16611

Some Microbiological Properties of Synthetic Nucleosides.*

KARL DITTMER, IRVING GOODMAN, DONALD VISSER,† AND HESTER P. MCNULTY.
From the Department of Chemistry, University of Colorado, Boulder, Colo.

Increasing interest in the roles of pyrimidines, purines, nucleosides, and other nucleic acid derivatives in the living cell has directed the attention of many investigators to various biological systems which utilize these compounds. Through X-ray irradiation, Beadle and co-workers[1] obtained mutants of *Neurospora crassa* which required added uracil or uridine for growth. Likewise, mutants of *Escherichia coli* which require uracil for normal growth have been developed and studied.[2]

The *L. casei* factor (pteroylglutamic acid) requirements for the growth of *Lactobacillus casei, Streptococcus faecalis R,* and certain other microorganisms can be fulfilled by thymine.[3]

Because specific metabolite antagonists have been useful in determining some of the biological functions of metabolites as well as changing the growth characteristics of living cells, we synthesized a number of derivatives of uracil[4] and thymine[5] as possible antagonists of these metabolites. The possibility that these derivatives might enhance the activity of the corresponding natural metabolite was equally interesting.

Emerson and co-workers[6] found that a specific antagonist of riboflavin was obtained when the ribityl group was replaced by a dulcityl group. This suggested the possibility of producing an antagonist of uridine by substituting galactose or other sugars for ribose of the nucleoside molecule.

Kuhn and co-workers[7] reported the 6,7-dichloro derivative of riboflavin as a specific antagonist of riboflavin. Hitchings, Falco and Sherwood[8] found that 5-chlorouracil and 5-bromouracil were antagonists of thymine. In both of these instances the substitution of a chlorine atom for a methyl group produced an antagonist. Since Loring and Pierce[9] found natural uridine from 10 to 60 times more effective than uracil as a growth promotor for a uracil-less mutant of *Neurospora,* it seemed possible that the ribosyl and other glycosyl derivatives of 5-chloro- and 5-bromouracil might be more effective thymine antagonists than 5-chlorouracil or 5-bromouracil.

To test these various possibilities, the ribosyl, arabinosyl, glucosyl, and galactosyl nucleosides of uracil[4] and thymine[5] were synthesized, as well as the corresponding 5-chloro- and 5-bromouracil derivatives. Although synthetic 1-D-ribosyluracil is chemically similar to naturally occurring uridine it has different physical properties.[10] Recent work of Todd and co-workers[11] suggests that,

* This work was started with the aid of a University of Colorado Research Grant and continued in part by a research contract with the Office of Naval Research.

† Present address: Department of Biochemistry and Nutrition, University of Southern California School of Medicine, Los Angeles, California.

1 Tatum, E. E., and Beadle, G. W., *Growth—4th growth symposium,* 1942, **6**, 27.

2 Lampen, J. O., and Roepke, R. R., Unpublished data. See Roepke, R. R., Libby, R. L., and Smith, M. H., *J. Bact.,* 1944, **48**, 401.

3 Stokes, J. L., *J. Bact.,* 1944, **48**, 201.

4 Visser, D., Dittmer, K., and Goodman, I., *J. Biol. Chem.,* 1947, **171**, 377.

5 Visser, D., Goodman, I., and Dittmer, K., *J. Am. Chem. Soc.,* 1948, **70**, 1926.

6 Emerson, G. A., Wurtz, E., and Johnson, O. H., *J. Biol. Chem.,* 1945, **160**, 165.

7 Kuhn, R., Weygand, F., and Möller, E. F., *Ber.,* 1943, **76**, 1044.

8 Hitchings, G. H., Falco, E. A., and Sherwood, M. B., *Science,* 1945, **102**, 251.

9 Loring, H. S., and Pierce, J. G., *J. Biol. Chem.,* 1944, **153**, 61.

10 Hilbert, G. E., and Johnson, T. B., *J. Am. Chem. Soc.,* 1930, **52**, 4489.

11 Davoll, J., Lythgoe, B., and Todd, A. R., *J. Chem. Soc.,* 1946, 833.

although natural and synthetic nucleosides are beta-glycosides, the sugars differ in their ring structures. For these reasons the synthetic nucleosides might be expected to show microbiological properties different from uridine and from the uracil and thymine derivatives.

