TABLE II. Influence of 16 Epi-oestriol on Uterine Growth in Ovariectomized Rats.

Treatment	No. of animals	Uterine wet wt (mg)	Uterine dry wt (mg)
Control, 10 full days after ovariectomy	25	115.5 ± 3.3*	22.4 ± .65
.1 μg oestradiol 17β in Sesame oil	36	227.3 ± 6.1	42.3 ± 1.8
.1 " in prop. glycol	15	236.0 ± 1.0	44.4 ± 2.5
.01 16 epi-oestriol	8	143.0 ± 7.2	27.0 ± 2.1
.1	15	126.4 ± 5.2	27.2 ± 1.5
1	8	150.0 ± 8.6	26.1 ± .8
5	8	142.7 ± 6.8	25.8 ± 1.7
10	9	171.4 ± 8.7	28.5 ± 1.6
25	9	164.3 ± 5.1	30.5 ± 1.1
30	7	167.2 ± 8.3	32.8 ± 2.0
40	7	181.6 ± 8.0	33.0 ± 2.0
50	7	175.1 ± 8.1	32.6 ± 1.9
100	6	180.2 ± 7.3	30.6 ± 1.5

* Avg ± stand. error.

as on the uterus. Three daily doses of 40.0 μg of 16 epi-oestriol produced a maximal effect on the vagina (full cornification) and the uterine weight. This substance seems to be the weakest of all the known naturally-occurring oestrogens described thus far.

1. Marrian, G. F., and Bauld, W. S., *Biochem. J.*, 1955, v59, 136.

2. Huffman, M. N., *J. Amer. Chem. Soc.*, 1942, v64, 2235.

3. Sondern, C. W., and Sealey, J. L., *Endocrinology*, 1940, v27, 670.

4. Sealey, J. L., and Sondern, C. W., *ibid.*, 1941, v29, 356.

5. Hisaw, F. L., Velardo, J. T., and Goolsby, C. M., *J. Clin. Endo. and Metab.*, 1954, v14, 1134.

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Pentose Dependence of *Lactobacillus gayonii* and Related Species for Early Growth.* (21976)

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Requirements for early growth of *Lactobacillus gayonii* and the closely related species, *buchneri, lycopersici, fermenti, mannitopoeus* and *brevis* have been investigated recently in the authors' laboratory because preliminary screening experiments showed this group of organisms to be particularly well suited for studies of D-α-hydroxy fatty acid and related lipid metabolism. The latter investigations, which were prompted by the finding that D-α-hydroxy fatty acids are essential in the nutrition of *Lactobacillus casei* 280-16(1,2), are to be described in subsequent reports. The effects of pentoses were studied because it had been shown previously that these sugars promote better growth and acid production than does glucose for this particular group of microorganisms(3), and it has now been found that although glucose may be utilized adaptively, an appropriate pentose is essential for early growth of most of these species.

Methods. The sugars tested were D-glucose (C.P. dextrose, J. T. Baker Chemical Co.), L-arabinose (Pfanstiehl Chemical Co.), D-arabinose, L-xylose, D-xylose, and D-ribose (products of Nutritional Biochemicals Corp.). Sugar solutions of appropriate concentration were added to 13 x 100 mm soft glass test tubes together with sufficient water to make 1.0 ml

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per tube and sterilized in the autoclave. The test medium, consisting of enzymatically digested casein (N-Z-Case, Sheffield Farms Co.) 0.6%, yeast extract (Difco) 0.2%, ammonia-hydrolysed yeast nucleic acid(4) 0.02%, glucose 0.5%, vitamin-mineral mixture(5) 20.6 mg %, and buffer solution(5) 5.0 ml %, was made to two-thirds final volume, autoclaved 15 minutes at 15 lb and added (2 ml per tube) aseptically to the previously sterilized tubes. The test microorganisms, described previously (6),† were maintained for the 10 years preceding the present experiments by serial subculture at 3-week intervals in Bacto-tomato juice agar (Difco) (the fresh stabs were incubated 40 hours then stored in a refrigerator). An inoculum medium was prepared by making a uniform suspension of dehydrated Bacto-tomato juice agar in the amount of water (cold) recommended by the manufacturer, stirring it for 10 minutes and then filtering to remove the agar, which, under these conditions, remained mostly undissolved. Fifteen ml aliquots of the filtrate were distributed into 50-ml centrifuge tubes and sterilized. Eighteen- to 24-hour cultures of the test organisms in this medium were centrifuged and resuspended in sufficient sterile saline to yield approximately 10^8 cells per ml. One-tenth ml of suspension per tube was employed as the inoculum. The inoculated tubes were incubated in a precision constant temperature water bath at 35°C.

