JOURNAL OF BACTERIOLOGY, Mar. 1978, p. 1163-1174
0021-9193/78/0133-1163$02.00/0

Vol. 133, No. 3
Printed in U.S.A.

Catabolite Inhibition and Sequential Metabolism of Sugars by *Streptococcus lactis*

JOHND THOMPSON,* KEITH W. TURNER, AND TERENCE D. THOMAS
New Zealand Dairy Research Institute, Palmerston North, New Zealand

Received for publication 11 October 1977

Growth of galactose-adapted cells of *Streptococcus lactis* ML_3 in a medium containing a mixture of glucose, galactose, and lactose was characterized initially by the simultaneous metabolism of glucose and lactose. Galactose was not significantly utilized until the latter sugars had been exhausted from the medium. The addition of glucose or lactose to a culture of *S. lactis* ML_3 growing exponentially on galactose caused immediate inhibition of galactose utilization and an increase in growth rate, concomitant with the preferential metabolism of the added sugar. Under nongrowing conditions, cells of *S. lactis* ML_3 grown previously on galactose metabolized the three separate sugars equally rapidly. However, cells suspended in buffer containing a mixture of glucose plus galactose or lactose plus galactose again consumed glucose or lactose preferentially. The rate of galactose metabolism was reduced by ~95% in the presence of the inhibitory sugar, but the maximum rate of metabolism was resumed upon exhaustion of glucose or lactose from the system. When presented with a mixture of glucose and lactose, the resting cells metabolized both sugars simultaneously. Lactose, glucose, and a non-metabolizable glucose analog (2-deoxy-D-glucose) prevented the phosphoenolpyruvate-dependent uptake of thiomethyl-β-D-galactopyranoside (TMG), but the accumulation of TMG, like galactose metabolism, commenced immediately upon exhaustion of the metabolizable sugars from the medium. Growth of galactose-adapted cells of the lactose-defective variant *S. lactis* 7962 in the triple-sugar medium was characterized by the sequential metabolism of glucose, galactose, and lactose. Growth of *S. lactis* ML_3 and 7962 in the triple-sugar medium occurred without apparent diauxie, and for each strain the patterns of sequential sugar metabolism under growing and nongrowing conditions were identical. Fine control of the activities of preexisting enzyme systems by catabolite inhibition may afford a satisfactory explanation for the observed sequential utilization of sugars by these two organisms.

The capacity of group N streptococci (*Streptococcus lactis*, *S. cremoris*, and *S. diacetylactis*) to ferment lactose to lactic acid is of major economic importance in the manufacture of dairy products. However, it is only recently that the biochemical pathways (see Fig. 1) involved in the transport and metabolism of lactose by this important group of organisms have been established (20). The transport of lactose is mediated via the phosphoenolpyruvate (PEP): lactose phosphotransferase system (lac-PTS) and the disaccharide is phosphorylated simultaneously with translocation (24, 25; for a review, see reference 33). The accumulated lactose phosphate [6-phosphogalactosyl-(β-1 → 4)-glucose] is subsequently cleaved by β-D-phosphogalactoside galactohydrolase (12, 27) to yield glucose and galactose-6-phosphate, which are further metabolized by the Embden-Meyerhof and D-tagatose-6-phosphate pathways (3-5), respectively. Possession of a functional lac-PTS and β-D-phosphogalactoside galactohydrolase appears to be a prerequisite for rapid growth and homolactic fermentation of lactose by group N streptococci (42).

Preliminary studies (K. W. Turner and T. D. Thomas, unpublished data) showed that, during growth of *S. lactis* ML_3 on lactose, there was no release of glucose or galactose into the medium; this demonstrated that, intracellularly, both hexose moieties were fermented simultaneously to lactic acid. However, in contrast to this balanced fermentation of the disaccharide, the growth of *S. lactis* in a medium containing a mixture of the component monosaccharides (glucose plus galactose) was characterized by the preferential utilization of the glucose fraction. In addition, the cells grew relatively slowly on free galactose, and fermentation was heterolactic (42).

The present investigation was undertaken to examine the mechanisms involved in the preferential utilization of glucose and in the more general problem of sequential metabolism of sugars by *S. lactis*. To this end we have: (i) monitored sugar utilization by cells growing in mixed-sugar media (i.e., media containing various combinations of glucose, galactose, and lactose), (ii) studied sugar utilization by nongrowing cells, and (iii) monitored transport of the non-metabolizable sugar analogs 2-deoxy-D-glucose (2-DG) and thiomethyl-β-D-galactopyranoside (TMG) by intact cells in an attempt to characterize the interactions of the different transport systems at the membrane level.

The characteristics of sugar metabolism by *S. lactis* ML_3 (which possesses the lac-PTS and β-D-phosphogalactoside galactohydrolase and which grows rapidly on lactose with homolactic fermentation) have been contrasted with those exhibited by *S. lactis* 7962. The latter strain does not possess β-D-phosphogalactoside galactohydrolase, is defective in the lac-PTS, and grows poorly on lactose; lactic acid forms only a minor end product (~15%) of lactose fermentation (42). Our results have been discussed in the light of current knowledge of transport and regulation of sugar metabolism in the lactic streptococci (for a review, see reference 20) and in other bacterial systems (16, 17, 22, 32, 33).

MATERIALS AND METHODS

Organisms. *S. lactis* ML_3 and 7962 were obtained from the culture collection of the New Zealand Dairy Research Institute.

Culture maintenance and growth of organisms. The methods of culture maintenance have been described previously (43); unless otherwise stated, the organisms were grown and maintained in a defined medium containing galactose (0.5% [wt/vol]) as the fermentable energy source.

Preparation of cell suspensions. Cells were harvested by centrifugation of cultures (13,000 × *g* for 1 min) in the mid-logarithmic phase of growth. The cell pellets were washed twice by suspension in (and centrifugation from) a 0.01 M $MgSO_4$ solution at room temperature. A thick cell suspension containing about 20 to 25 mg (dry weight) of cells per ml was prepared as described previously (43).

Transport studies with nongrowing cells. Accumulation of non-metabolizable sugar and amino acid analogs was determined by using a basal transport medium of 10 ml of 0.1 M tris(hydroxymethyl)aminomethane-maleate (Tris-maleate) buffer (pH 7.0) that contained, when necessary, 0.1 mM [^{14}C]TMG (specific activity, 0.2 μCi/μmol), 0.1 mM [^{3}H]2-DG (specific activity, 0.5 μCi/μmol), or 0.2 mM α-[^{14}C]aminoisobutyric acid (AIB; specific activity, 0.2 μCi/μmol). An 80- to 100-μl sample of the previously prepared thick cell suspension was introduced to the transport system (maintained at 30°C) to obtain a final cell density of 200 μg (dry weight) of cells per ml. Sampling and counting techniques have been described in a previous report (43).

Sugar metabolism by nongrowing cells. Washed cells were suspended at a density of 200 μg (dry weight) per ml in 20-ml volumes of either 0.1 M Tris-maleate (pH 7.0) or 0.1 M sodium phosphate (pH 7.0) buffer containing the appropriate sugar(s) at a 0.3 mM final concentration. The cell suspensions were gently agitated at 30°C with a water bath shaker. At intervals, 1- to 2-ml volumes were withdrawn, and the cells were quickly removed (ca. 10 s) by vacuum filtration through 0.45-μm-pore size membrane filters (type HA; Millipore Corp., Bedford, Mass.). The filtrates were collected in small glass tubes, sealed, immediately frozen with a solid CO_2-ethanol freezing mixture, and maintained frozen until required for sugar analyses.

