were repeatedly evaporated with water on the steam-bath to constant weight to remove all traces of polymeric formaldehyde, they became somewhat colored, their specific rotation decreased to as little as $-15°$ and crystallization could no longer be induced.

Summary

The condensation of nitromethane with D-erythrose and with 2,4-benzylidene-D-erythrose has been studied. The latter yielded the corresponding benzylidene nitroalcohols in the crystalline state in a combined yield of 64%. D-*erythro*-Triacetoxy-1-nitropentene-1, prepared from either of the two nitroalcohols, or directly from D-erythrose without the isolation of intermediates, was converted to D-*erythro*-2-desoxypentose, the sugar occurring naturally in certain nucleic acids.

SAINT LOUIS, MISSOURI RECEIVED JULY 15, 1949

[CONTRIBUTION FROM THE DEPARTMENTS OF NUTRITION AND VIROLOGY, MEDICAL RESEARCH DIVISION, SHARP AND DOHME, INC.]

Competitive Antagonism of Ribonucleic and Desoxyribonucleic Acids in the Nutrition of *Lactobacillus bifidus*[1]

BY HELEN R. SKEGGS, JOHN SPIZIZEN AND LEMUEL D. WRIGHT

Recent studies with lactobacilli have demonstrated instances of a relationship between their nutritive requirements and desoxyribonucleic acid or certain of its constituents. Thymine desoxyriboside was reported by Shive, *et al.*,[1a] to replace vitamin B_{12} in the nutrition of *Lactobacillus lactis*. Skeggs, *et al.*,[2] reported similar findings for *Lactobacillus leichmannii*. Further studies by Kitay, *et al.*,[3] indicated that thymidine is not specific in its ability to substitute for essential factors in the nutrition of various lactobacilli. *Lactobacillus bifidus*[1a] (ATCC 4963) has been found[4] to be capable of utilizing either vitamin B_{12} or thymidine for growth in an otherwise complete medium. However, *Lactobacillus bifidus* also has been found to respond to intact desoxyribonucleic acid in the absence of either vitamin B_{12} or thymidine. While the growth factor requirements of this organism are not specific, it was felt that it might prove to be a useful instrument in a search for a compound that would inhibit utilization of desoxyribonucleic acid (DNA).

Experimental and Results

The basal medium employed is shown in Table I. *Lactobacillus bifidus* was carried in skim milk medium (Difco) containing 1% Bacto tryptose. Inocula were prepared by suspending 0.1 ml. of a twenty-four-hour culture in 10 ml. of sterile physiological saline. Each tube received one drop of this suspension as seed. All tests were carried out in 10-ml. volumes, of which 5 ml. was the double-strength medium. Tests were autoclaved at 120° for fifteen minutes prior to aseptic addition of samples and seeding, then incubated at 37° for from twenty-four to seventy-two hours. Results were obtained at twenty-four hours by measuring the turbidity produced with a Klett–Summerson photoelectric colorimeter or at seventy-two hours by titration of acid production with 0.1 N sodium hydroxide and brom thymol blue as indicator.

TABLE I

COMPOSITION OF DOUBLE STRENGTH BASAL MEDIUM

Casein hydrolysate[a]	1.0 g.
Tryptophan	20 mg.
Cystine	20 mg.
Adenine	1 mg.
Guanine	1 mg.
Xanthine	1 mg.
Uracil	1 mg.
Salts A[b]	1 ml.
Salts B[b]	1 ml.
Na acetate (anhyd.)	1.2 g.
Glucose	4 g.
Biotin	1 γ
Pyridoxine	400 γ
Pyridoxal	400 γ
Riboflavin	200 γ
Nicotinic acid	200 γ
Pantothenic acid	200 γ
Thiamine	200 γ
Folic acid	100 γ
p-Aminobenzoic acid	100 γ
Tween 80	0.2 ml.
Distilled water to 100 ml.	

[a] Univ. Texas Pub. No. **4137**, 82, 1941. [b] E. E. Snell and L. D. Wright, *J. Biol. Chem.*, **139**, 675 (1941).

Lactobacillus bifidus responded to DNA over a range of from 5 to 50 micrograms per tube as shown in Fig. 1. Increased amounts of DNA allowed no further growth. The response of the organism to DNA was the same whether the DNA was autoclaved in the test medium or added aseptically prior to seeding of the test. Various com-

(1) This organism has been reclassified as *Lactobacillus acidophilus* and is now designated as such by the American Type Culture collection. However, other strains of *L. acidophilus* do not, in our hands, exhibit the characteristics described here for culture 4963.

(1a) W. Shive, R. E. Eakin, W. M. Harding, J. M. Ravel and J. E. Sutherland, THIS JOURNAL, **70**, 2299 (1948).