Results obtained in testing these synthetic nucleosides as possible metabolite antagonists for various microorganisms are reported in this paper.

Tests with Escherichia coli. Two strains of *E. coli* were used: a uracil-requiring mutant strain (550-460)‡ and a strain (American Type Culture Collection No. 9723) which synthesizes its own uracil. Good growth of the uracil mutant was obtained when uracil (0.005 to 0.04 mg per 7.5 ml) or natural uridine§ (0.01 to 0.1 mg per 7.5 ml) were added to the medium described by MacLeod.[12] The tests with this mutant were carried out in the presence of 0.015 or 0.02 mg of uracil per 7.5 ml of medium. These levels of uracil permitted about 50% of normal growth. When the uracil and thymine derivatives were tested in concentrations up to 1 mg per 7.5 ml of medium, only 5-chlorouracil was slightly stimulatory, while none of the following showed any growth-promoting or growth-inhibiting properties: 1-D-ribosyluracil, 1-D-arabinosyluracil, 1-D-glucosyluracil, 1-D-galactosyluracil, 1-D-ribosyl-5-chlorouracil, 1-D-arabinosyl-5-chlorouracil, 1-D-glucosyl-5-chlorouracil, 1-D-galactosyl-5-chlorouracil, 1-D-ribosyl-5-bromouracil, 1-D-arabinosyl-5-bromouracil, 1-D-glucosyl-5-bromouracil, 1-D-galactosyl-5-bromouracilthymine, 1-D-ribosylthymine, 1-D-arabinosylthymine, 1-D glucosylthymine, and 1-D-galactosylthymine. None of these compounds, when tested in concentrations up to 20 mg per 7.5 ml of medium, inhibited the growth of the American Type Culture Collection No. 9723 strain of *E. coli.*

Tests with Neurospora crassa.‖ The wild strain of *Neurospora crassa, Abbott 4A* was grown in the medium described by Horowitz and Beadle.[13] With the uracil-less mutant, *Neurospora crassa, 1298,* a typical growth curve was obtained when either uracil or natural uridine[9] was added to the medium. To obtain high enough concentrations without using too much of the various compounds, all tests with *Neurospora* were carried out in 10 ml of medium. In this amount of medium maximum growth was equivalent to 20 to 30 mg of dried mycelia. All tests with the uracil-less mutant were carried out in the presence of 0.1 mg uridine in 10 ml of medium. Of all the compounds listed in the previous section, when tested in concentrations up to 0.8 mg per 10 ml of medium, only 5-chlorouracil slightly stimulated the growth of the uracil-less mutant. None of the compounds in concentrations up to 20 mg per 10 ml inhibited the growth of either the wild or uracil-less strains of *Neurospora.*

Tests with Lactobacillus casei. All tests with *L. casei* (American Type Culture Collection, No. 7469) were carried out in the medium and according to the procedure described by Luckey, Briggs, and Elvehjem.[14] In the absence of added pteroylglutamic acid the growth reached a level of 20 turbidity units, whereas the addition of 0.010 mg of thymine permitted maximum growth equivalent to 130 turbidity units. The following compounds consistently stimulated the growth of *L. casei*: (The percentage values given in the parentheses indicate the relative molar activity as compared with thymine taken as 100%): 1-D-ribosylthymine (1.0%), 1-D-glucosylthymine (1.4%), 1-D-galactosylthymine (0.5%), and 1-D-arabinosyl-5-chlorouracil (0.3%). In the presence of 0.002 mg of thymine per 10 ml medium the follow-

‡ The cultures of *E. coli,* American Type Culture Collection No. 9723 and the uracil mutant, *E. coli,* (550-460), were obtained through the courtesy of Dr. J. O. Lampen of the American Cyanamid Company of Stamford, Conn.

§ The authors are indebted to Dr. Hubert Loring of Stanford University for a generous sample of natural uridine.