Growth of *S. lactis* ML_3 and 7962 in mixed-sugar media. The chemically defined medium described previously (43) that contained the appropriate sugar concentrations (see Results) was used for the growth of *S. lactis* ML_3. The same medium, supplemented with 0.5% (wt/vol) Trypticase (Baltimore Biological Laboratories, Cockeysville, Md.) and 1% (wt/vol) tryptone (Oxoid Ltd., London), was used for *S. lactis* 7962, because in the defined medium alone the growth rate of 7962 was considerably slower than that of *S. lactis* ML_3. The triple-sugar media (300 ml) were inoculated (0.75% [vol/vol]) from overnight, galactose-grown cultures of *S. lactis* ML_3 or 7962. Duplicate samples were withdrawn from each culture at appropriate intervals; growth (optical density) was measured in the first sample with a Unicam SP 500 spectrophotometer (Pye Unicam Ltd., Cambridge, England) operated at 600 nm with a 1-cm light path. Samples were diluted where necessary to make optical density proportional to cell mass. Cells were removed from the second sample by Millipore filtration, and filtrates were assayed for residual sugars.

Sugar analyses. Glucose and galactose were determined by using Glucostat and Galactostat reagent kits (Worthington Biochemicals Corp., Freehold, N.J.). Lactose was assayed indirectly by determination of the glucose formed after incubation with β-D-galactoside galactohydrolase (β-galactosidase, EC 3.2.1.23). Control experiments conducted with standard lactose and glucose solutions showed that the hydrolysis of 1 mol of lactose was accompanied by the formation of 1 mol of glucose. The activity of β-galactosidase in Tris-maleate buffer was consistently lower and more variable than that observed in phosphate buffer. For this reason, phosphate buffer was employed in those experiments concerned with lactose metabolism by nongrowing cells.

Intracellular G6P determination. Extracts were prepared from *S. lactis* ML_3 growing exponentially in the presence of excess carbohydrate, and intracellular glucose-6-phosphate (G6P) levels were determined by minor modification of previously described procedures (8, 31). To halt glycolysis as rapidly as possible, two extraction procedures were employed. Method 1 (filter plus trichloroacetic acid) was as follows. Cells from 25 ml of mid-log-phase culture (ca. 8 mg total dry weight of cells) were collected by filtration through a 47-mm-diameter (0.8-μm pore diameter) Millipore membrane filter and then placed in 5 ml of 10% (wt/vol) trichlo-

roacetic acid solution at 0°C. The total elapsed time between commencement of filtration and immersion of the cells in trichloroacetic acid was 10 to 15 s. Method 2 (trichloroacetic acid direct) proceeded as follows. A 5-ml portion of exponentially growing culture was placed on a Vortex mixer, and 1 ml of 60% (wt/vol) trichloroacetic acid was quickly added. The time taken for the culture to reach a trichloroacetic acid concentration of 10% (wt/vol) with this method was <1 s. The trichloroacetic acid concentration was removed from the cell extracts by ether extraction (8, 31), and G6P in the neutralized extracts was determined by fluorescence spectrophotometry (Perkin-Elmer, model MPF-2A) with G6P dehydrogenase and a nicotinamide adenine dinucleotide phosphate-coupled indicator system (31). The instrument was calibrated with standard solutions of G6P covering a concentration range from 0 to 1.5 nmol per 2.5-ml assay system.

Trace amounts of G6P were released into the medium during exponential growth of *S. lactis* (T. D. Thomas, unpublished data). The culture filtrates arising from method 1 were assayed for G6P, and the values were subtracted from the total G6P determined by method 2 to obtain the intracellular concentration of the hexose phosphate. Intracellular G6P concentrations were calculated from the known dry weight of cells initially extracted, on the basis that 1 g (dry weight) of cells of *S. lactis* ML_3 was equivalent to 1.67 ml of intracellular (protoplast) fluid (43).

Reagents. The radioactive materials [^{3}H]2-DG and α-[^{14}C]AIB were obtained from the Radiochemical Centre, Amersham, England, and [^{14}C]TMG was obtained from New England Nuclear Corp., Boston, Mass. The non-metabolizable glucose analog, 6-deoxy-D-glucose (6-DG), was purchased from Koch-Light Laboratories, Bucks, England. Lactose, D-galactose (both substantially glucose-free), β-galactosidase, G6P dehydrogenase, and nicotinamide adenine dinucleotide phosphate were obtained from the Sigma Chemical Co., St. Louis, Mo.; D-glucose was purchased from B.D.H. Laboratories, Poole, England.

RESULTS

Characteristics of growth of *S. lactis* ML_3 and 7962. Cells of *S. lactis* ML_3, previously grown on galactose as the sole fermentable sugar, possess the necessary complement of enzymes (Fig. 1) for the metabolism of glucose, lactose, and galactose. When such cells were grown in a chemically defined medium containing a mixture of the three potential energy sources, a clear "preference" or sequential utilization of sugars was observed (Fig. 2). The logarithmically growing organisms (doubling time [T_D], ~81 min) fermented glucose and lactose simultaneously for an initial period of about 3.7 h. During this interval, the glucose concentration in the medium decreased by about 80% and the lactose concentration decreased by about 40%. Galactose, the sugar on which the inoculum had been grown initially, and which was present in the medium at a concentration threefold greater than either glucose or lactose, was not significantly metabolized (<5%) by the cells. After approximately 3.7 h, an increase in growth rate occurred (T_D, ~62 min), and this continued until the point of lactose exhaustion from the medium (~5 h). At this time, a decrease in growth rate occurred (T_D, ~120 min), and galactose served as the sole energy source for growth. In defined media containing glucose or lactose as the sugar, exponentially growing cells of *S. lactis* ML_3 have similar doubling times (T_D, ~55 min; see reference 43 and Fig. 7 and 8). However, when the growing cells metabolized these sugars simultaneously, the doubling time increased considerably, to 81 min (Fig. 2).

When the lactose-defective variant *S. lactis* 7962 was grown under similar conditions, a different sequence of sugar utilization was observed (Fig. 3). Though previously induced for galactose metabolism, the cells again showed preferential fermentation of glucose (T_D, ~45 min) from the sugar mixture. Three hours after inoculation, 80% of the original glucose had been utilized, the concentration of galactose had decreased by only 5%, and no significant metabolism of lactose had occurred. After 3 h, and without apparent diauxie, a change in growth rate occurred (T_D, ~55 min) that was coincident with the fermentation of galactose as the principal energy source. Finally, upon exhaustion of galactose from the medium (5.25 h), very slow growth occurred on lactose (T_D, ~310 min).

Metabolism of sugars by nongrowing cells of *S. lactis* ML_3 (galactose grown). The data presented in Fig. 2 showed that, during growth of *S. lactis* ML_3 in the triple-sugar medium, the utilization of sugars proceeded according to the following sequence: glucose plus lactose (simultaneously), lactose, and galactose. The following experiments showed that a similar sequence of sugar metabolism by the cells occurred under nongrowing conditions.