(2) H. R. Skeggs, J. W. Huff, L. D. Wright and D. K. Bosshardt, *J. Biol. Chem.*, **176**, 1459 (1948).

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(4) H. R. Skeggs, J. Spizizen and L. D. Wright, "The Use of *Lactobacillus bifidus* in the Study of Antagonists of Desoxyribonucleic Acid," Abstract 3rd Meeting-in-Miniature, Philadelphia, p. 27, 1949.

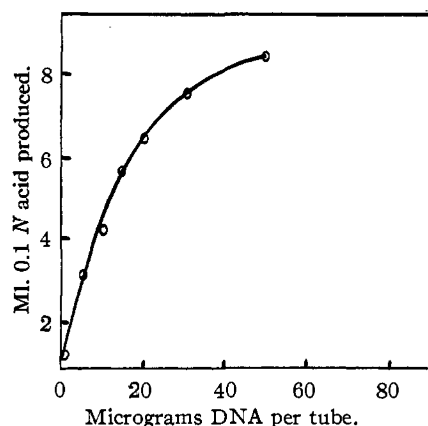

Fig. 1.—Response of *Lactobacillus bifidus* to desoxyribonucleic acid.

mercial samples of DNA showed comparable activity when tested by the chemical[5] and microbiological methods.

Lactobacillus bifidus proved to be more sensitive to DNA as an essential nutrient than to vitamin B_{12}. The organism would not survive repeated transfer in the basal medium containing vitamin B_{12}, but it remained viable and sensitive when transferred serially in basal medium supplemented with DNA.

In testing compounds for their ability to inhibit the utilization of DNA by *L. bifidus*, all of the compounds to be tested were filtered through ultra-fine sintered-glass filters and added aseptically to previously sterilized tubes containing 10 micrograms of DNA in an appropriate amount of distilled water and 5 ml. of double strength basal medium. By using tenfold dilutions of the compound, a large number of compounds could be screened readily and those showing a significant degree of inhibition then were subjected to further study. Streptomycin, nitroacridine, protamine, 8-hydroxyquinoline and penicillin, for example, were among those found to be inhibitory in the preliminary screening. When tested over a wide range against various levels of DNA, however, none of these compounds could be reversed by addition of more DNA. Therefore, none could be considered as specifically blocking the utilization of DNA by the organism. Reversible inhibition was obtained, however, with ribonucleic acid (RNA). Seven commercial preparations of RNA have been tested and in all cases an inhibition ratio of approximately 10 was obtained (Table II). One sample of RNA was highly purified according to the method of Elmore, *et al.*,[6] and found to retain its ability specifically to block the utilization of DNA by *L. bifidus*.

TABLE II

EFFECT OF RIBONUCLEIC ACID (RNA) ON UTILIZATION OF DESOXYRIBONUCLEIC ACID (DNA) BY *L. bifidus*

DNA γ/tube	RNA γ/tube	Ml. 0.1 *N* acid produced	Inhibition ratio
0	0	1.35	
5	0	2.55	
10	0	3.20	
15	0	4.10	
20	0	5.55	
30	0	7.60	
50	0	8.70	
100	0	9.70	
1000	0	10.25	
10	10	3.60	
10	20	3.60	
10	30	3.80	
10	50	3.00	
10	100	1.60	*ca.* 10
10	200	1.00	
10	300	1.00	
10	500	1.00	
100	10	9.20	
100	20	9.30	
100	30	8.10	
100	50	9.05	
100	100	5.45	
100	200	6.60	
100	300	4.35	*ca.* 10
100	500	3.20	
100	1000	2.00	
100	2000	1.20	
100	3000	1.10	
100	5000	1.10	
1000	1000	10.50	
1000	2000	10.60	
1000	3000	7.10	*ca.* 10
1000	5000	4.60	

Discussion

Lactobacillus bifidus has been found to grow in the presence of vitamin B_{12}, thymidine or DNA. The mechanism of its utilization of intact DNA is probably by enzymatic release of the component desoxyribosides from the nucleic acid. It is felt that the ability of the organism to utilize the intact DNA is of primary interest because of the fundamental role of DNA in all living cells. DNA has been supposed to display some directing role in the transmission of genetic characters, in cellular multiplication, and in maturation. DNA is a main constituent of many animal and bacterial viruses and is responsible for directed artificially induced mutations in pneumococci and other bacteria. It also is present in very high concentrations in tumor tissue, suggesting a possible role in the pathogenesis of cancer. In view of this essential and widespread nature of DNA, a compound that would block specifically the utilization of DNA could be of considerable importance.

