil in the medium did not inhibit the utilization of uracil. For this reason most of these growth studies with 2-thiouracil were performed using U-*E. coli* in minimal medium containing uracil.

2-Thiouracil produced two easily detectable stimulatory effects on the growth of U-*E. coli:* it increased growth density and reduced the lag period. Each of the sulfur-containing amino acids stimulated an increase in growth density equal to the stimulation produced by 2-thiouracil. Each sulfur-containing amino acid would reduce the lag period but none were as effective as 2-thiouracil. From these results it was concluded that the stimulation of growth density by 2-thiouracil was probably produced by sulfur donation in the biosynthesis of sulfur-containing amino acids. Reduction of the lag period may not be produced entirely by sulfur donation.

An inspection of the structural formula of 2-thiouracil indicated that it could affect metabolic processes either by donating sulfur and producing uracil or by its conversion to a biologically active metabolite. Sulfur-containing amino acids were not found to reduce the lag period as effectively as 2-thiouracil. It was deduced that 2-thiouracil either donated sulfur to a sulfur-containing metabolite other than an amino acid, or the 2-thiouracil moiety was utilized unchanged in the biosynthesis of some essential metabolite. In order to test these two possibilities other antithyroid compounds were tested. These compounds were similar in their biological properties but the differences in their chemical structures varied. For example, 6-methyl-2-thiouracil is quite similar to 2-thiouracil but 1-methyl-2-mercaptoimidazole is not. It is unlikely that 1-methyl-2-mercaptoimidazole would replace 2-thiouracil as a constituent of tRNA where steric requirements are rigid, but it could replace 2-thiouracil as a nonspecific donor of a reactive thiol group.

The results in Fig. 3 show that stimulation of growth density was

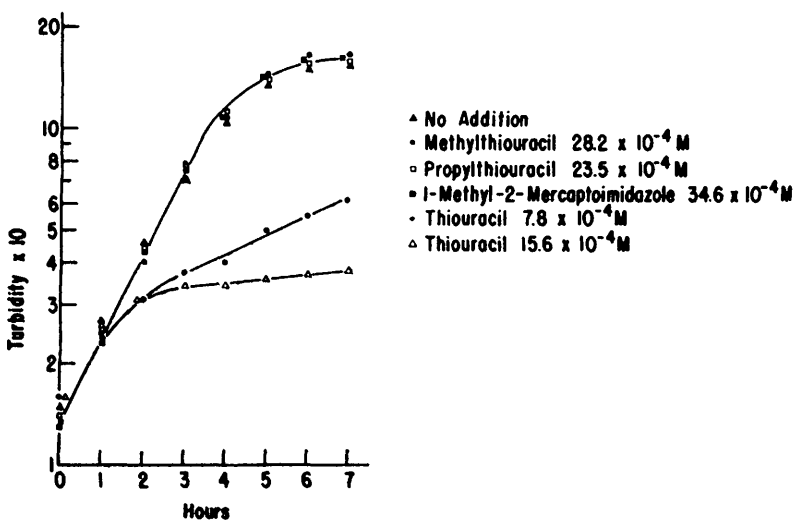


**Figure 3.** Growth stimulation of wild *E. coli* by 2-thiouracil, 6-methyl-2-thiouracil, 6-propyl-2-thiouracil and 1-methyl-2-mercaptoimidazole. The cultures were incubated at 37 C without aeration.

approximately equal for all the antithyroid compounds but that 2-thiouracil was slightly more effective in reducing the lag period. The possibility that 2-thiouracil enters the cell more readily than the other antithyroid compounds should not be excluded. However, the results suggest that 2-thiouracil may be more effective in reducing the lag period because in addition to the donation of active sulfur, it has another physiological activity. 2-Thiouracil in the reduced state has been found in tRNA and appears to be necessary for biological activity (Carbon, *et al.*, 1965). One may hypothesize that a portion of the lag period observed for *E. coli* grown in minimal medium resulted from the biosynthesis of a 2-thiouridine nucleotide component in certain tRNA molecules.

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