7. Robinson, R. R., Schmidt-Nielsen, B., ibid., 1963, v62, 147.

8. Schmidt-Nielsen, B., Shrauger, C. R., Am. J. Physiol., 1963, v205, 483.

9. Walker, A. M., Hudson, C. L., ibid., 1937, v118, 153.

10. Uranga, J., Sawyer, W. H., ibid., 1960, v198, 1287.

11. Fawcett, J. K., Scott, J. E., J. Clin. Pathol., 1960, v13, 156.

12. Rolf, D. A., Surtshin, A., White, H. L., Proc. Soc. Exp. Biol. & Med., 1949, v872, 351.

13. Shepherd, R. G., Howard, K. S., Bell, P. A., Caccioli, A. R., Child, R. G., Davies, M. C., English, J. P., Finn, B. M., Misenhelder, J. H., Mayer, A. W., Van Der Schen, J., J. Am. Chem. Soc., 1954, v78, 5051.

14. Appelboom, J. W., Brodsky, W. A., Tuttle, W. S., Diamond, I., J. Gen. Physiol., 1958, v41, 1153.

15. Brown, G. W., Jr., Cohen, P. P., J. Biol. Chem., 1959, v234, 1769.

16. Nazario, M., Córdoba, Argentina, Doctoral Thesis of Univ. of Córdoba, 1964, 17.

17. Archibald, R. M., J. Biol. Chem., 1944, v156, 121.

18. Mora, J., Tauab, R., Martuscelli, J., Soberon, G., Biochem. J., 1965, v96, 588.

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A Toxic Effect of 2-Thiouracil on Pyrimidine Metabolism.* (32182)

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2-Thiouracil, an antithyroid compound, is also an inhibitor in pathways of nucleic acid metabolism(1,2). It has been reported that the antithyroid effect results from an interference with the iodination of thyroxine precursors rather than as a result of any effect on nucleic acid metabolism(2,3,4).

The effect of 2-thiouracil on nucleic acid metabolism in animals seems to result in an inhibition of growth and development. This inhibition is suggested because the compound inhibits plant protein and RNA synthesis, has an antiviral effect against numerous plant and animal viruses, and interferes with the development of sea urchin eggs(5,6,7,8). The present study presents evidence for a precise effect of 2-thiouracil on nucleic acid metabolism detected by following growth response in an organism while nucleic acid metabolism was influenced by the presence of 2-thiouracil. *Escherichia coli* was chosen as the organism for study to eliminate thyroid effects. An *E. coli* auxotroph requiring uracil or orotic acid for growth was used to determine the effect of 2-thiouracil on utilization of uracil and orotic acid.

Materials and methods. E. coli cells were grown at 37° without aeration in a 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. Uracil (3.9×10^{-4} M) was added to the medium to maintain the uracil-requiring *E. coli* auxotroph. The two *E. coli* organisms used in these experiments were isolated as revertants from an *E. coli* auxotroph obtained from Dr. Norman Durham, Oklahoma State University. One organism required uracil or orotic acid for growth and was designated as the uracil-requiring (Ura^-) *E. coli* strain. The uracil requirement was then eliminated from the Ura^- strain, and the resulting organism requiring no uracil addition to the minimal salt medium for growth was designated as the non-uracil-requiring (Ura^+) *E. coli* strain. L-aspartic acid, DL-ureidosuccinic acid, and dihydro-L-orotic acid would not support the growth of the Ura^- strain. Biochemical compounds used in these studies were purchased from Sigma Chemical Co., St. Louis, Mo.

Growth of the organism was followed by

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increases in turbidity measured by means of a Klett-Summerson photo-electric colorimeter with a 540 mμ filter. Direct measurement with a stage micrometer of large numbers of the Ura+ strain grown in minimal salt medium and of the Ura− strain grown in the presence of uracil or 2-thiouracil showed no detectable difference in cell size. Viable cell counts of both strains were approximately equal for a given turbidity value even when a high concentration of 2-thiouracil was present in the medium. These growth properties show that viable cells of a uniform size were produced in the experiments.