Nongrowing cells: glucose and lactose metabolism. Galactose-grown cells of *S. lactis* ML_3 metabolized glucose at a rapid and linear rate of 6.9 μmol/mg (dry weight) of cells per hour (Fig. 4A). Lactose was consumed at the rate of 3.4 μmol/mg per h. Since lactose-phosphate is hydrolyzed to glucose and Gal6P moieties within the cell, the observed fermentation rate of the disaccharide was thus equivalent to 6.8 μmol of total hexose per mg per h. When present together (Fig. 4B), the sugars were metabolized simultaneously, but at rates slower than those observed with the individual sugars. However, the combined rate of glucose plus lactose utilization (2.8 and 2.1 μmol/mg per h,

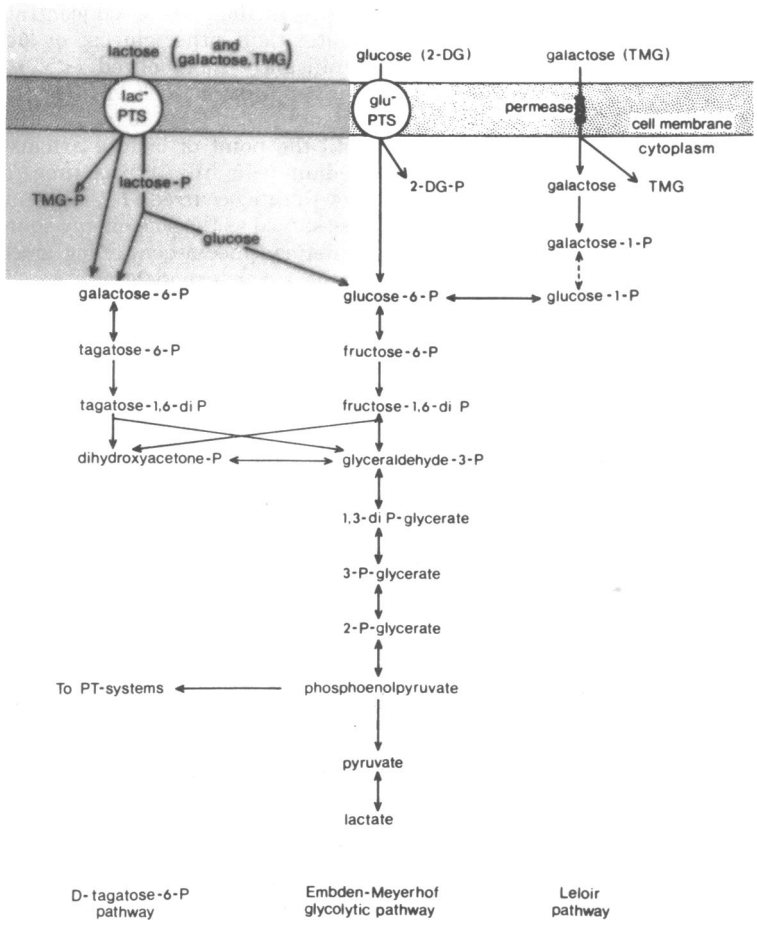

FIG. 1. *Major pathways for the transport and metabolism of sugars in galactose-grown cells of S. lactis ML₃. "Permease" refers to an ATP-dependent, nonphosphotransferase, active transport system. The shaded area indicates absent or defective elements in galactose-grown cells of S. lactis 7962.*

respectively) from the mixture was equivalent to an overall rate of hexose fermentation of ~7 μmol/mg per h.

Nongrowing cells: inhibition of galactose metabolism by glucose and lactose. Both glucose and lactose dramatically depressed the rate of galactose metabolism by galactose-adapted resting cells. As shown in Fig. 5A, glucose and galactose were utilized individually at similar rates (6.2 and 5.6 μmol/mg per h), though the rate of galactose fermentation decreased after 10 to 12 min at the lower sugar concentrations. When presented with the same concentrations of the two sugars together (Fig. 5B), the cells fermented glucose at a near-normal rate (5.8 μmol/mg per h), but galactose metabolism was reduced by 96% to 0.2 μmol/mg per h. Rapid fermentation of galactose (4.1 μmol/mg per h) was resumed only when glucose had been exhausted from the system.

Lactose also depressed the rate of galactose metabolism (Fig. 6A). Separately, galactose and lactose were metabolized at rates of 5.0 and 3.4 μmol/mg per h, respectively, but when they were present simultaneously (Fig. 6B), lactose was utilized first (3.8 μmol/mg per h), and the rate of galactose metabolism decreased by ~94% to 0.3 μmol/mg per h. After exhaustion of the disaccharide from the system (~24 min), galactose metabolism increased to 1.5 μmol/mg per h.

Growth of *S. lactis* ML₃ on galactose: effect of glucose or lactose addition to the medium. The presence of glucose or lactose (Fig. 5B and 6B, respectively) depressed the rate of galactose metabolism by galactose-grown cells of *S. lactis* ML₃ suspended in buffer. The effect of the addition of these sugars to cultures of *S. lactis* ML₃ growing in a defined medium in

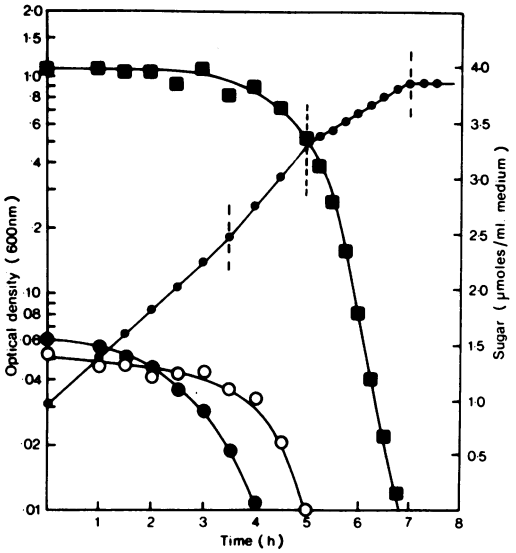

FIG. 2. *Growth of galactose-adapted cells of S. lactis ML_3 in triple-sugar medium. Symbols and initial concentrations (millimolar) of sugars (read on right-hand axis): ●, glucose (1.5); ○, lactose (1.5); and ■, galactose (4). read on left-hand axis: ●, cell growth; dotted lines indicate transition points in sugar metabolism and growth rate.*

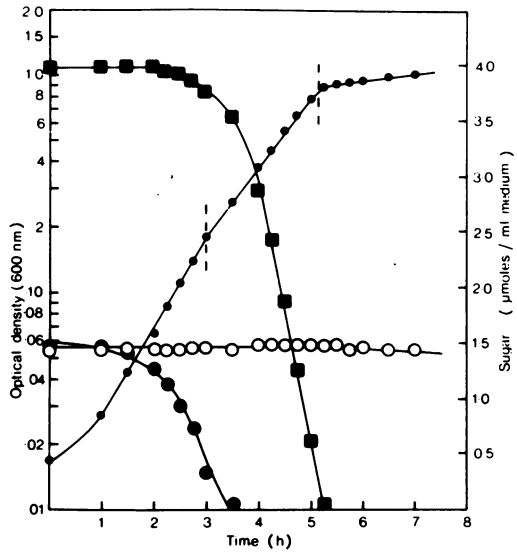

FIG. 3. *Growth of galactose-adapted cells of S. lactis 7962 in triple-sugar medium. Symbols: ●, glucose; ○, lactose; and ■, galactose (all read on right-hand axis); ●, cell growth (read on left-hand axis). Dotted lines indicate transition points in sugar metabolism and growth rate.*

which galactose served initially as the sole energy source was then investigated. The growth of the organism on galactose alone (T_D, ~80 min) ceased after 7 h upon exhaustion of the sugar from the medium (Fig. 7). The addition of glucose to a duplicate culture at 4.5 h, so that

