hydroxymethylglyoxal). It is of interest that reductone was active whereas ascorbic acid was ineffective since both owe their reducing activity to an ene-diol structure.

Schuhardt *et al.*(11) and Rode *et al.*(12) found that cystine when autoclaved in neutral solution (but not when autoclaved at pH 1.5) was toxic for brucellae, and explained the toxicity of some lots of tryptose by the formation of oxidation products of cystine on standing. Under the conditions of our experiments cystine was toxic after autoclaving in acid as well as in neutral solution. Recently Schuhardt *et al.*(13,14) have identified the substance toxic to brucellae as elemental sulfur. Following this report the toxicity of colloidal sulfur‡ for *L. bifidus* was tested. Growth of the organism was inhibited by sulfur added to the autoclaved medium but the amount required for complete inhibition was large, about 10 γ per ml, whereas the growth of brucellae had been inhibited by 0.1 to 0.2 γ of sulfur per ml. The toxic effect of the sulfur could be reversed by autoclaving it with the complete sugar-containing medium or by addition of cysteine with the sulfur.

These experiments emphasize the importance and variety of interactions among the components of the medium, particularly under the severe conditions imposed by autoclaving. With a high level of cystine in the medium autoclaving with sugar was beneficial, with a low level of cystine, detrimental. Low concentrations of sugar were not tested but would surely modify the picture. An apparent complete failure of sucrose to support growth in the usual medium was a reflection of the fact that it is not a reducing sugar.

Summary. Autoclaved cystine is toxic to *L. bifidus*. Autoclaving in the presence of reducing sugar prevents development of the toxic product. The toxicity may be counteracted by addition, after autoclaving, of cysteine, thioglycollic or thiomalic acids, reductone, or cystine itself with ascorbic acid, but not by addition of ascorbic acid alone.

‡ The method by which the colloidal sulfur used in his experiments was prepared was kindly furnished to the authors by Dr. V. T. Schuhardt.

1. Rose, C. S., Luchi, R. J., Avery A., Norris, R. F., and György, P., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 712.

2. Orla-Jensen, A. D., *J. Soc. Chem. Ind.*, 1933, v52, 374.

3. Smiley, K. L., Niven, C. F., Jr., and Sherman, J. M., *J. Bact.*, 1943, v45, 445.

4. Rabinowitz, J. C., and Snell, E. E., *J. Biol. Chem.*, 1947, v169, 631.

5. Snell, E. E., Kitay, E., and Hoff-Jorgensen, E., *Arch. Biochem.*, 1948, v18, 495.

6. Maillard, L. C., *Ann. Chim.*, 1916, v5, 258.

7. Lankford, C. E., Swausch, C., and Ravel, J. M., *J. Bact.*, 1947, v53, 368.

8. Camien, M. N., and Dunn, M. S., *J. Biol. Chem.*, 1950, v183, 561.

9. Norris, R. F., Flanders, T., Tomarelli, R. M., and György, P., *J. Bact.*, 1950, v60, 681.

10. Lugg, J. W. H., *Biochem. J.*, 1933, v27, 1022.

11. Schuhardt, V. T., Rode, L. J., Oglesby, G., and Lankford, C. E., *J. Bact.*, 1950, v60, 655.

12. Rode, L. J., Oglesby, G., and Schuhardt, V. T., *J. Bact.*, 1950, v60, 661.

13. Schuhardt, V. T., Rode, L. J., Jr., Oglesby, G., and Lankford, C. E., *Bact. Proc.*, 1951, p. 36.

14. ———, *J. Bact.*, 1952, v63, 123.

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Mono- and Disaccharides in Growth of *Lactobacillus bifidus* and Its Mutants. (19997)

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Wright(1) described a strain of *Streptococcus thermophilus* which grew more readily with lactose or sucrose than with any other sugar. Since that time, several workers have discussed organisms which utilize disaccharides more readily than their constituent

monosaccharides. Hestrin(2) has reviewed the literature and discussed possible mechanisms for this direct utilization of disaccharides.

Recent studies in this laboratory indicate that *Lactobacillus bifidus* is a member of the group of organisms which utilize disaccharides preferentially. Norris, Flanders, Tomarelli, and György(3) have reported the isolation and maintenance of a number of strains of a microorganism with the morphologic and metabolic characteristics of *L. bifidus* which was first described by Tissier in 1899(4). These authors discussed also unbranched strains of lactobacilli which appeared to be derived by mutation from the branched strains and resembled certain standard strains of *L. acidophilus,* in particular, the strains of Weiss and Rettger which were originally designated *L. bifidus*. In the present communication a difference between the bifid and unbranched strains in carbohydrate utilization is reported. Whereas *L. bifidus* fermented lactose or maltose more rapidly than glucose or galactose, the unbranched strains, as well as various standard strains of lactobacilli utilized monosaccharides as well as or better than the disaccharides.