Results. 2-Thiouracil additions to the medium inhibited growth density of the Ura+ strain in a linear fashion until the 2-thiouracil concentration reached 10^{-3} M. At this concentration growth of the organism was severely inhibited (70%-90%), and higher concentrations of 2-thiouracil produced no further inhibition. Growth which could not be inhibited (10%-30%) was approximately equal to the amount of growth of the Ura−

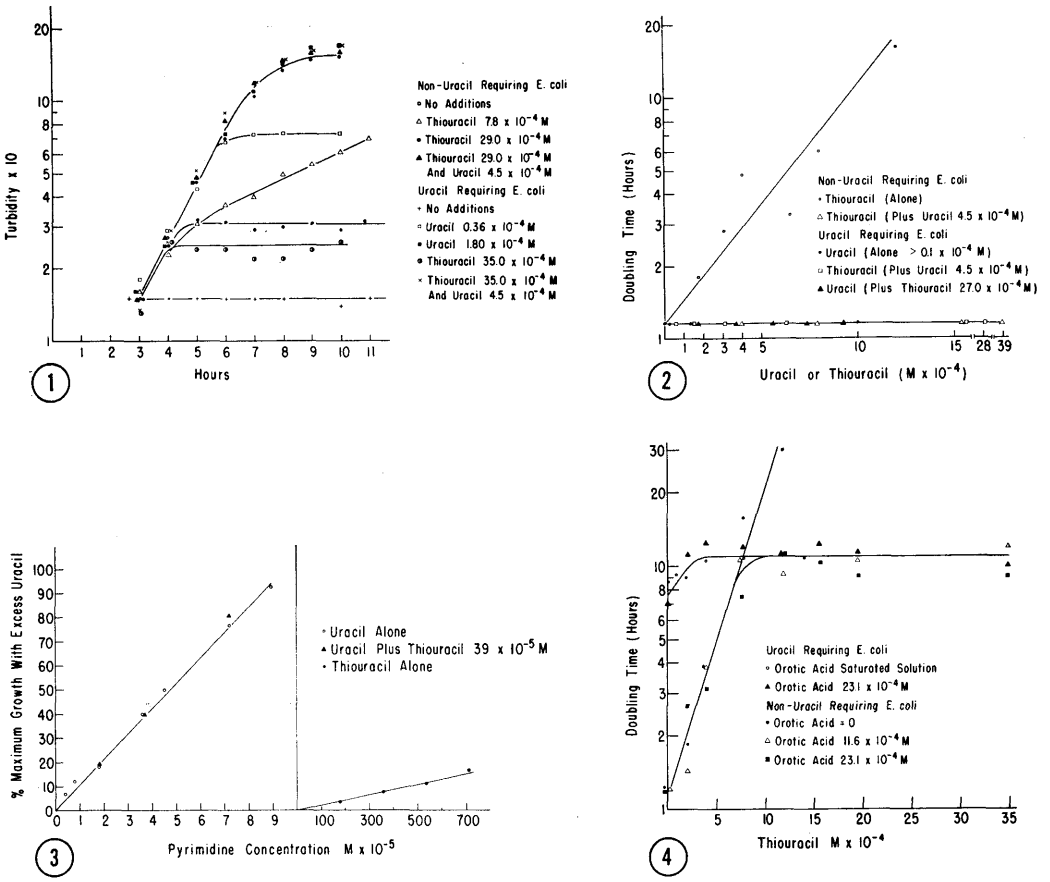

FIG. 1. Growth of Ura+ *E. coli* and Ura− *E. coli* at 37° without aeration in minimal salt medium containing uracil, 2-thiouracil, or uracil and 2-thiouracil mixtures.

FIG. 2. Effect of 2-thiouracil or 2-thiouracil mixed with uracil on the growth rate of Ura+ *E. coli* and the effect of uracil or uracil plus 2-thiouracil on the growth rate of Ura− *E. coli*. Growth rates for Ura− *E. coli* assume that sufficient uracil (0.9×10^{-4} M) was present to permit measurement of growth since at 0 concentration there is no growth. Incubations were carried out at 37° without aeration, and pyrimidines were added as indicated.

