Hill, S. R., Jr., Roche, M., Staehelin, D., and Wilson, D. L., *New Eng. J. Med.*, 1950, v242, 783, 824, 865.

3. Elkington, J. R., Hunt, A. D. Jr., Godfrey, L., McCrory, W. W., Rogerson, A. G., and Stokes, J. Jr., *J.A.M.A.*, 1949, v141, 1273.

4. Sprague, R. G., Power, M. H., Mason, H. L., Albert, A., Mathieson, D. R., Hench, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F., *Arch. Int. Med.*, 1950, v85, 199.

5. Sayers, G., Burns, T. W., Tyler, F. H., Jager, B. V., Schwartz, B., Smith, E. L., Samuels, L. T., and Davenport, H. W., *J. Clin. Endocrin.*, 1949, v9, 503.

6. Conn, J. W., and Louis, L. H., *J. Clin. Endocrin.*, 1950, v10, 12.

7. Wallace, W. M., *Am. J. Dis. Child.*, 1949, v77, 104.

8. Wallace, W. M., Holliday, M., Cushman, M., and Elkington, J. R., *J. Lab. Clin. Med.*, 1951, v37, 621.

9. Hald, P. M., in Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Williams and Wilkins, Baltimore, 1932, v2, 838.

10. Danowski, T. S., and Gilmore, G., *J. Lab. Clin. Med.*, 1950, v35, 67.

11. Berger, E. Y., and Steele, J. M., *J. Clin. Invest.*, 1952, v31, 451.

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Factors Related to Growth of Psittacosis Virus (Strain 6BC) III. Uracil and Related Compounds.* (19906)

HERBERT R. MORGAN. (With the technical assistance of John P. Bader.)

From the Department of Bacteriology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Previous observations(1-3) indicate that pteroylglutamic acid and or citrovorum factor are required for the intracellular proliferation of psittacosis virus and that certain purines (adenine, guanine) and other compounds are important for the intracellular multiplication of psittacosis virus (6BC). Since these various substances are concerned with the intracellular synthesis of nucleic acids, of which desoxyribonucleic acid (DNA) has been shown to be a prominent constituent of the psittacosis group of viruses(4), it was of interest to see if evidence could be obtained that the pyrimidine uracil, a material substituent of ribonucleic acid (RNA), is related to the intracellular proliferation of psittacosis virus.

Methods. Tissue cultures were prepared by planting whole minced tissues of 9- to 11-day-old chick embryos on perforated cellophane discs(5) in the bottoms of 10 ml Erlenmeyer flasks(2). Two ml of nutrient solution composed of 2 volumes of Hanks's(6) balanced solution and one volume of ox serum ultrafiltrate, to which virus was added, were then placed into each flask. The psittacosis virus (6BC) had been cultivated in the yolk sacs of embryonated eggs for a long period of time. Tissue cultures were incubated at 36°C. After 24 hours the infected nutrient fluid was withdrawn and replaced with 2 ml of fresh nutrient solution to which one of the various compounds had been added. The compound was added to the fluids for 2 successive changes after which normal nutrient solution was employed. Fluid changes were carried out every 4 days and the fluid removed was promptly tested for its virus content by injection into embryonated eggs. A single dilution of the virus-containing fluid from each tissue culture was prepared in broth and 0.25 ml of the material was injected into the yolk sacs of 12 or more 7-day-old embryonated eggs. Eggs were candled daily, deaths recorded, and the average day of death calculated. The virus titer was determined from this value by the method described(7).

To evaluate the possible toxic action of the test compounds on chick embryo tissues, 8

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fragments of heart tissue from 12- to 14-day-old embryos were planted in the plasma clot lining the walls of roller tubes which were then incubated for 4 days at 36°C while revolving in a roller-tube apparatus. Daily examinations were made to note the capacity of the fragments to pulsate and the outgrowth of fibroblasts at the periphery of the explants.

Results. Of the compounds tested, thiouracil† produced significant suppression of virus growth as shown in the growth curve (Fig. 1). This inhibition was demonstrable at a concentration (0.5 mg/ml) which produced no obvious toxic effects on the chick embryonic heart tissue as measured by contractility and fibroblastic proliferation. 6-Methylthiouracil at the tolerated concentration of 0.1 mg/ml produced slight inhibition of virus growth (Table I). The other compounds, 6-methyluracil, diazouracil, and uracil had no significant influence on virus growth at the concentrations used.

Discussion. Previous studies(1-3) have suggested that the synthesis of DNA is an important event in the multiplication of psittacosis virus (6BC) as would be anticipated, since these viruses contain significant amounts of this substance. These earlier experiments gave direct evidence that interference with intracellular metabolism of the purines adenine and guanine would result in the suppression of virus growth. The data presented here indicate that thiouracil blocks some aspect of uracil metabolism which is normally essential for the formation of virus. Experiments with tobacco mosaic virus(8) indicate that this may be due to interference with the incorporation of uracil into the virus particle. The demonstration that interference with the intracellular metabolism of its substituent uracil suppresses virus growth suggests that the synthesis of RNA is involved in the growth of psittacosis virus unless the uracil has some other role unrelated to RNA.