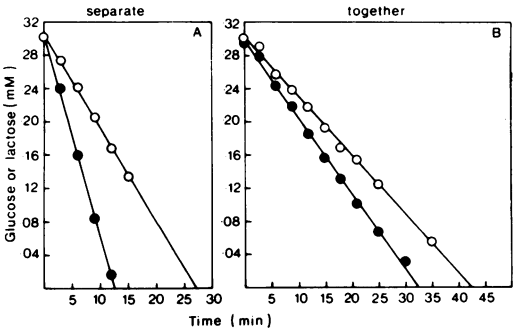

FIG. 4. *Metabolism of glucose and lactose by S. lactis ML_3 (previously grown on galactose) when washed cells were placed in buffer containing (A) individual sugars separately or (B) both sugars together. Experimental conditions are described in the text. Symbols: ●, glucose; ○, lactose.*

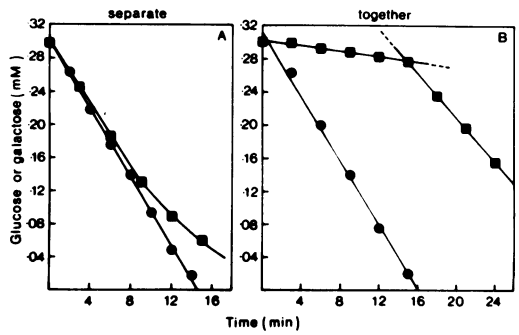

FIG. 5. *Metabolism of glucose and galactose by S. lactis ML_3 (galactose grown) when incubated in buffer with (A) individual sugars separately or (B) both sugars together. Symbols: ●, glucose; ■, galactose.*

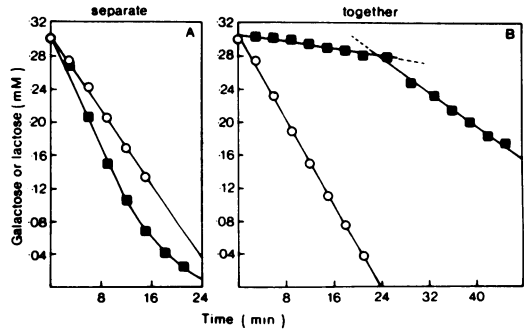

FIG. 6. *Metabolism of lactose and galactose by S. lactis ML_3 (galactose grown) when incubated in buffer with (A) individual sugars separately or (B) both sugars together. Symbols: ■, galactose; ○, lactose.*

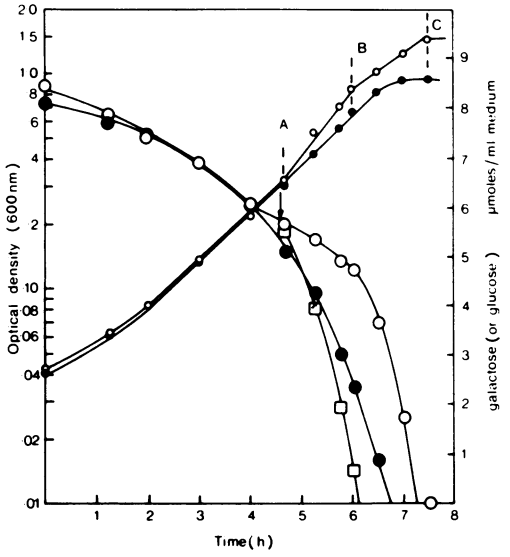

FIG. 7. *Effect of glucose addition to cultures of S. lactis ML_3 growing on galactose. Symbols: ●, cell growth (optical density) in control system: ●, galactose utilization in control system; In the test system: ○, cell growth in test system; ○, galactose utilization in test system; and □, glucose metabolism in test system; ↓, time of glucose addition to the test culture. Dotted lines at A, B, and C show transition points of sugar metabolism and growth rate.*

glucose and residual galactose were present at equivalent concentrations (~5.5 mM), resulted in an immediate increase in growth rate (T_D, ~55 min) and a marked inhibition of galactose utilization when compared with the control, glucose-free system. The increased growth rate was maintained until all the added glucose had been consumed (~6 h), at which time the rate of growth decreased (T_D, ~110 min), coincident with a resumption of galactose metabolism by the cells. Growth ceased (Fig. 7) after about 7.5 h because of sugar exhaustion. No discernible diauxie occurred during transition from growth on galactose to glucose or from glucose back to galactose (Fig. 7).

The results of a similar experiment in which lactose was added to the growing culture are presented in Fig. 8. Addition of the disaccharide (4.5 h) caused an immediate increase in growth rate from a doubling time of about 80 min (on galactose) to about 56 min (on lactose). The preferential metabolism of lactose resulted in pronounced inhibition of galactose metabolism. Growth continued at the faster rate until the point of lactose exhaustion (7 h), when a change to a slower growth rate took place, coincident with the growth of the organism on residual galactose as the sole energy source. Growth rate transitions accompanying the changeover in cellular metabolism from galactose to lactose and back to galactose once more proceeded without apparent diauxie, and growth of *S. lactis* ML_3 finally ceased after 7.5 h because of the exhaustion of sugar from the medium (Fig. 8).

Transport of sugar analogs by intact cells of *S. lactis* ML_3. In a recent report from this laboratory (44), we showed that starved cells of *S. lactis* ML_3 had the capacity to maintain an intracellular PEP pool for many hours, and this unexpected endogenous energy source could support the accumulation of sugar analogs to intracellular levels of 40 to 50 mM. These findings, together with the use of isotopically labeled non-metabolizable analogs TMG and 2-DG, have enabled us to study the transport of solutes mediated by the lac-PTS and glucose (glu)-PTS, respectively, in the intact cell. In the absence of an exogenous energy source, nongrowing cells accumulated TMG via the PEP-dependent lac-PTS to an eventual concentration of 40 to 50 mM (Fig. 9 and 10; also see reference 44). The presence of glucose caused complete inhibition of β-galactoside accumulation (Fig. 9), but glucose was rapidly metabolized (6.3 μmol/mg per h), and the ensuing ATP formation supported the active transport of AIB by the cells (43). At the point of glucose exhaustion (~14 min), indicated by cessation of AIB uptake and verified by direct assays for glucose, an immediate and

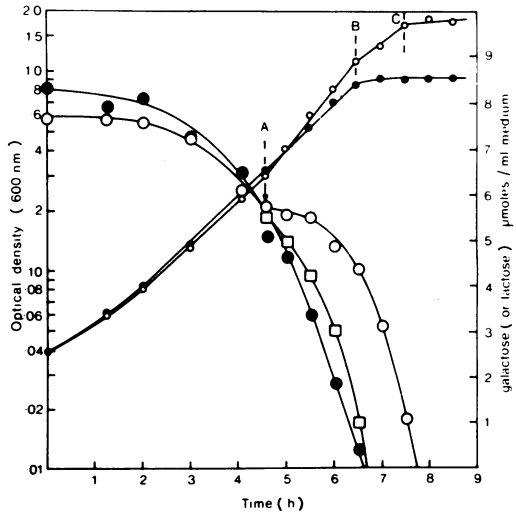

FIG. 8. *Effect of lactose addition to cultures of S. lactis ML_3 growing on galactose. Symbols: ●, cell growth (optical density) in control system; ●, galactose utilization in control system; ○, cell growth in test system; ○, galactose utilization in test system; □, lactose metabolism in test system; ↓, time of lactose addition to test culture. Dotted lines at A, B, and C show transition points of sugar metabolism and growth rate.*

