INTERRELATIONSHIPS BETWEEN RIBOSE AND DESOXYRIBOSE COMPONENTS OF NUCLEIC ACIDS

HELEN R. SKEGGS, HELGA M. NEPPEL, JOHN SPIZIZEN, AND LEMUEL D. WRIGHT

Medical Research Division, Sharp and Dohme, Inc., Glenolden, Pennsylvania

Received for publication September 21, 1950

Lactobacillus bifidus (Lactobacillus acidophilus ATCC 4963) was previously reported (Skeggs et al., 1949) to require for growth, in an otherwise complete medium, either vitamin B12, thymine desoxyriboside, or intact desoxyribonucleic acid (DNA). The growth of L. bifidus in the presence of DNA was found (Skeggs et al., 1950) to be inhibited competitively by yeast ribonucleic acid (RNA). Subsequent investigations (Skeggs, Wright, et al., 1950) revealed that the ribose nucleotides, adenylic acid and guanylic acid, effectively replaced yeast RNA in preventing utilization of DNA by L. bifidus. Highly purified preparations of adenosine-3-phosphoric acid and adenosine-5-phosphoric acid were equally inhibitory. The isomeric adenylic acid described by Carter (1950) had little or no inhibitory activity. When vitamin B12 replaced DNA, inhibition with RNA or guanylic acid was not reproducible, but adenylic acid was somewhat inhibitory although less effective than it was in the presence of DNA.

Through the courtesy of Dr. Waldo Cohn, samples of thymidylic acid, desoxyadenylic acid, desoxyctydyllic acid, and desoxyguanylic acid (VoIkin et al., 1951) were made available to us. Hypoxanthine and thymine desoxyribosides were provided through the courtesy of Dr. J. O. Lampen. All the desoxyribonucleotides and the available desoxyribosides proved capable of replacing DNA or vitamin B12 in the nutrition of L. bifidus. The effect of RNA and the ribose nucleotides on the growth of L. bifidus in the presence of these compounds is the subject of this communication.

EXPERIMENTAL PROCEDURE

The basal medium employed was described some time ago for the assay of "animal protein factor" (since identified as vitamin B12) with Lactobacillus leichmannii (Skeggs et al., 1948), except that in the present experiments the tryptic digest of casein was omitted. The omission of the tryptic digest of casein resulted in greater reproducibility of the inhibitory effects observed with RNA and the purine nucleotides and made possible the demonstration of some inhibition by the pyrimidine nucleotides in the presence of DNA.

The usual microbiological assay procedures were employed. L. bifidus was carried by daily transfer in skim milk (Difco) containing 1 per cent Difco tryptic

1 Through the courtesy of Drs. J. Baecher and F. W. Allen (J. Biol. Chem., 183, 641, 1950) a sample of pentose nucleic acid isolated from pancreas was made available and was found to replace yeast RNA in inhibiting the utilization of DNA.

2 Generously supplied to us by Dr. Charles E. Carter.

3 Generously supplied to us by Dr. Henry Lardy.
tose, with a return to stock culture (carried in the same medium) at monthly intervals. Tests were conducted in 10-ml volumes (5 ml double-strength medium and 5 ml test solution) in 20-by-150-mm acid-cleaned test tubes. Sterilization was at 121 C for 15 minutes. The inoculum was prepared by suspending 0.1 ml of a 24-hour milk culture in 10 ml sterile physiological saline. Tests were incubated at 37 C for 72 hours. Acid production, measured by titration with 0.1 N NaOH with bromthymol blue as an indicator, was used as the index of growth.

Tests were observed carefully at 24 and 48 hours so that transient inhibitory effects, which were apparent only during early growth, could be recorded.

