# Studies on Deoxyribosidic Growth Requirement of *Lactobacillus acidophilus* R-26. (23092)

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(Introduced by A. J. Dalton)
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Recent independent studies of acid soluble extracts of liver(1,2) and thymus(3) have demonstrated the presence of different types of deoxyribose compounds in these tissues. In liver, the nucleoside, deoxycytidine, was isolated and shown to account for an average of 92% of the total deoxyribosidic compounds present, while 5′ mono-, di-, and triphosphates of deoxycytidine and thymidine were isolated from thymus. The relative proportions of the latter compounds have not been established. Since in the study of liver, deoxyribosidic compounds were determined by growth of *L. acidophilus* R-26, the results were contingent upon specificity of the deoxynucleosidic requirement of this organism. To resolve these differences, the ability of the organism to utilize di- and triphosphates of pyrimidine deoxynucleosides was tested. The results indicate that the organism was unable to grow in the presence of these compounds.

*Materials and methods.*† Ability of various deoxyribose containing compounds to support growth of the organism, *L. acidophilus* R-26, was determined essentially as described previously(1,4) using a modified medium suggested by Dr. E. Hoff-Jorgensen (personal communication). Pure thymidine served as standard growth supplement for this organism. Ability of other compounds to support growth was expressed in terms of micromoles of thymidine. The highest concentration at which compounds with low activity were tested, however, was usually limited to 100 times the concentration of pure thymidine required to produce growth. Compounds tested for activity included the following: commercial calcium TMP‡ and barium 3′, 5′-TDP,‡ TDP, TTP, D-CDP, and D-CTP isolated from thymus(2) or synthesized(5) using modifications of the method of Hall and Khorana(6), and 3 samples of TMP isolated from enzymic or acid hydrolysates of TDP or TTP. Concentration of these compounds was calculated from absorption of neutral solutions at 260 mμ using the constant 8400 for thymidine containing solutions and 7400 for deoxycytidine containing solutions(7). Deoxyribose content of solutions was determined by the cysteine-sulfuric acid reaction(8). In our experience, the results with this method can vary as much as ±

TABLE I. Deoxyribose Content and Microbiological Activity of Deoxynucleotides.

| Compound | Method of sterilization* | Deoxyribose content† | Microbiological activity‡ Before hydrolysis | Microbiological activity‡ After hydrolysis§ |
|---|---|---|---|---|
| TTP | A | .81 | .13 | .69 |
| " | F | | .011 | .69 |
| D-CTP | F | .86 | <.005 | .95 |
| D-CDP | F | .87 | .052 | .84 |
| TDP | F | .78 | .48 | .67 |
| 3′, 5′-TDP | A | | .14 | |
| " | F | | .055 | |
| TMP¶ | A | | 1.02 | |
| " ¶ | A | .88 | .94 | |
| " ** | A | 1.02 | .88 | |
| " †† | A | .68 | .83 | |

* A = autoclaving; F = filtration.
† Micromoles/micromole nucleotide.
‡ Expressed as micromoles thymidine/micromole nucleotide.
§ Hydrolysis was for 10 min. at 100° in 1 N HCl.
Commercial sample; 100 ± 3% pure.
¶ Isolated from apyrase hydrolysate of thymus TTP.
** Isolated from apyrase hydrolysate of thymus TDP.
†† Isolated from acid soluble fraction of thymus.

* Supported by Atomic Energy Commission contract.

† The following abbreviations will be used: TMP, TDP, and TTP, the 5′ mono-, di-, and triphosphates of thymidine; 3′,5′-TDP, thymidine 3′,5′ diphosphate; D-CDP and D-CTP, the 5′ di- and triphosphates of deoxycytidine; DNA, deoxyribonucleic acid.

‡ Obtained from California Foundation for Biochemical Research, Inc.

TABLE II. Deoxyribose and Phosphate Content and Microbiological Activity of Natural and Synthetic Deoxynucleotides.

| Compound* | Deoxyribose content† | After sterilization by filtration | | After hydrolysis in 1 N HCl | |
|---|---|---|---|---|---|
| | | Thymidine‡ | Inorganic phosphorus§ | Thymidine‡ | Inorganic phosphorus§ |
| TDP-I | .93 | .11 | .14 | .81 | .99 |
| " -S | .96 | .04 | .03 | .79 | .96 |
| D-CDP-I | .84 | .15 | .17 | .77 | .89 |
| " -S | 1.01 | .02 | .03 | .81 | .88 |
| TTP-I | .98 | <.01 | .061 | .79 | 1.95 |
| " -S | .97 | " | .099 | .83 | 1.98 |
| D-CTP-I | .80 | " | .088 | .82 | 1.77 |
| " -S | 1.00 | " | .079 | .94 | 1.90 |
| TMP | | .95 | | .77 | |

* I = isolated from thymus; S = synthetic. † See Table I. ‡ Micromoles/micromole nucleotide as measured microbiologically. § Micromoles/micromole nucleotide as measured by method of Soyenkoff(9).

10%. Phosphorus was determined colorimetrically by the method of Soyenkoff(9).