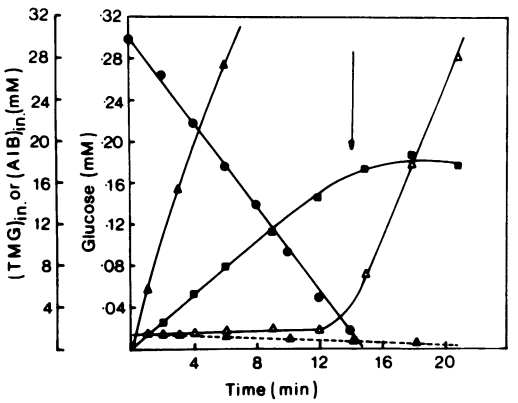

FIG. 9. *Effect of glucose on [^{14}C]TMG and α-[^{14}C]AIB uptake by S. lactis ML$_3$. Cells were suspended at a concentration of 200 μg (dry weight) per ml in 0.1 M Tris-maleate buffer (pH 7.0) containing 0.3 mM glucose. The substrates [^{14}C]TMG (0.1 mM, 0.2 μCi/μmol) and α-[^{14}C]AIB (0.2 mM, 0.2 μCi/μmol) were added to the appropriate system. Filtrates were retained and assayed for residual glucose. Symbols: ▲——▲, TMG uptake (no glucose present); ▲- - -▲, TMG uptake in presence of 2-DG (0.1 mM); △, TMG uptake and, ■, AIB accumulation from systems initially containing glucose; ●, glucose metabolism; ↓, point of glucose exhaustion, cessation of AIB uptake, and commencement of TMG accumulation (~14 min).*

rapid accumulation of TMG occurred. The duration of the inhibitory period before commencement of TMG uptake was directly proportional to the initial concentration of glucose in the system; at 0.1 and 0.2 mM levels, accumulation of TMG did not occur until after 4 to 5 min and 9 min of incubation, respectively (data not shown).

The non-metabolizable glucose analog 2-DG also prevented uptake of the β-galactoside (Fig. 9). The mechanism of 2-DG (and glucose) inhibition was suggested by an experiment presented in Fig. 10. Maximum TMG uptake by the non-growing cells occurred after ~10 to 12 min, but, at an equivalent concentration (0.1 mM), maximum uptake of 2-DG was attained within 20 s. When both analogs were present simultaneously, no uptake of TMG occurred, whereas 2-DG rapidly attained the high level observed under control conditions. These data suggested that the failure of the cells to accumulate TMG was a consequence of a more rapid and preferential utilization of available PEP by substrates of the glu-PTS. TMG exclusion does not appear to be a consequence of direct inhibition of the lac-PTS by 2-DG (and glucose), because we have found that, as the concentration of 2-DG was increased, the extent of TMG uptake decreased accordingly, whereas the activity of the lac-PTS (determined by initial rates of TMG uptake over 1 min of incubation) remained constant (data not shown). Furthermore, the presence of 6-DG, which cannot be transported by the glu-PTS (34, 36, 37), had no significant inhibitory effect upon either the initial rate or final extent of TMG accumulation by the starved cells, even when present at 100-fold greater concentration than the 2-DG analog.

Lactose inhibition of TMG uptake by *S. lactis* ML$_3$. Lactose depressed the metabolism of galactose by resting cells (Fig. 6); furthermore, the disaccharide proved to be a potent inhibitor of TMG accumulation (Fig. 11). In the absence of lactose, the resting cells rapidly accumulated TMG to a maximum concentration of ~45 mM. When lactose was included in the systems at 0.1 mM or 0.2 mM, no accumulation of TMG occurred until after 8 to 9 and 18 min of incubation, respectively. Subsequent analyses of the filtrates (data not shown) showed accumulation of the analog by the cells to be coincident with the points of lactose exhaustion from the system.

Intracellular G6P in *S. lactis* ML$_3$ growing on different sugars. Fructose-adapted cells of *Escherichia coli*, when transferred to a fresh medium containing equimolar concentrations of fructose plus glucose, exhibit a strong preference for glucose as the energy source for growth. Kornberg (15) suggested that the intracellular concentrations of hexose phosphates, particularly G6P, might play an important role in regulating the activity ("fine" control; see references 16 and 18) of previously induced sugar PTS. In the present investigation, glucose and lactose were metabolized in preference to galac-

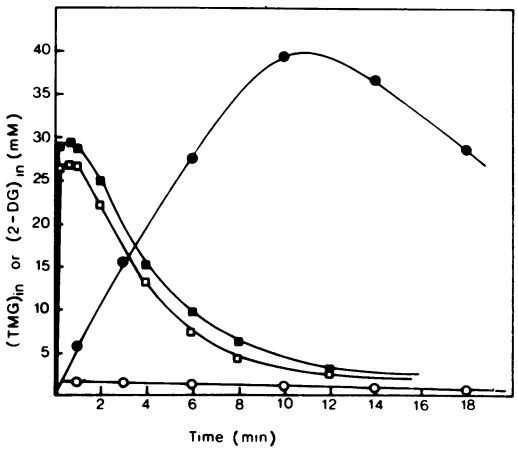

FIG. 10. *2-DG inhibition of [^{14}C]TMG uptake by S. lactis ML$_3$. The basal transport systems, described in Materials and Methods, contained the following substrates at a final concentration of 0.1 mM: ●, [^{14}C]TMG; ○, [^{14}C]TMG plus 2-DG; ■, [^{3}H]2-DG; and □, [^{3}H]2-DG plus TMG.*

tose by galactose-adapted *S. lactis* ML_3 (Fig. 5 and 6); furthermore, the addition of either glucose or lactose to cultures of cells growing solely on galactose (Fig. 7 and 8, respectively) caused immediate inhibition of galactose fermentation but continued growth on the added hexose or disaccharide. By the use of two different extraction procedures, similar intracellular G6P concentrations were found (Table 1) in cells growing solely on either glucose, galactose, or lactose. It is unlikely, therefore, that variation in intracellular G6P concentration is the regulatory factor that permits the cells to utilize lactose in preference to galactose. Attempts to study the possibility that G6P inhibits PTS activity in intact cells exposed to various "decryptifying" procedures (6, 10) were unsuccessful. Only a fraction of the expected PTS activity could be detected with the spectrophotometric assay procedure of Kornberg and Reeves (19).

Transport and metabolism of sugars by *S. lactis* 7962. Galactose-grown cells of *S. lactis* 7962 do not possess a functional lac-PTS, and galactose is apparently metabolized via an ATP-energized permease:Leloir pathway route (Fig. 1). Resting cells of *S. lactis* 7962 metabolized the individual sugars glucose and galactose at similar rates (5.3 and 5.2 μmol/mg per h, respectively). When the sugars were present simultaneously, glucose was utilized preferentially, and the rate of galactose metabolism (~0.8 μmol/mg per h) was depressed by ~80%. Galactose metabolism was resumed at a near-normal rate (4.8 μmol/mg per h) after the exhaustion of glucose from the medium.