Experimental. The organisms studied included 5 strains of *L. bifidus* isolated in this laboratory and maintained in subculture over a period of 2 years, 2 unbranched strains, "A" and "I" which may be considered as mutants of the bifid strains, and 5 cultures of *L. acidophilus* obtained from the American Type Culture Collection: No. 9857, Döderlein's bacillus; Nos. 4355 and 4357, a murine and a human strain isolated by Kulp; and Nos. 4962 and 4963, strains which were originally classified by Rettger as *L. bifidus* and which are very similar to strain "A"(3). All of these strains were carried on a modification of the Teply and Elvehjem medium as described by Norris *et al.*(3). The inoculum, medium, and general procedures used in these studies have been discussed by Tomarelli *et al.*(5). The minimal medium satisfactory for growth of the bifid strain was modified to provide optimal conditions for the various organisms studied. One unbranched strain, "I", required desoxyriboside for growth, and strains "A" and "I" as well as several of the ATCC strains required unsaturated fatty acid. The first of these needs was met by use of an enzymatic digest of casein as the source of nitrogen. The pancreatin present in this preparation contains desoxyriboside. Unsaturated fatty acid was supplied as Tween 80, a polyoxyethylene derivative of sorbitan monooleate which, unlike free unsaturated fatty acids, was not toxic to the bifid organisms (6). When sodium citrate was used as the buffer in the medium 100 mg of dicalcium phosphate, 10 mg of manganese sulfate and 5 mg of magnesium sulfate were added to each tube to compensate for the binding of bivalent ions by citrate. Only the calcium salt was necessary for most of the unbranched strains, but the branched strain required the additional supplement of manganese and magnesium ions for regular and consistent growth. The concentration of sugar in the medium was 3.5%. Growth of the organisms was measured by the amount of acid produced.

All of the strains of *L. bifidus* studied utilized lactose more readily than glucose in marked contrast to the unbranched variants derived from them. In Table I are given data of an experiment in which the acid production of a bifid strain is compared with that of Strain A, a straight mutant, when they were grown in media containing lactose or one or both of its constituent monosaccharides. In the medium buffered with sodium acetate there was good growth of both strains after 64 hours, regardless of the type of sugar present. However, in 20 hours the bifid strain grew well only with lactose, the straight strain only with glucose. When the sodium acetate of the medium was replaced by sodium citrate, a buffer which is less satisfactory for the growth of these organisms(6), the difference between the 2 strains was more marked. In this medium the bifid strain grew excellently on lactose, but very poorly on glucose, even after 64 hours. The straight strain, on the contrary, grew negligibly on lactose in 64 hours, while with glucose, it produced half the maximum amount of acid in 20 hours. Growth with galactose was intermediate between that with glucose and lactose for both strains. An equimolecular mixture of glucose

TABLE I. Utilization of Sugars by *L. bifidus* and an Unbranched Variant.

Buffer of medium	Sugar (350 mg/tube)	Acid production (ml .1 N per 10 ml culture) Bifid strain 20 hr	Bifid strain 40 hr	Bifid strain 64 hr	Unbranched strain (A) 20 hr	Unbranched strain (A) 40 hr	Unbranched strain (A) 64 hr
Acetate	L*	9	23.6	23.7	3.6	10.1	13.1
	Gl	1.6	14.6	17.1	11	16.8	17.1
	Ga	1.1	14.7	19.5	3.5	8.2	9.3
	Gl + Ga†	1.9	15.9	19.3	10.5	12.5	12.4
Citrate	L	.8	19.5	23.6	0	.7	1.1
	Gl	0	4.1	6.2	7	12.4	10.6
	Ga	0	7.7	16.5	1.1	2.8	7.8
	Gl + Ga†	0	10.3	13.1	6.9	8.9	8.7
Acetate	L	3.2	17.1	24.1			
	M	1.8	14.2	18.2			
	S	0	0	0			
Acetate	L added aseptically	4.3	20.4	24	Cysteine, 6 mg, added asep.		
	M " "	3	14.1	16.9	"	"	" "
	S " "	0	6.6	18.3	"	"	" "

* L = Lactose, Gl = Glucose, Ga = Galactose, M = Maltose, S = Sucrose.
† 175 mg of each.

and galactose was not equivalent to lactose in supporting growth of the bifid organism.

All of the unbranched strains of lactobacilli studied resembled strain A in their utilization of glucose and lactose. None of them grew better on lactose than on glucose. Two strains used glucose and lactose at the same rate; the other strains metabolized glucose faster than lactose. This advantage of glucose persisted even though the strains had been carried for at least several months in medium containing lactose as the only sugar.

In other experiments the relative growth promoting activity for the bifid strain of the disaccharides, sucrose, lactose and maltose was compared (Table I). Maltose was almost as effective as lactose, but under the usual experimental conditions sucrose was inactive. It was found, however(7), that medium which did not contain reducing sugar acquired toxic properties during autoclaving. This difficulty was circumvented by sterilizing the sugar together with cysteine, by seitz-filtration and adding the mixture to medium autoclaved without sugar. When this procedure was followed, there was some growth with sucrose, but the rate was much slower than with lactose or maltose.