The ribose nucleotide preparations were all obtained commercially from either the Schwarz Laboratories or the Nutritional Biochemicals Corporation. Paper strip chromatography in the tertiary butyl alcohol system described by Smith and Markham (1950) revealed no gross contamination of uridylic or cytidylic acids. The guanylic acid and adenyl acid preparations, when developed in the isoamyl alcohol and KH$_2$PO$_4$ solvent system described by Carter (1950), showed no gross contamination with other nucleotides.
RESULTS AND DISCUSSION

The growth response of *L. bifidus* to the various desoxyribose compounds and vitamin B12 is shown in figure 1. Although, when expressed on a weight basis, the desoxyribosides and desoxyribonucleotides appear to be more effective than DNA, *L. bifidus* responds to equimolar concentrations of DNA and its compo-

![Figure 1](http://jb.asm.org/)

*Figure 2.* Effect of ribose nucleotides and RNA on utilization by *L. bifidus* of desoxyribose compounds and vitamin B12. The broken lines at the top of each curve represent the acid production obtained with the indicated desoxyribose compound in the absence of the ribose compounds, and those at the bottom represent the blank tubes. The ribose compounds are identified as follows: squares—ribonucleic acid; triangles—adenylic acid; crosses—guanylic acid; open circles—uridylic acid; closed circles—cytidylic acid.

nent desoxyribosides and desoxyribonucleotides. An explanation for the differences observed in the shape of the curves and the extent to which the organisms grow on the various compounds is not at once apparent.

The effects of RNA and the ribose nucleotides on the growth of *L. bifidus* in the presence of the various desoxyribose compounds and vitamin B12 are
shown in figure 2. Studies with the ribose nucleosides, ATP, and purines and pyrimidines in addition to those contained in the basal medium were not conducted since previous studies with these compounds in the presence of vitamin B12 or DNA had shown them to be neither stimulatory nor inhibitory.

Inhibition of growth by RNA is pronounced in the presence of DNA and the desoxyribonucleotides but not in the presence of the available desoxyribosides or vitamin B12. Inhibition with uridylic acid is most pronounced in the presence of desoxycytidyllic acid. Marked inhibition by cytidylic acid occurs only in the presence of the pyrimidine desoxyribonucleotides. Adenylic acid and, to a lesser extent, guanylic acid inhibit growth markedly in the presence of DNA and the desoxyribonucleotides. Inhibition by the ribose nucleotides is much less evident in the presence of the available nucleosides of thymine and hypoxanthine. When vitamin B12 is present, partial inhibition of growth with adenylic acid can be observed at 24 hours, but the extent of the growth retardation in no way compares with that observed in the presence of the desoxyribonucleotides or DNA, where, at 24 hours, tubes containing adenylic acid or guanylic acid are completely blank.

In view of the fact that inhibition by the ribose nucleotides as well as by RNA is well defined in the presence of the phosphorylated desoxyribose compounds, DNA and the nucleotides, but not in the presence of the dephosphorylated desoxyribosides, it may be postulated that the ribose nucleotides interfere with a phosphate transfer mechanism. It would follow that L. bifidus is able to utilize DNA and the desoxyribonucleotides only by converting them to the desoxyribose. Ribonucleic acid and its component acids, adenylic, guanylic, cytidylic, and uridylic, in that order of activity, may interfere with growth in the presence of the foregoing compounds by competing for a nucleotide phosphatase.

SUMMARY

In addition to DNA, thymidine, or vitamin B12, *Lactobacillus bifidus* is able to utilize for growth desoxyadenyllic acid, desoxycytidylic acid, desoxyguanylic acid, thymidylic acid, or hypoxanthine desoxyriboside.

Utilization of DNA or the desoxyribonucleotides is inhibited by adenylic acid and ribonucleic acid, and to a lesser degree by guanylic acid, cytidylic acid, and uridylic acid.

Utilization of the desoxyribo-sides of hypoxanthine and thymine is not markedly inhibited by the ribose compounds.

Competition between the desoxyribose and ribose nucleotides for a nucleotide phosphatase is offered as a possible explanation for the observed inhibitory effects.

REFERENCES


Volkin, E., Khy, J. X., and Cohn, W. E. 1951 To be published.