Cells of *S. lactis* 7962 grown previously on galactose were unable to ferment lactose, and, in contrast to *S. lactis* ML_3 (Fig. 6), the presence of lactose had no significant inhibitory effect upon the rate of galactose metabolism. The sequence of sugar utilization predicted from the resting cell experiments—glucose, galactose, lactose—was the sequence actually observed when galactose-adapted cells were grown in a mixed-sugar medium (Fig. 3).

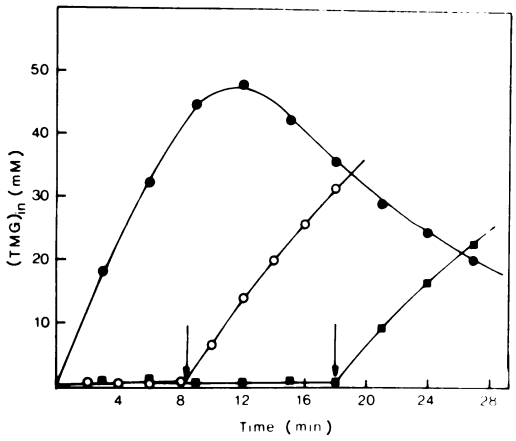

FIG. 11. *Lactose inhibition of [^{14}C]TMG accumulation by nongrowing cells of S. lactis ML_3 in basal transport media containing: ●, no lactose (control); ○, 0.1 mM lactose; and ■, 0.2 mM lactose. Arrows indicate points of lactose exhaustion (data not shown) and commencement of TMG uptake.*

DISCUSSION

Galactose-grown cells of *S. lactis* ML_3 had, on resuspension in buffer, the capacity to metabolize the separate sugars—glucose, galactose, and lactose—by the probable pathways shown in Fig. 1. Subsequent growth of the galactose-adapted cells in a medium containing a mixture of the three sugars was characterized initially by the simultaneous fermentation of glucose and lactose; galactose was not significantly metabolized until the former sugars had been exhausted from the medium (Fig. 2). Preferential utilization of glucose over galactose has previously been reported in other strains of streptococci, including *S. lactis* strains C2 (9) and C10 (11), *S. diacetylactis* (28), and *S. thermophilus* (30), but little is known of the mechanisms by which this selectivity is controlled in these organisms.

The capacity of *E. coli* and some other bacteria to utilize glucose preferentially from mixed-sugar systems is well known (1, 15–18, 22). Orig-

TABLE 1. *Intracellular G6P concentrations in log-phase cells of S. lactis ML_3 growing in batch culture with excess sugar.*

Sugar in medium	Intracellular G6P concn (mM)					
	Extraction method 1[a]			Extraction method 2[a]		
	Expt 1	Expt 2	Mean	Expt 1	Expt 2	Mean
Lactose	3.3	3.8	3.55	2.9	3.1	3.0
Glucose	6.4	5.7	6.05	7.7	5.9	6.8
Galactose	2.8	2.7	2.75	1.9	3.7	2.8

[a] Extraction methods are described in the text. The difference between the values obtained by the two extraction methods was not statistically significant for any specific sugar in the growth medium.

inally termed the "glucose effect," this phenomenon is now recognized as having at least two quite separate possible mechanisms involved in its expression. (i) Glucose, or some product of its catabolism, may repress the synthesis of enzymes required for the metabolism of the second sugar. This "coarse" control (16–18), which affects the level of enzymes within the cell, is termed catabolite repression (26, 29, 32). (ii) Alternatively, glucose may inhibit the utilization of a particular sugar even by cells preinduced for metabolism of that sugar. This latter effect ("fine" control; 16–18), which regulates the activity of existing enzymes, has been called catabolite inhibition (22, 23, 32).

The preferential utilization of glucose (and lactose) over galactose by *S. lactis* ML_3 was clearly demonstrated by the addition of glucose or lactose (Fig. 7 and 8, respectively) to cultures growing exponentially on galactose, when an immediate increase in the growth rate and marked inhibition of galactose metabolism occurred. Catabolite inhibition was evident, because the inoculum and the actively growing cells had not previously been exposed to either glucose or lactose. The rapid and preferential utilization of the added sugars was clearly a consequence of the reduced activity of enzymes available for galactose metabolism and not of a failure of the cells to induce the necessary enzymes for galactose utilization. In nongrowing cells, the inhibitory effects of glucose and lactose (Fig. 5 and 6) were not caused by catabolite repression, because, again, the cells were already fully induced for the necessary complement of enzymes for galactose metabolism. Neither was the preferential utilization a consequence of more rapid metabolism of the inhibitory sugars than of galactose by the cells. The prompt resumption of galactose metabolism by the nongrowing cells after exhaustion of glucose or lactose from the system was further evidence of catabolite inhibition by the latter sugars.

Mechanism of glucose inhibition of galactose metabolism and TMG uptake. Group N streptococci have the enzymatic potential to metabolize galactose via the Leloir or D-tagatose-6-phosphate pathways, but the relative participation of the two routes is not yet known (4, 9, 21). However, data obtained with *S. lactis* C2 (9) suggested that metabolism of the sugar occurs predominantly via the lac-PTS:D-tagatose-6-phosphate sequence. The transport of lactose, galactose, and TMG in *S. lactis* ML_3 is most probably mediated by the PEP-dependent lac-PTS (see below and Fig. 1). Glucose fermentation generates PEP, but the inability of the cells to accumulate TMG in the presence of glucose (Fig. 9) showed that the intracellular PEP was not available to the TMG (and galactose) transport system. These data suggest that a preferential utilization of intracellular PEP may accompany the transport of solutes via the glu-PTS. Further evidence to support this contention was obtained by using starved cells that contained a high endogenous pool of PEP (44) with 2-DG and TMG to monitor activities of the glu- and lac-PTS, respectively. When the nonmetabolizable analogs were present simultaneously, a preferential and extremely rapid uptake of 2-DG occurred that depleted the cells of the available PEP pool (J. Thompson, unpublished data) and resulted in the exclusion of TMG. As the concentration of 2-DG was increased, the extent of TMG accumulation decreased accordingly, but the initial rate of uptake of the β-galactoside remained constant. The data suggested that 2-DG did not directly inhibit at the TMG recognition site (enzyme 11-B^{lac}), but such a result would have been predicted on the basis of competition between the glu- and lac-PTS for a common and finite concentration of energy source (PEP, 40 to 50 mM; reference 44). The glucose analog 6-DG, which cannot be transported by the glu-PTS, failed to inhibit TMG uptake by the cells. We suggest that, in *S. lactis* ML_3, the process of glucose (or 2-DG) translocation, with attendant preferential utilization of PEP, results in the exclusion of both TMG and galactose from the cells. McGinnis and Paigen (23) also concluded that catabolite inhibition in *E. coli* occurred during the transport of glucose across the cell membrane, whereas Kornberg (15) suggested some years ago that either the process of glucose phosphorylation or the intracellular concentration of G6P was the cause of preferential utilization of glucose over fructose. Recent data obtained by Clark and Holms (7) suggest that an interaction occurs between the two PTSs such that glucose denies access of fructose to its own specific transport system, thereby ensuring glucose dominance in the mixed-sugar medium (see also reference 2).