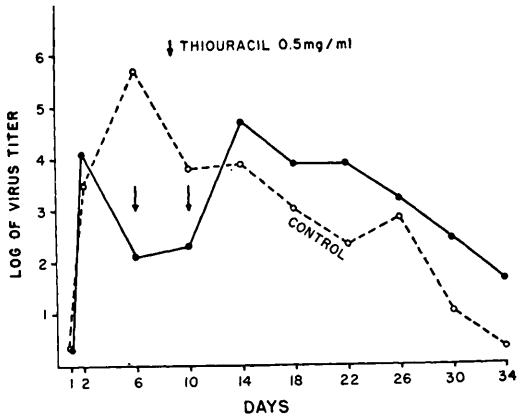

FIG. 1. Inhibition of growth of psittacosis virus (6BC) in tissue culture by thiouracil.

† Provided through the courtesy of Dr. J. M. Ruegsegger, Lederle Laboratories Division, American Cyanamid Co.

TABLE I. Effect of Various Uracil Derivatives on Growth of Psittacosis Virus (6BC) in Tissue Culture.

Compound	Conc., mg/ml	Virus inhibition	Tissue toxicity
Thiouracil	.5	+++	0
6-Methylthiouracil	.5	NT	+++
	.1	+	0
6-Methyluracil	1	+	±
	.5	±	0
Diazouracil	.5	0	0
Uracil	.5	0	0

NT = Not tested.

It is important to note in the experiments here reported that the suppression of virus growth in the tissue cultures was exhibited under conditions which gave no evidence of serious toxic effects on the host tissue cells since fibroblastic growth occurred and fragments of heart tissue continued to pulsate, indicating that their energy metabolism was not seriously impaired.

Summary. Thiouracil inhibited the growth of psittacosis virus (6BC) in tissue cultures at concentrations which had no obvious toxic effects on the host tissues. In tolerated amounts 6-methylthiouracil, 6-methyluracil, diazouracil, and uracil had no effect on virus growth. These experiments suggest that some aspect of the intracellular metabolism of uracil is essential for the multiplication of psittacosis virus (6BC).

1. Morgan, H. R., *J. Exp. Med.*, 1948, v88, 285.
2. ———, *J. Exp. Med.*, 1952, v95, 269.
3. ———, *J. Exp. Med.*, 1952, v95, 277.
4. Crocker, T. T., *Fed. Proc.*, 1952, v11, 464.
5. Evans, V. J., and Earle, W. R., *J. Nat. Cancer*

Inst., 1947, v8, 103.
6. Hanks, J. H., and Wallace, R. E., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 196.
7. Golub, O. J., *J. Immunol.*, 1948, v59, 71.
8. Commoner, B., and Mercer, F., *Nature*, 1951, v168, 113.

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Metabolic Demethylation of 3,5,5-Trimethyl-2,4-Oxazolidinedione (Trimethadione, Tridione).* (19907)

THOMAS C. BUTLER, DARIEN MAHAFFEE, AND COLLINS MAHAFFEE.
From the Department of Pharmacology, University of North Carolina, Chapel Hill, N. C.

Methyl groups are now known to be removed metabolically from nitrogen in a wide variety of chemical types of N-methyl compounds. As some of these compounds find extensive use as drugs and as the metabolic products are in some cases themselves pharmacologically active, the reaction of demethylation has important practical implications in therapeutics. From analogy with the closely related derivatives of barbituric acid and hydantoin, it could be expected that the antiepileptic drug, trimethadione, would undergo demethylation in the mammalian body. The product of the reaction would be 5,5-dimethyl-2,4-oxazolidinedione (henceforth to be designated "DMO"). The present report concerns the isolation of this substance from the urine of dogs receiving trimethadione.

Materials. Samples of DMO were supplied through the kindness of Dr. Roger W. Stoughton of Mallinckrodt Chemical Works and Dr. M. A. Spielman of Abbott Laboratories. The compound melts at 76-78°C. The ionic form absorbs strongly in the ultraviolet, the absorption peak lying at wavelengths below 210 mμ. The molar extinction coefficient is 1.1×10^4 at 215 mμ and diminishes sharply with increasing wavelength. In this spectral region the absorption of the undissociated molecule in solution in negligible. At 37°C and at an ionic strength of 0.1 the pK′ of DMO as calculated from spectrophotometric data is 6.2.†

Trimethadione was isolated from commercial capsules and recrystallized from water and from petroleum ether. It melted at 44-46°C. At wavelengths longer than 215 mμ the absorption of trimethadione in aqueous solution is negligible in comparison with that of the ionic form of DMO. A test was made for the possibility of contamination with significant amounts of DMO by examination of the ultraviolet absorption of a buffer of pH 8 shaken with an ether solution of trimethadione. In this way it was demonstrated that the sample of trimethadione could not contain as much as 0.2 per cent DMO.

Administration of drugs. Both dogs of Table I were females. The drugs were given in aqueous solution intraperitoneally, the total dose being divided into two portions, which were administered several hours apart. The solution of trimethadione contained 5 g and that of DMO 10 g per 100 ml. With each dog the trimethadione experiment preceded the DMO experiment by at least 3 weeks.

Isolation of DMO from urine. Urine was collected from the dogs for seven days after the drug injection. The urine was acidified to pH about 2 and extracted with ethyl ether. A batch of urine would be divided into four portions, which would be successively shaken with two portions of ether, each five times the volume of each portion of urine. The ether extract was distilled to a volume of 100 ml and was then extracted with four 25 ml portions of a saturated aqueous solution of $NaHCO_3$. The bicarbonate extract was acidified with hydrochloric acid to pH about 2 and shaken with two 50 ml portions of benzene, which were discarded. The aqueous phase was then

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† The pK′ as determined by potentiometric titration has been reported by Erlenmeyer *et al.*(1) as 6.1.