*Results.* Hoff-Jorgensen(4) demonstrated previously that the organism *L. acidophilus* R-26 would produce the same amount of growth when supplemented with equimolar amounts of any of the 5 known deoxynucleosides. On the basis of an experiment in which amount of growth obtained with a deoxyribonuclease digest of DNA was the same as its deoxynucleotide content, he also concluded that deoxynucleotides were utilized to the same extent by the organism as deoxynucleosides. Although the correctness of this conclusion was placed in doubt by the experiments of Sinsheimer(10) which showed that deoxyribonuclease digests of DNA contained only 1% of their nucleotide content as simple mononucleotides, any of the pure natural deoxynucleotides gave the same growth of the organism on a molar basis as the deoxynucleosides(1) (see also Tables I and II).§

In contrast to the ability of deoxynucleosides and deoxynucleotides to permit growth of *L. acidophilus* R-26, a sample of TTP isolated from thymus permitted only 13% as much growth as thymidine on the basis of nucleotide content (Table I). Since the compound had been autoclaved with the medium in this experiment, and since triphosphate solutions are known to be unstable at elevated temperatures, the TTP solution was sterilized by filtration‖ and added to the autoclaved medium. Under these conditions, the compound exhibited only 1.1% of the activity anticipated (Table I). A solution of sample of D-CTP had even less activity while D-CDP and commercial 3′, 5′-TDP had about 5% of the activity expected from their nucleotide content when sterilized by filtration. On the other hand, a sample of TDP isolated from thymus had 48% of the growth activity expected after sterilization by filtration. The latter result was entirely unexpected since the other 4 di- or triphosphates gave much lower activity when tested under these conditions, and it was suspected that the thymus TDP had undergone extensive breakdown before it was tested.

To determine whether phosphorylated deoxynucleotides isolated from thymus had the potential to promote growth of *L. acidophilus* R-26, they were hydrolyzed in 1 N HCl to remove the labile phosphate groups. After this treatment, the compounds gave 67-95% of the activity expected from their nucleotide content (Table I). These values were in rough agreement with the deoxyribose content determined colorimetrically (Table I).

§ In preliminary experiments with Dr. R. L. Sinsheimer, 2 dinucleotides isolated from a deoxyribonuclease digest of DNA gave the same growth as if the nucleotides they contained were completely utilized. These results and also results of tests of other polynucleotide components of deoxyribonuclease digests of DNA will be reported later.

‖ Sterilization was carried out with ultra fine fritted glass filter (Corning No. 33992).

Although results of the experiments reported in Table I strongly suggested that deoxynucleoside di- and triphosphates were unable to support growth of the organism, the high activity observed with the TDP sample and the slight activity of the other 2 diphosphates left some doubt as to whether these compounds might possess some intrinsic ability to promote growth of the organism. In a second series, new samples of deoxynucleoside di- and triphosphates were tested. These included samples purified from thymus extracts as well as synthetic preparations. The results, Table II, show that diphosphates isolated from thymus had activities of 11 and 15% while corresponding synthetic samples had activities of only 4 and 2%, respectively. Both natural and synthetic samples of the deoxynucleoside triphosphates were inactive when tested at concentrations sufficient to detect activity at the 1% level.

The variable activities of the deoxynucleoside diphosphates (Tables I and II) could best be explained on the assumption that the different samples had decomposed in varying degree to inorganic phosphate and the corresponding deoxynucleotides, which would, of course, be fully utilized by the organism. That this was the case was strongly indicated by the close correlation between the inorganic phosphate content of the diphosphate samples and their ability to promote growth of the organism (Table II). Decomposition would be expected to be a less serious problem with the triphosphate samples, as far as growth promoting activity was concerned, since 2 phosphate groups would have to be lost before microbiological activity could occur. The fact that the triphosphates (Table II) had less than 1% of the anticipated activity although their inorganic phosphate contents varied from 6.1 to 9.9% indicated that decomposition had proceeded only to the diphosphate stage.

After hydrolysis in acid to remove the labile phosphate groups, the natural and synthetic phosphorylated deoxynucleotide samples exhibited high activity, ranging from 77-94% of that expected from their nucleotide contents (Table II). In this series of experiments the effect of acid hydrolysis on microbiological activity of TMP was also tested. A 20% reduction in the ability of this compound to support growth occurred. This loss in activity may account in part for the discrepancies between microbiological activities of the hydrolyzed compounds and their deoxyribose contents.

*Discussion.* Although our results clearly show that pyrimidine deoxynucleoside polyphosphates cannot satisfy the deoxyribosidic growth requirement of the organism, *L. acidophilus* R-26, it should be possible to convert these compounds enzymatically to deoxynucleosides or deoxynucleotides so that the organism could be used to determine these polyphosphates in biological material.

Additional experiments will be required to determine whether failure of the organism to utilize deoxynucleoside polyphosphates was due to impermeability of the bacterial cell wall or to inability of the organism to convert the compounds to a usable form.

*Summary.* Our experiments indicate that *L. acidophilus* R-26 could not use either 3', 5'-TDP, TDP, TTP, D-CDP, or D-CTP for growth, although the compounds were fully utilized after hydrolysis. It was concluded that the organism could not be used directly to detect deoxynucleoside polyphosphates in tissue extracts.

1. Schneider, W. C., *J. Biol. Chem.*, 1955, v216, 287.

2. Schneider, W. C., and Brownell, L. W., *J. Nat. Cancer Inst.*, 1957, v16, 000.

3. Potter, R. L., and Schlesinger, S., *J. Am. Chem. Soc.*, 1955, v77, 6714.

4. Hoff-Jorgensen, E., *Biochem. J.*, 1952, v50, 400.

5. Potter, R. L., Schlesinger, S., Buettner-Janusch, V., and Thompson, L., *J. Biol. Chem.*, in press.

6. Hall, R. H., and Khorana, H. G., *J. Am. Chem. Soc.*, 1954, v76, 5056.

7. Beaven, G. H., Holiday, E. R., and Johnson, E. A., in Chargaff, E., and Davidson, J. N., *The Nucleic Acids*, 1955, v1, 493, Academic Press, New York.

8. Brody, S., *Acta Chem. Scand.*, 1953, v7, 502.

9. Soyenkoff, B. C., *J. Biol. Chem.*, 1952, v198, 221.

10. Sinsheimer, R. L., *idem.*, 1954, v208, 445.

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