Mechanism of lactose inhibition of galactose metabolism and TMG uptake. The inhibition of galactose metabolism (Fig. 6) and TMG uptake by lactose (Fig. 11), though superficially similar to the effect produced by glucose, may be a reflection of differing affinities of the potential galactoside substrates for a common binding component (enzyme 11-B^{lac}) of the lac-PTS. The results of complementation studies (24) suggest a close similarity between the lac-PTS in *S. aureus* (39–41) and *S. lactis*. By analogy with *S. aureus*, the transport of galactosides (lactose, galactose, TMG, and isopropyl-β-thio-D-galactopyranoside) by cells of *S. lactis* ML_3 may be represented as follows:

$$\text{PEP} + \text{HPr} \xrightleftharpoons{\text{enzyme 1, } Mg^{2+}} \text{P-HPr} + \text{pyruvate} \quad (1)$$

$$\text{P-HPr} + \tfrac{1}{3}\ \text{factor III}^{lac} \rightleftharpoons \tfrac{1}{3}\ \text{P-factor III}^{lac} + \text{HPr} \quad (2)$$

$$\tfrac{1}{3}\ \text{P-factor III}^{lac} + \text{galactoside} \xrightleftharpoons{\text{enzyme 11-B}^{lac}, Mg^{2+}} \text{galactoside-6-P} + \tfrac{1}{3}\ \text{factor III}^{lac} \quad (3)$$

Enzyme 1 is a soluble protein, and enzyme 11-B^{lac} is a membrane-bound component that exhibits binding specificity toward galactosides. Factor III^{lac} is a sugar-specific factor found in the soluble fraction of the cell, and HPr is a low-molecular-weight phospho-carrier protein. In the group N streptococci, enzyme 1 and HPr are constitutive, whereas enzyme 11-B^{lac}, factor III^{lac}, and the enzymes associated with the tagatose-6-phosphate pathway are induced by galactose-6-phosphate (24) to high levels when the cells are grown on galactose (or lactose).

In *S. aureus*, the substrate specificity (relative K_m) of several galactosides with respect to the reconstituted lac-PTS was studied, with TMG as the standard for affinity. It was found that lactose had the greatest affinity and was by far the best substrate for the binding and recognition protein enzyme 11-B^{lac} (39, 40). If, in *S. lactis* ML_3, the affinity of enzyme 11-B^{lac} for lactose is much greater than for galactose or TMG, then the preferential utilization of the disaccharide from a mixture of lactose and galactose (Fig. 6), as well as the inhibition of TMG uptake by lactose (Fig. 11), may be explained. The PEP-dependent accumulation of TMG (and galactose) occurred only after lactose exhaustion from the system (Fig. 6, 11), when presumably the PEP generated from endogenous glycolytic intermediates (44) could be used for the transport of TMG and galactose in the absence of any competitive effect from exogenous lactose for the enzyme 11-B^{lac} protein.

Paigen and Williams (32) suggested that the concentration of the glucose-enzyme 11 complex formed and its affinity for P-HPr, compared with the amounts and affinity of other enzyme 11-sugar complexes, may determine the intensity with which glucose inhibits the entry of other sugars into the cell. In this context, we found that although glucose prevented the lac-PTS-mediated uptake of TMG and galactose, cells of *S. lactis* ML_3 had the capacity to transport and metabolize glucose and lactose simultaneously under nongrowing conditions and during exponential growth in a mixed-sugar medium. The simultaneous metabolism of glucose and lactose by growing cells of *S. lactis* C2 has been reported by Schifsky and McKay (38).

Catabolite inhibition in *S. lactis* 7962. The "glucose effect" discussed previously for *S. lactis* ML_3 cannot be invoked in the case of the lactose-defective variant *S. lactis* 7962, because, in the absence of a functional lac-PTS (24, 25), the entry and accumulation of TMG and galactose by cells of this strain is mediated by an adenosine 5'-triphosphate-dependent, active transport system (13, 14). Though galactose and TMG are transported by the same non-phosphotransferase carrier system, the presence of glucose (a PTS sugar; J. Thompson, unpublished data) prevented the metabolism of galactose but not the uptake of TMG (13, 44; also see Fig. 1). Saier and Roseman (35) have discussed three possible mechanisms whereby the utilization of non-PTS sugars may be controlled by their PTS counterparts, namely: (i) regulation of uptake of the non-PTS sugar (inducer exclusion), (ii) regulation of the activities of induced catabolic enzyme systems (catabolite inhibition), and (iii) control of induction or synthesis of enzymes of a particular catabolic system. Of the three postulated mechanisms, only (ii)—catabolite inhibition—offers a plausible explanation for our observations with pre-induced cells of *S. lactis* 7962. Glucose (or a subsequent metabolite), by regulating the activity of enzyme(s) of the Leloir sequence, may control the flux of galactose through this pathway.

In contrast to *S. lactis* ML_3, accumulation of TMG and the metabolism of galactose by *S. lactis* 7962 were unaffected by the inclusion of lactose in the incubation medium. The transport system for galactosides in *S. lactis* 7962 was therefore specific for galactose and TMG, having little affinity for the disaccharide. Data obtained previously by Kashket and Wilson (13) support this conclusion. Postma and Roseman (33) have suggested that the M protein in *E. coli* may be a modified form of enzyme 11-B^{lac}, but whether this is the case for the galactoside "permease" in *S. lactis* 7962 is not known.

The patterns of sequential sugar utilization for each *S. lactis* strain under growing and nongrowing conditions were identical, and we suggest that catabolite inhibition in various forms may play an important role in this process. Fine control of the activities of pre-existing enzyme systems may therefore permit *S. lactis* ML_3 and *S. lactis* 7962 to make an immediate and effective choice between alternative energy sources during growth in an environment containing a mixture of potentially fermentable sugars.