FIG. 3. Growth of Ura− *E. coli* at 37° without aeration supported by addition of uracil, 2-thiouracil, or uracil and 2-thiouracil mixtures to minimal salt medium.

FIG. 4. Reversal by orotic acid of 2-thiouracil inhibition of the growth of Ura+ *E. coli* and effect of 2-thiouracil on the growth of Ura− *E. coli* when orotic serves as the source of pyrimidine. Incubations were carried out in minimal salt medium at 37° without aeration, and additions were made as indicated.

TABLE I. Reversal of 2-Thiouracil Inhibition to Growth of *E. coli* by Precursors of Uracil in Pyrimidine Metabolism.

Precursor	Concentration, M × 10^{-4}	2-Thiouracil, 17.6 × 10^{-4} M	Total growth (turbidity)	Inhibition reversal
None (control)		—	177	
" "		+	44	
Orotic acid	35.9	—	199	
" "	35.9	+	167	++++
Dihydro-L-orotic acid	35.4	—	162	
" "	35.4	+	65	+
DL-Ureidosuccinic acid	31.8	—	179	
" "	31.8	+	140	+++
L-Aspartic acid	42.1	—	207	
" "	42.1	+	59	±

The organism was grown at 37° without aeration in minimal salt medium, as described in the text. The media were saturated at the indicated concentrations of orotic acid and dihydro-L-orotic acid.

strain which was supported by commercial 2-thiouracil (Fig. 1). This small amount of growth in both cases may be due to the ability of the organisms to convert some 2-thiouracil to uracil or to a minor contamination of the 2-thiouracil with uracil. A logarithmic slowing of growth rate or doubling time of the Ura+ strain resulted from linear increases in 2-thiouracil concentration (Fig. 2). The linear decrease in growth density and logarithmic decrease in growth rate resulted from a decreased growth rate with increased periods of growth in the presence of 2-thiouracil.

Growth of the Ura− strain supported by uracil or uracil mixed with 2-thiouracil is seen in Fig. 3. A linear response in growth density was obtained with additions of uracil or uracil mixed with 2-thiouracil. Ura− *E. coli* completely utilized growth-limiting quantities of uracil in the presence of 2-thiouracil at 10 times the uracil concentration (Fig. 2). It was concluded that 2-thiouracil does not inhibit the utilization of uracil. Since inhibition by 2-thiouracil can be completely reversed by uracil, 2-thiouracil must inhibit the synthesis of uracil in the Ura+ strain.

Other precursors of uracil in pathways of pyrimidine metabolism in *E. coli*(9) have the ability to reverse 2-thiouracil inhibition of growth of the Ura+ strain (Table I). Of the compounds examined orotic acid and DL-ureidosuccinic acid showed a marked ability to reverse 2-thiouracil inhibition.

After a 25% initial slowing of the growth rate, the addition of 2-thiouracil at concentrations up to 35 × 10^{-4} M did not further change the doubling time of the Ura− strain utilizing orotic acid (23.1 × 10^{-4} M) in the medium as the source of uracil (Fig. 4). 2-Thiouracil in the presence of orotic acid initially inhibited the growth rate of the Ura+ strain just as in the absence of orotic acid. However, the growth rate could not be inhibited beyond a rate approximately equal to the growth rate of the Ura− strain, utilizing orotic acid as the source of uracil (Fig. 4). The inhibition of Ura+ *E. coli* by 2-thiouracil in the presence of orotic acid represents an inhibition of the production of uracil by the cell. Inhibition of uracil production by 2-thiouracil in the presence of orotic acid forced the Ura+ strain to utilize orotic acid in the medium as the source of uracil. The high concentration of orotic acid required to stimulate the growth of the Ura− strain may indicate that *E. coli* has little or no ability to actively transport orotic acid into the cell. Lack of an active transport system and slow diffusion of the negatively charged orotic acid into the cell would explain the slow growth rate of the Ura− strain utilizing orotic acid as a source of uracil. Slow diffusion of orotic acid into the cell would also explain why orotic acid will only reverse 2-thiouracil inhibition of the Ura+ strain to a growth rate about equal to the growth rate of the Ura− strain utilizing orotic acid as the source of uracil.