Results. Bacto-tomato juice agar was employed as the inoculum medium (agar removed) to avoid adaptations during growth of the inoculum which might not have already occurred during maintenance of the test microorganisms. It is of interest in this respect that the composition of Bacto-tomato juice agar (tomato juice solids 2%, Bacto-peptone 1%, Bacto-peptonized milk 1%, agar 1.1%) includes tomato juice as the sole source of utilizable carbohydrate for most of these organisms since lactose, presumably present in peptonized milk, is not utilized appreciably by this group with the exception of *L. fermenti*(3). No report has been found concerning the types of sugar present in tomatoes, but it seems likely that pentoses are present in Bacto-tomato juice agar since the test organisms, as will be shown further on, remain unadapted to glucose utilization. The total sugars present in tomatoes average about 3.4% according to Chatfield and Adams(7).

Yeast extract was included in the test medium since it has been shown previously to stimulate growth of lactic acid bacteria(3), and ammonia-hydrolysed yeast nucleic acid was employed as a nucleotide supplement, since the latter is known to stimulate growth of *L. gayonii*(4). The growth rates of *L. brevis, L. buchneri, L. gayonii* and *L. mannitopoeus* were determined in the test medium with L-arabinose as the sugar source with and without the yeast extract and ammonia-hydrolysed yeast nucleic acid, and the stimulatory effects of these supplements (data not shown) were found to be independent and additive except with *L. buchneri,* for which the ammonia-hydrolysed yeast nucleic acid appeared to be inert. The complete test medium (containing both supplements) was used in subsequent experiments unless otherwise specified.

Glucose (0.5%) was autoclaved with the medium in all of the tests (unless otherwise noted) since Rogers *et al.*(8) have shown that an unidentified substance essential for early growth of *L. gayonii* is formed in glucose containing media upon heating. The concentrations of the various sugars tested (including glucose) were in addition to this basal glucose level, but the test sugars were sterilized separately from the medium to avoid alteration through "browning".

The data shown in Fig. 1 illustrate that *L. brevis* (B), *L. gayonii* (G), and *L. mannitopoeus* (M) respond markedly to L-arabinose (a) in 16 hours, but negligibly to glucose (g) under these conditions. Similar effects were noted with *L. buchneri* and *L. lycopersici* (data not shown), but *L. fermenti* exhibited a considerable 16-hour response to glucose, exceeded only by that to D-xylose and D-ribose.

All test organisms responded ultimately to glucose, although *L. buchneri* was slowest in doing so. The data presented in Fig. 2 show

† The American Type Culture Collection No. of *L. brevis* is incorrectly given as 8257 in the reference cited. The correct number is 8287.

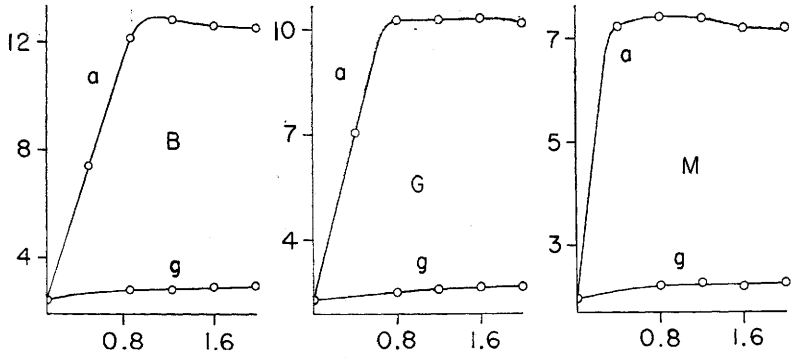

FIG. 1. 16 hr response of *L. brevis* (B), *L. gayonii* (G) and *L. mannitopoeus* (M) to L-arabinose (a) and D-glucose (g). Values on horizontal scales are sugar concentrations (%); those on vertical scales are titrations (calculated as ml of 0.01 *N* NaOH required to neutralize 1 ml of culture).

that the response of *L. buchneri* to glucose (g) was still only slight after 145 hours, whereas D-xylose (x) was nearly completely fermented in 145 hours and L-arabinose (a) in 50 hours.

Eighteen-hour responses of each of the test organisms to 1% L-arabinose, D-ribose, D-xylose and D-glucose, respectively, are given in Table I (the responses to D-arabinose and L-xylose were excluded from the table because they were negligible in all cases). L-Arabinose yielded the best or nearly the best response for all the test organisms except *L. fermenti,* and D-ribose and D-xylose were generally much better utilized than D-glucose (although some utilization of the latter was already in evidence).

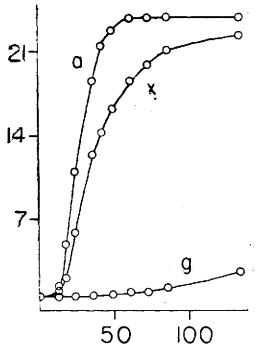

FIG. 2. Growth of *L. buchneri* with 2% L-arabinose (a), 2% D-xylose (x), and 2% glucose (g). Values on horizontal scale are hours of incubation; those on vertical scale are titrations (calculated as ml of 0.01 *N* NaOH required to neutralize 1 ml of culture). The yeast extract, the ammonia-hydrolysed yeast nucleic acid and the initial supplement of glucose were omitted from the test medium in this experiment.