The ability of RNA to inhibit competitively the utilization of DNA by *Lactobacillus bifidus* suggests that it may have a similar function in cellular metabolism. It might be postulated that one of the functions of RNA in the cell is to regulate

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(6) D. T. Elmore, J. M. Gulland, D. O. Jordan and H. F. W. Taylor, *Biochem. J.*, **42**, 308 (1948).

cellular multiplication by maintaining a normal balance between the two types of nucleic acid. Further speculation might lead one to suppose that in tumor cells this balance is disturbed.

Summary

Lactobacillus bifidus (ATCC 4963) has been found to grow in response to increasing amounts of desoxyribonucleic acid (DNA) over a range of from 5 to 50 micrograms per tube. The ability of the organism to utilize intact DNA has been used to search for a substance that specifically interferes with the utilization of DNA. Ribonucleic acid (RNA) has been found specifically to inhibit the utilization of DNA by *L. bifidus*. The application of this finding to cellular metabolism has been discussed briefly.

GLENOLDEN, PA. RECEIVED JUNE 24, 1949

[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY[1]]

Gelation Properties of Partially Acetylated Pectins[2]

BY E. L. PIPPEN, R. M. MCCREADY AND H. S. OWENS

The presence of acetic acid in pectins from various sources has been reported by many investigators.[3-11] In some cases the acetic acid seems to be an artifact, but there is agreement that sugar beet pectin contains at least four per cent.[3,4,6,9,10] The lack of jelling power of sugar beet pectin has been attributed to the presence of acetyl groups.[12b] Roboz and van Hook[12a,b] have also suggested that low molecular weight of sugar beet pectin as found by Schneider and Bock[13] is a possible explanation. There is also the possibility that its high non-uronide content might be a factor.

To resolve this problem and to obtain further information on the mechanism of jelly formation, several partially acetylated pectins now have been prepared. Their jelly power has been measured before and after deacetylation. This resulting knowledge has been applied to the preparation of a jellying sugar beet pectin.

Materials and Methods

Preparation of Pectin Acetates.—A commercial citrus pectin (sample 444-P-42, 9.8% methoxyl; 1.03% ash, m. f. b.) was used throughout for the preparation of the acetates according to the procedure of Carson and Maclay.[14] The amount of acetyl in the resulting pectin was controlled by the amount of acetic anhydride used. Isolation of the pectin acetates was accomplished by pouring the reaction mixture into four volumes of alcoholic hydrochloric acid (47.9 ml. of concd. hydrochloric acid/1180 ml. of 95% comm. ethanol). The collected precipitates were washed with three portions of the alcoholic hydrochloric acid, washed free of chloride with 95% ethanol, and finally washed free of ethanol with acetone. The samples were then air dried, ground, dried overnight *in vacuo* at 60° and stored over calcium chloride. The analyses for these samples are given in Table I.

Viscosity Determinations.—For viscosity measurements, 0.6 g. of sodium chloride, 1.0 g. of sodium polymetaphosphate and the sample being investigated were dissolved in 75 ml. of distilled water. The *p*H of the solution was then adjusted to 6.0 with 0.1 *N* sodium hydroxide and the resulting solution, after dilution to 100.0 ml. was used for viscosity measurements in an Ostwald–Cannon–Fenske viscometer.[15] All measurements were made in a water-bath at 25 ± 0.05°. For each sample a plot of log η_{sp}/c *vs.* C was made and the antilog of intercept at $c = 0$ was taken as the intrinsic viscosity.

Preparation of Jellies.—Pectin–sucrose–acid jellies (65% solids) were prepared according to the method of Cox and Higby[16] except that a citric acid solution containing 600 g. of citric acid monohydrate per liter of solution was used instead of a solution of tartaric acid.

Testing of Jellies.—Jellies were tested within forty-eight hours after their preparation. The shear moduli were determined by the method of Owens, Porter and Maclay.[17] Breaking strengths were determined by measuring the force required to break the surface of the jelly with a plunger having an area of 3.14 sq. cm.

Acetyl Determinations.—The percentage of acetyl in the pectin samples was determined by a modification of Clark's method[18] and will be discussed in greater detail elsewhere. The sample was dispersed in and permitted to stand overnight at room temperature in *N*/8 aqueous sodium hydroxide (50 ml. alkali/g. of pectin). The solution was then diluted to 100.0 ml., a 20.0-ml. aliquot withdrawn and acidified with 20 ml. of Clark's sulfuric acid–magnesium sulfate solution, and the mixture steam distilled until 100 ml. of distillate was collected. The acetic acid in the distillate was determined by titration with 0.05 *N* sodium hydroxide using phenol red as the indicator.

Methoxyl Determinations.—Methoxyl analyses were performed by the Zeisel method as described by Shriner.[19]

(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture. Article not copyrighted.

(2) Presented before the Sugar Division, American Chemical Society, April 7, 1949, San Francisco, California.

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