LITERATURE CITED

1. **Adhya, S., and H. Echols.** 1966. Glucose effect and the galactose enzymes of *Escherichia coli*: correlation between glucose inhibition of induction and inducer transport. J. Bacteriol. **93**:601-608.
2. **Bag, J. 1974.** Glucose inhibition of the transport and phosphoenolpyruvate-dependent phosphorylation of galactose and fructose in *Vibrio cholerae*. J. Bacteriol. **118**:764-767.
3. **Bissett, D. L., and R. L. Anderson.** 1973. Lactose and D-galactose metabolism in *Staphylococcus aureus*: pathway of D-galactose 6-phosphate degradation. Biochem. Biophys. Res. Commun. **52**:641-647.
4. **Bissett, D. L., and R. L. Anderson.** 1974. Lactose and D-galactose metabolism in group N streptococci: presence of enzymes for both the D-galactose 1-phosphate and D-tagatose 6-phosphate pathways. J. Bacteriol. **117**:318-320.
5. **Bissett, D. L., and R. L. Anderson.** 1974. Genetic evidence for the physiological significance of the D-tagatose 6-phosphate pathway of lactose and D-galactose degradation in *Staphylococcus aureus*. J. Bacteriol. **119**:698-704.
6. **Citti, J. E., W. E. Sandine, and P. R. Elliker.** 1965. β-Galactosidase of *Streptococcus lactis*. J. Bacteriol. **89**:937-942.
7. **Clark, B., and W. H. Holms.** 1976. Control of the sequential utilization of glucose and fructose in *Escherichia coli*. J. Gen. Microbiol. **95**:191-201.
8. **Collins, L. B., and T. D. Thomas.** 1974. Pyruvate kinase of *Streptococcus lactis*. J. Bacteriol. **120**:52-58.
9. **Cords, B. R., and L. L. McKay.** 1974. Characterization of lactose-fermenting revertants from lactose-negative *Streptococcus lactis* C2 mutants. J. Bacteriol. **119**:830-839.
10. **Gachelin, G.** 1969. A new assay of the phosphotransferase system in *Escherichia coli*. Biochem. Biophys. Res. Commun. **34**:382-387.
11. **Gilliland, S. E., M. L. Speck, and J. R. Woodard.** 1972. Stimulation of lactic streptococci in milk by β-galactosidase. Appl. Microbiol. **23**:21-25.
12. **Johnson, K. G., and I. J. McDonald.** 1974. β-D-Phosphogalactoside galactohydrolase from *Streptococcus cremoris* HP: purification and enzyme properties. J. Bacteriol. **117**:667-674.
13. **Kashket, E. R., and T. H. Wilson.** 1972. Role of metabolic energy in the transport of β-galactosides by *Streptococcus lactis*. J. Bacteriol. **109**:784-789.
14. **Kashket, E. R., and T. H. Wilson.** 1974. Protonmotive force in fermenting *Streptococcus lactis* 7962 in relation to sugar accumulation. Biochem. Biophys. Res. Commun. **59**:879-886.
15. **Kornberg, H. L.** 1972. Nature and regulation of hexose uptake by *Escherichia coli*, p. 157-180. *In* J. F. Woessner and F. Huijing (ed.), The molecular basis of biological transport. Miami Winter Symposia, vol. 3. Academic Press Inc., New York.
16. **Kornberg, H. L.** 1973. Fine control of sugar uptake by *Escherichia coli*, p. 175-193. *In* D. D. Davis (ed.), Rate control of biological processes. Symp. Soc. Exp. Biol. XXVII.
17. **Kornberg, H. L.** 1976. The nature and control of carbohydrate uptake by *Escherichia coli*. FEBS Lett. **63**:3-9.
18. **Kornberg, H. L., and M. C. Jones-Mortimer.** 1977. The phosphotransferase system as a site of cellular control, p. 217-240. *In* B. A. Haddock and W. A. Hamilton (ed.), Microbial energetics. Symposium of the Society for General Microbiology. Cambridge University Press, Cambridge.
19. **Kornberg, H. L., and R. E. Reeves.** 1972. Inducible phosphoenolpyruvate-dependent hexose phosphotransferase activities in *Escherichia coli*. Biochem J. **128**:1339-1344.
20. **Lawrence, R. C., T. D. Thomas, and B. E. Terzaghi.** 1976. Reviews of the progress of dairy science: cheese starters. J. Dairy Res. **43**:141-193.
21. **Lee, R., T. Molskness, W. E. Sandine, and P. R. Elliker.** 1973. Carbohydrate metabolism in lactic streptococci: fate of galactose supplied in free or disaccharide form. Appl. Microbiol. **26**:951-958.
22. **McGinnis, J. F., and K. Paigen.** 1969. Catabolite inhibition: a general phenomenon in the control of carbohydrate utilization. J. Bacteriol. **100**:902-913.
23. **McGinnis, J. F., and K. Paigen.** 1973. Site of catabolite inhibition of carbohydrate metabolism. J. Bacteriol. **114**:885-887.
24. **McKay, L., A. Miller III, W. E. Sandine, and P. R. Elliker.** 1970. Mechanisms of lactose utilization by lactic acid streptococci: enzymatic and genetic analyses. J. Bacteriol. **102**:804-809.
25. **McKay, L. L., L. A. Walter, W. E. Sandine, and P. R. Elliker.** 1969. Involvement of phosphoenolpyruvate in lactose utilization by group N streptococci. J. Bacteriol. **99**:603-610.
26. **Magasanik, B.** 1961. Catabolite repression. Cold Spring Harbor Symp. Quant. Biol. **26**:249-256.
27. **Molskness, T. A., D. R. Lee, W. E. Sandine, and P. R. Elliker.** 1973. β-D-Phosphogalactoside galactohydrolase of lactic streptococci. Appl. Microbiol. **25**:373-380.
28. **Moustafa, H. H., and E. B. Collins.** 1968. Role of galactose or glucose-1-phosphate in preventing the lysis of *Streptococcus diacetilactis*. J. Bacteriol. **95**:592-602.
29. **Neidhardt, F. C., and B. Magasanik.** 1956. Inhibitory effect of glucose on enzyme formation. Nature (London) **178**:801-802.
30. **O'Leary, V. S., and J. H. Woychik.** 1976. Utilization of lactose, glucose, and galactose by a mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in milk treated with lactase enzyme. Appl. Environ. Microbiol. **32**:89-94.
31. **Opheim, D., and R. W. Bernlohr.** 1973. Purification and regulation of glucose-6-phosphate dehydrogenase from *Bacillus licheniformis*. J. Bacteriol. **116**:1150-1159.
32. **Paigen, K., and B. Williams.** 1970. Catabolite repression and other control mechanisms in carbohydrate utilization, p. 251-324. *In* A. H. Rose and J. F. Wilkinson (ed.), Advances in microbial physiology, vol. 4. Academic Press Inc., New York.
33. **Postma, P. W., and S. Roseman.** 1976. The bacterial phosphoenolpyruvate: sugar phosphotransferase system. Biochim. Biophys. Acta **457**:213-257.
34. **Romano, A. H., S. J. Eberhard, S. L. Dingle, and T. D. McDowell.** 1970. Distribution of the phosphoenolpyruvate:glucose phosphotransferase system in bacteria. J. Bacteriol. **104**:808-813.
35. **Saier, M. H., and S. Roseman.** 1976. Sugar transport. Inducer exclusion and regulation of the melibiose, maltose, glycerol and lactose transport systems by the phosphoenolpyruvate:sugar phosphotransferase system. J. Biol. Chem. **251**:6606-6615.
36. **Schachtele, C. F.** 1975. Glucose transport in *Streptococcus mutans*: preparation of cytoplasmic membranes and characteristics of phosphotransferase activity. J. Dent. Res. **54**:330-338.
37. **Schachtele, C. F., and J. A. Mayo.** 1973. Phosphoenolpyruvate-dependent glucose transport in oral streptococci. J. Dent. Res. **52**:1209-1215.
38. **Schifsky, R. F., and L. L. McKay.** 1974. Isolation of *Streptococcus lactis* C_2 mutants with high phospho-β-galactosidase activity. J. Dairy Sci. **58**:482-493.
39. **Simoni, R. D., J. B. Hays, T. Nakazawa, and S. Roseman.** 1973. Sugar transport VI. Phosphoryl transfer in the lactose phosphotransferase system of *Staphylococ-*

cus aureus. J. Biol. Chem. **248**:957–965.

40. **Simoni, R. D., T. Nakazawa, J. B. Hays, and S. Roseman.** 1973. Sugar transport IV. Isolation and characterization of the lactose phosphotransferase system in *Staphylococcus aureus*. J. Biol. Chem. **248**:932–940.
41. **Simoni, R. D., and S. Roseman.** 1973. Sugar transport VII. Lactose transport in *Staphylococcus aureus*. J. Biol. Chem. **248**:966–976.
42. **Thomas, T. D.** 1976. Regulation of lactose fermentation in group N streptococci. Appl. Environ. Microbiol. **32**:474–478.
43. **Thompson, J.** 1976. Characteristics and energy requirements of an α-aminoisobutyric acid transport system in *Streptococcus lactis*. J. Bacteriol. **127**:719–730.
44. **Thompson, J., and T. D. Thomas.** 1977. Phosphoenolpyruvate and 2-phosphoglycerate: endogenous energy source(s) for sugar accumulation by starved cells of *Streptococcus lactis*. J. Bacteriol. **130**:583–595.