12 MacLeod, C. M., *J. Exp. Med.,* 1940, **72**, 217.

‖ The authors wish to thank Dr. Herschel Mitchell and Dr. Hubert Loring for kindly supplying cultures of *Neurospora.*

13 Horowitz, N. H., and Beadle, G. W., *J. Biol. Chem.,* 1943, **150**, 325.

14 Luckey, T. D., Briggs, G. M., Jr., and Elvehjem, C. A., *J. Biol. Chem.,* 1944, **152**, 157.

ing compounds were slightly stimulatory, whereas in the absence of thymine they were inactive: 1-D-glucosyl-5-chlorouracil, 1-D-galactosyl-5-chlorouracil, 1-D-arabinosyl-5-bromouracil, and 1-D-glucosyl-5-bromouracil. The following compounds were completely inactive when tested up to concentrations of 1.0 mg per 10 ml of medium: 1-D-arabinosylthymine, uracil, 5-chlorouracil, natural uridine, 1-D-ribosyluracil, 1-D-arabinosyluracil, 1-D-glucosyluracil, 1-D-galactosyluracil, 1-D-ribosyl-5-chlorouracil, I-D-ribosyl-5-bromouracil, and 1-D-galactosyl-5-bromouracil. At concentrations of 1.0 mg per 10 ml of medium none of the compounds showed any growth inhibitory properties, except uracil and 5-chlorouracil[5] which inhibited the growth in the presence of 0.002 mg of thymine. At concentrations of 20 mg per 10 ml of medium some of these synthetic nucleosides were slightly inhibitory.

Tests with Streptococcus faecalis R. The tests with *Strep. faecalis R*[†] (American Type Culture Collection, No. 8043) were made in the medium and according to the procedure referred to above.[14] The results obtained were almost identical with those obtained with *L. casei.* The compounds which showed some stimulation in the absence of thymine were: 1-D-ribosylthymine (2.2%), 1-D-galactosylthymine (0.7%), and 1-D-glucosylthymine (1.1%). The following compounds were slightly stimulatory in the presence of 0.002 mg of thymine per 10 ml of medium but were inactive alone: 1-D-ribosyl-5-chlorouracil, 1-D-arabinosyl-5-chlorouracil, 1-D-glucosyl-5-chlorouracil, 1-D-galactosyl-5-chlorouracil, 1-D-ribosyl-5-bromouracil, 1-D-arabinosyl-5-bromouracil, 1-D-glucosyl-5-bromouracil, and 1-D-galactosyl-5-bromouracil. None of the other compounds showed any growth-promoting activity.

At a concentration of 0.020 mg per 10 ml of medium 5-chlorouracil inhibited the growth to 100% in the presence of 0.002 mg of thymine. None of the other compounds up to a concentration of 1.0 mg per 10 ml showed any growth-inhibitory properties. However, when the concentration of these compounds was greatly increased, some of them were toxic. Galactosyluracil and 5-chlorogalactosyluracil, at levels of 10 and 5 mg, respectively, inhibited the growth to 50% of that obtained with 0.002 mg of thymine.

Summary. A number of synthetic nucleosides, and chloro- and bromo- derivatives of these nucleosides, were tested for growth-promoting and growth-inhibiting properties on *Escherichia coli,* strain 9723, and its uracil-requiring mutant, *E. coli,* strain 550-460; *Neurospora crassa,* wild strain Abbott 4A, and a uracil-less strain of *Neurospora crassa,* No. 1298; and on the growth of *Lactobacillus casei* and *Streptococcus faecalis R.*

The compounds tested were the 1-D-ribosyl-, 1-D-arabinosyl-, 1-D-glucosyl-, and 1-D-galactosyl- nucleosides of uracil, thymine, 5-chlorouracil and of 5-bromouracil. Of these 16 compounds, only a few at high concentrations showed inhibitory properties on the growth of the microorganisms tested. Some showed slight, but insignificant, growth-stimulatory activity.

The authors wish to show their appreciation to Miss Ann Coblenz, Miss Janet Tozer, Miss Evelyn Wyatt, and Mrs. Charmion McMillan for carrying out some of the microbiological tests.

[†] The authors wish to thank Dr. C. A. Elvehjem for a culture of *Strep. faecalis R.*