INTERRELATIONSHIPS BETWEEN RIBOSE AND DESOXYRIBOSE COMPONENTS OF NUCLEIC ACIDS

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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 B_{12}, thymine desoxyriboside, or intact desoxyribonucleic acid (DNA). The growth of *L. bifidus* in the presence of DNA was found (Skeggs *et al.*, 1950)[1] to be inhibited competitively by yeast ribonucleic acid (RNA). Subsequent investigations (Skeggs, Wright, *et al.*, 1950) revealed that the purine 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[2] and adenosine-5-phosphoric acid[3] were equally inhibitory. The isomeric adenylic acid described by Carter (1950)[2] had little or no inhibitory activity. When vitamin B_{12} 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, desoxycytidylic acid, and desoxyguanylic acid (Volkin *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 B_{12} 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 B_{12}) 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 tryp-

[1] Through the courtesy of Drs. J. Bacher 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.

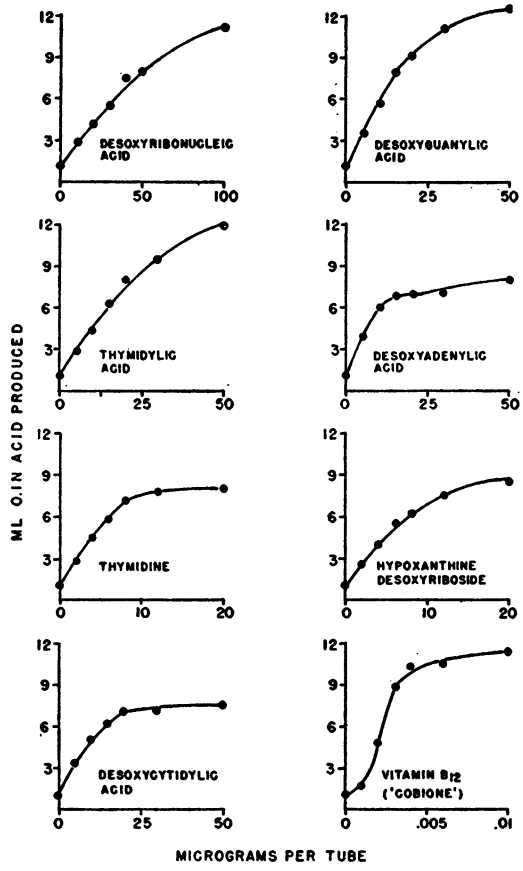

Figure 1. Response of *L. bifidus* to desoxyribose compounds and vitamin B_{12}.

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 adenylic acid preparations, when developed in the isoamyl alcohol and KH_2PO_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 B_{12} 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-

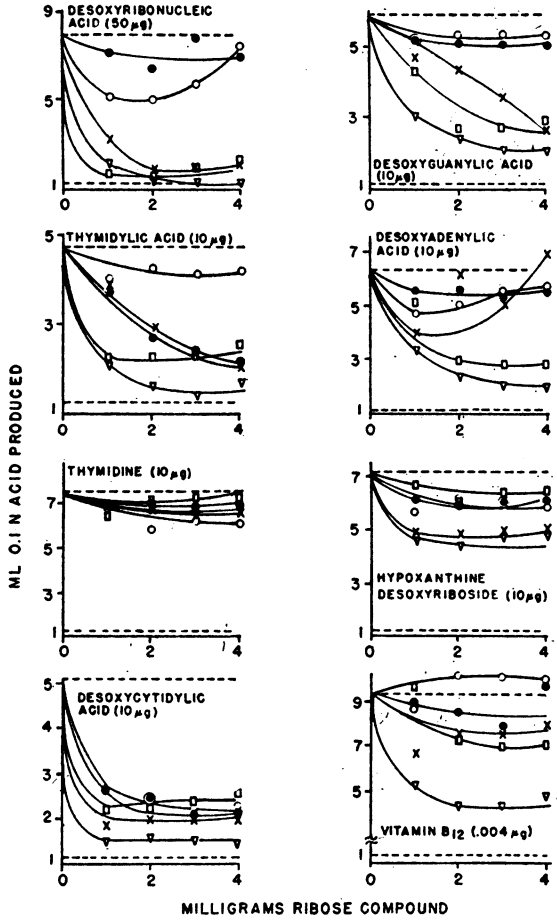

Figure 2. Effect of ribose nucleotides and RNA on utilization by *L. bifidus* of desoxyribose compounds and vitamin B_{12}. The broken lines at the top of each curve represent the acid production obtained with the indicated desoxyribose compound in the absence of the ribose compounds, and those at the 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 B_{12} 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 B_{12} 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 B_{12}. Inhibition with uridylic acid is most pronounced in the presence of desoxycytidylic 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 B_{12} 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 desoxyribosides. 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 B_{12}, *Lactobacillus bifidus* is able to utilize for growth desoxyadenylic 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 desoxyribosides 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.

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