Results in the test medium solidified with agar and supplemented with D-glucose and L-arabinose, respectively, showed the same trends as in liquid medium. Colonies of *L. brevis, L. gayonii* and *L. mannitopoeus* in L-arabinose-supplemented agar medium were large (approximately 3 mm diameter) after 40 hours incubation under carbon dioxide, whereas those in D-glucose-supplemented agar medium were still nearly microscopic after this length of time. Colonies in the latter medium ultimately reached normal size, however, and the final number of colonies per unit volume of inoculum suspension were approximately the same with either D-glucose or L-arabinose, indicating that probably each cell in the inoculum suspension possessed an adaptive mechanism for the utilization of D-glucose.

The relatively large amounts of pentose required to promote growth of the test organisms (Fig. 1) suggest that the pentose functions as an energy source, rather than as a vitamin-like nutrient. A study of the enzyme systems involved has not been attempted, but a reasonable hypothesis appears to be that these pentose-requiring organisms possess a constitutive pentokinase together with an adaptive hexokinase. It seems unlikely that the growth-promoting effects of the pentoses were due to contamination with vitamin-like growth factors since the test medium contained yeast extract, which is generally found to be an adequate source of such unidentified nutrients. This possibility was rendered still less likely by the use of recrystallized L-arabinose, which

TABLE I. 18-Hour Response of the Test Organisms to 4 Sugars (at 1% Concentration).

	Growth response (acid production)*			
	L-Arabinose	D-Ribose	D-Xylose	D-Glucose
L. brevis	14.25	14.13	12.42	2.80
L. buchneri	10.28	4.87	4.80	.67
L. fermenti	2.38	9.81	8.17	5.33
L. gayonii	11.28	11.92	6.42	2.60
L. lycopersici	3.40	1.30	2.53	2.10
L. mannitopoeus	13.48	8.42	10.13	2.79

* Calculated as ml of 0.01 N NaOH required to neutralize 1 ml of culture.

was found to have retained all of its original growth-promoting activity.

That D-glucose has been employed with general success as a carbohydrate source for these and related microorganisms may be accounted for by adaption to D-glucose resulting under the test conditions employed. Cheldelin and Riggs(9), for example, carried their culture of *L. gayonii* (A.T.C.C. 8289) in a medium containing 1% glucose. 1% yeast extract, and 2% agar and, like most other researchers, employed an inoculum medium also containing glucose as the sole carbohydrate source. Such conditions would, of course, be certain to maintain this organism in its maximally adapted state.

Summary. Evidence has been presented indicating that an appropriate pentose is essential as an energy source for early growth of *L. brevis, L. buchneri, L. gayonii, L. lycopersici* and *L. mannitopoeus* if passage through a pentose-free glucose-containing medium, which would promote adaptive utilization of glucose, is avoided.

1. Camien, M. N., and Dunn, M. S., *J. Biol. Chem.*, 1953, v201, 621.

2. ———, *ibid.*, 1954, v211, 593.

3. Camien, M. N., Dunn, M. S., and Salle, A. J., *ibid.*, 1947, v168, 33.

4. Hutchings, B. L., Sloane, N. H., and Boggiano, E., *ibid.*, 1946, v162, 737.

5. Camien, M. N., and Dunn, M. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 177.

6. Dunn, M. S., Shankman, S., Camien, M. N., and Block, H., *J. Biol. Chem.*, 1947, v168, 1.

7. Chatfield, C., and Adams, G., *U. S. Dept. Agr.*, 1931, *Circ. No.* 146.

8. Rogers, D., King, T. E., and Cheldelin, V. H., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 140.

9. Cheldelin, V. H., and Riggs, T. R., *Arch. Biochem.*, 1946, v10, 19.

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Effectiveness of Phenoxymethyl Penicillin V, and Sodium Penicillin G Against Hemolytic Streptococcus Infection in White Mice. (21977)

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The following experiments were conducted to determine the comparative effectiveness on a weight basis of phenoxymethyl penicillin V, and sodium penicillin G against hemolytic streptococcus infection in white mice.

Materials and methods. Two groups totaling 640 white mice in 2 tests were injected intraperitoneally with a 10^{-5} dilution of a 16-hour blood broth culture of hemolytic streptococcus C-203. This is usually somewhat more than 1000 LD_{50}, but less than 10.-000 LD_{50}. In one test indicated below it was actually 4,050 LD_{50}, and in the other test it was 5,900 LD_{50} according to virulence tests which utilized 100 mice each. Following injection of the virulent culture, subgroups of 20 mice each were injected immediately by the subcutaneous route with either one or the other of the above penicillins. Different subgroups of 20 mice received either 7 or 9 2-fold