The reversal by DL-ureidosuccinic acid of 2-thiouracil inhibition to the growth rate of the Ura+ strain is shown in Fig. 5. Loga-

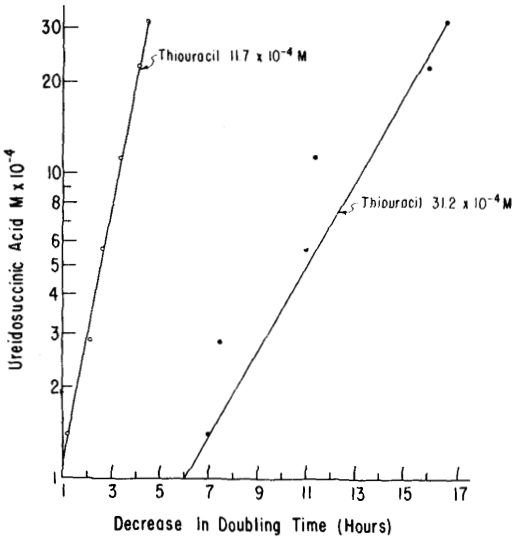

FIG. 5. Reversal of 2-thiouracil inhibition of the growth of Ura+ *E. coli* by DL-ureidosuccinic acid. Incubations were carried out in minimal salt medium at 37° without aeration, and additions were made as indicated.

rithmic increases in DL-ureidosuccinic acid were required to produce a linear increase in growth rate (a decrease in doubling time). Slopes of the lines in Fig. 5 represent the rates at which DL-ureidosuccinic acid reversed 2-thiouracil inhibition. The slopes are proportional to 2-thiouracil concentration, and by tripling the 2-thiouracil concentration the rate of reversal by DL-ureidosuccinic acid was also approximately tripled. This is opposite to the effect expected if 2-thiouracil were inhibiting the utilization of L-ureidosuccinic acid. It was concluded that 2-thiouracil inhibits the formation of L-ureidosuccinic acid or a precursor of L-ureidosuccinic acid.

L-ureidosuccinic acid is formed by the condensation of L-aspartic acid and carbamyl phosphate catalyzed by aspartate transcarbamylase(9). As seen in Table I, L-aspartic acid had only a slight ability to reverse 2-thiouracil inhibition of growth. Carbamyl phosphate was not tested because it is a labile compound.

Discussion. The logarithmic decreases in growth rate produced by linear increases in 2-thiouracil concentration would be expected if an enzyme were inhibited from catalyzing the formation of a metabolite essential for cell growth. Evidence that L-ureidosuccinic acid is the metabolite was found when logarithmic increases in DL-ureidosuccinic acid concentration in the medium produced a linear reversal of 2-thiouracil inhibition to growth rate. Production of L-ureidosuccinic acid or a precursor of L-ureidosuccinic acid was considered to be the major site for inhibition by 2-thiouracil of the growth of the Ura+ strain.

Precursors of L-ureidosuccinic acid are intermediates in metabolic pathways other than pyrimidine metabolism. L-aspartic acid is required in protein synthesis, and carbamyl phosphate is required for production of arginine and ornithine. One enzyme is reported to catalyze the production of all carbamyl phosphate in the cell(10). If 2-thiouracil severely inhibits carbamyl phosphate production, uracil would not be expected to completely reverse 2-thiouracil inhibition since arginine production would also be inhibited. High concentrations of L-aspartic acid in the medium did not reverse 2-thiouracil inhibition, and it is concluded that production of L-aspartic acid is not inhibited by 2-thiouracil. Dihydro-L-orotic acid would not reverse even a partial inhibition by 2-thiouracil of the growth of *E. coli*. This result has not been explained since L-ureidosuccinic acid is reported to be a precursor of dihydro-L-orotic acid(9). It is possible that the commercial dihydro-L-orotic acid was not an authentic sample or that dihydro-L-orotic acid did not penetrate the cell. It is also possible that free dihydro-L-orotic acid could not be utilized by the cell.

An evaluation of the results indicates that 2-thiouracil or a metabolite of 2-thiouracil inhibits the formation of L-ureidosuccinic acid by inhibiting aspartate transcarbamylase.