Since glucose was so poorly utilized by the bifid strain, cultures grown on lactose were tested for the presence of glucose by the glucose oxidase method of Keilin and Hartree (8). It was found that glucose accumulated slowly during the incubation period being 0.3% after 24 hours, 0.8% after 40 hours, and 1.0% after 64 hours. This result offered the possibility that the organism was making effective use only of the galactose portion of the molecule. However, assay for galactose and glucose by the procedure of Sprague and Bellamy(9) using *Streptococcus mastiditis* 68 showed that glucose and galactose were present in the culture in approximately equal amounts. An unbranched strain tested at intervals from 8 to 64 hours contained at no time sufficient glucose to be detected by the glucose oxidase method.

Discussion. The several strains of *Lactobacillus bifidus* studied were distinct from their unbranched variants and from the strains of *L. acidophilus* tested in that they metabolized lactose more readily than glucose. The straight rods utilized glucose at least as well as, and usually better than lactose even though they were routinely carried in a medium containing lactose. Adam(10) reported that lactose, maltose, and sucrose, in decreasing order of efficiency were superior to monosaccharides for the growth of *L. bifidus*. He based his conclusions on the number and morphology of organisms observed in gram-stained preparations. Orla-Jensen, Orla-Jensen, and Winther(11), and Olsen(12), who studied the fermentation of a large number of carbohy-

drates by *L. bifidus* did not observe significant differences among the sugars we have studied. However, they measured acid production after a 2-week incubation period so that differences in the rate of fermentation such as we observed in 20 to 64 hours were not apparent.

Organisms which grow preferentially on disaccharides vary in the specificity of their requirements. A strain of *L. bulgaricus* studied by Snell, Kitay, and Hoff-Jorgensen(13) used only lactose of the disaccharides but grew on a number of B-galactosides. The strain of *Streptococcus thermophilus* discussed by Wright(1) grew best on lactose and sucrose. *L. bifidus* used sucrose to only a limited extent but metabolized both maltose and lactose readily.

It has been noted by a number of workers that even though an organism used a disaccharide much better than one or both of the constituent monosaccharides there was no accumulation of monosaccharides during the growth period(13-15). Doudoroff *et al.*(15) using dried cell preparations of a mutant of *Escherichia coli* which utilized maltose did observe accumulation of glucose and his suggested mechanism accounts for its formation. However, glucose was never found in more than trace amounts when intact cells were used. *L. bifidus* on the contrary does show accumulation of both glucose and galactose indicating that it does contain an active lactase which converts a considerable portion of the lactose present to glucose and galactose but lacks the enzyme system to make effective use of these sugars.

Summary. 1. *Lactobacillus bifidus* utilizes the disaccharides lactose and maltose, but not sucrose, more readily than the monosaccharides, glucose and galactose. The reverse is true of straight rod variants of *L. bifidus*. Several strains of *L. acidophilus* utilize glucose or lactose equally well or use glucose more readily. 2. During growth of *L. bifidus* on lactose, glucose and galactose accumulated in the medium. The straight rod mutant of *L. bifidus*, on the other hand, utilizes lactose completely.

1. Wright, H. D., *J. Path. Bact.*, 1936, v43, 487.
2. Hestrin, S., *Wallerstein Lab. Communicat.*, 1948, v11, 193.
3. Norris, R. F., Flanders, T., Tomarelli, R. M., and György P., *J. Bact.*, 1950, v60, 681.
4. Tissier, H., *Compt. rend. soc. biol.*, 1899, v51, 943.
5. Tomarelli, R. M., Norris, R. F., and György, P., *J. Biol. Chem.*, 1950, v181, 879.
6. Tomarelli, R. M., Norris, R. F., Rose, C. S., and György, P., *J. Biol. Chem.*, 1950, v187, 197.
7. Rose, C. S., Feldbaum, E. P., Norris, R. F., and György, P., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 709.
8. Keilin, D., and Hartree, E. F., *Biochem. J.*, 1948, v42, 230.
9. Sprague, C. F., and Bellamy, W. D., *J. Bact.*, 1949, v57, 95.
10. Adam, A., *Kinderheilk.*, 1922, v31, 331.
11. Orla-Jensen, S., Orla-Jensen, A. D., and Winther, O., *Zent. Bakt. Parasitenk. II*, 1936, v93, 321.
12. Olsen, E., *Studies on the Intestinal Flora of Infants*. Ejnar Munksgaard, Copenhagen, 1949.
13. Snell, E. E., Kitay, E., and Hoff-Jorgensen, E., *Arch. Biochem.*, 1948, v18, 495.
14. Doudoroff, M., *J. Biol. Chem.*, 1945, v157, 609.
15. Doudoroff, M., Hassid, W. Z., Putnam, E. W., and Potter, A. L., *J. Biol. Chem.*, 1949, v179, 921.

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Studies on Phenylpyruvic Oligophrenia. Phenylpyruvic Acid Content of Blood. (19998)

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Phenylpyruvic oligophrenia (phenylketonuria) is a form of mental deficiency characterized by the urinary excretion of phenylpyruvic, phenyllactic, phenylacetic acids and phenylalanine(1). Although abnormally high amounts of phenylalanine have been observed