Summary. Inhibition of nucleic acid metabolism by 2-thiouracil was investigated using *Escherichia coli* and an auxotroph which required uracil or orotic acid for growth. Linear increases of 2-thiouracil concentration in minimal salt medium produced logarithmic decreases in the growth rate of *Escherichia coli*. Uracil reversed the inhibition, and neither growth rate nor growth density was reduced by high concentrations of 2-thiouracil in the presence of the minimum amount of uracil required for growth of the uracil-requiring

Escherichia coli. 2-Thiouracil did not inhibit the utilization of uracil and produced only partial inhibition (25%) of the utilization of orotic acid.

Dihydro-L-orotic acid, DL-ureidosuccinic acid, and L-aspartic acid were tested for ability to reverse 2-thiouracil inhibition. Only DL-ureidosuccinic acid clearly reversed this inhibition. An increase in 2-thiouracil concentration did not decrease the ability of DL-ureidosuccinic acid to reverse growth inhibition, indicating that 2-thiouracil does not inhibit L-ureidosuccinic acid utilization, but inhibits its formation or the formation of precursors. L-ureidosuccinic acid precursors are necessary for protein synthesis; thus aspartate transcarbamylase which catalyzes the formation of L-ureidosuccinic acid is considered to be the specific site of inhibition.

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1. Cardeilhac, P. T., Fed. Proc., 1967, v26, 540.
2. Lindsay, R. H., Nakagawa, H., Cohen, P. P., Endocrinology, 1965, v76, 728.
3. Maloof, F., Soodak, M., Pharmacol. Rev., 1963, v15, 43.
4. Wang, Shih-Chen, Lui, Hsiu-Ming, Yu, Ching-Hau, Fan, Chen-Fu, Shih Yen Sheng Wu Hsueh Pao., 1964, v9, 185. (Chem. Abstr., 1965, v62, 10760h).
5. Tupy, J., Biol. Plant., 1966, v8, 398.
6. Ralph, R. K., Matthews, R. E. F., Matus, A. I., Biochim. Biophys. Acta, 1965, v108, 53.
7. Amos, H., Vollmayer, E., Virology, 1955, v6, 337.
8. Tinacci, F., Boll. Soc. Ital. Biol. Sper., 1947, v23, 572.
9. Beckwith, J. R., Pardee, A. B., Austrian, R., Jacob, F., J. Mol. Biol., 1962, v5, 618.
10. Piérard, A., Science, 1966, v154, 1572.

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Renal Compensation During Mild Water Diuresis and its Inhibition by Vagotomy.* (32183)

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Vagal afferent nerve fibers are implicated in the reflex renal regulation of fluid volume, possibly by controlling the rate of secretion of antidiuretic hormone (ADH)(1). The inhibition of water diuresis(2) and increased blood antidiuretic activity(3) promoted by vagotomy support this concept. However, the increased water and salt excretion resulting from rapid expansion of extracellular fluid volume is not completely abolished(4,5) and mannitol diuresis is unaffected(6) by vagal section. If, indeed, ADH is released after vagotomy, this procedure would not be expected to inhibit a diuresis accompanied by significant natriuresis, since the hormone's activity on water reabsorption is inversely related to the magnitude of solute excretion (7). If fluid volume could be expanded in a manner which enhances only free-water excretion, then procedures which increase circulating ADH should conceivably inhibit the renal compensation. Functional exclusion of one kidney during mild water diuresis has been found to satisfy this condition. The characteristics of the renal response to this procedure and the effects of vagotomy thereon are presented here.

Methods. Mongrel dogs of both sexes (13.6-19.5 kg; mean body weight ± SE: 15.9 ± 0.5 kg), deprived of food for 18 hours, were anesthetized with pentobarbital sodium (30 mg/kg, with supplementation as needed). After a priming intravenous injection of creatinine and para-aminohippurate (PAH), warm 5% glucose, containing these substances, was infused continuously by pump at the rate of 2.5 ml/min. The ureters, exposed through a low mid-ventral incision, were cannulated with polyethylene tubing. A loose cupper clamp(8) was placed around the left renal artery, exposed retroperitoneally with minimal clearing of overlying tissue;

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