

# Malate Utilization by a Group D *Streptococcus*: Regulation of Malic Enzyme Synthesis by an Inducible Malate Permease

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Induction of an nicotinamide adenine dinucleotide-specific malic enzyme and a malate entry system permits *Streptococcus faecalis* to grow at the expense of malate. Evidence is presented which shows that biosynthesis of the permease, but not of the malic enzyme, is subject to catabolite repression by glucose. In contrast to the malic enzyme, the catalytic function of the entry system does not appear to be inhibited by intermediate products of glycolysis. Although the induction of the entry system does not appear to be coordinated with the induction of the malic enzyme, the latter process is dependent upon the permease for the transport and accumulation of inducer.

In a previous publication (5), we discussed the kinetics of induction and biochemical characteristics of a streptococcal malic enzyme [L-malate: nicotinamide adenine dinucleotide (NAD) oxidoreductase (decarboxylating) E.C. 1.1.1.39]. Addition of 0.020 to 0.075% glucose to cultures of *Streptococcus faecalis* growing at the expense of malate did not significantly repress the synthesis of the malic enzyme. However, malate dissimilation was completely inhibited at the lowest glucose concentration tested. Utilization of malate was resumed after the disappearance of glucose from the medium. It was subsequently demonstrated that fructose-1,6-diphosphate (FDP) and 3-phosphoglycerate (3-PGA) were inhibitors of the malic enzyme (6). We postulated that the accumulation of one or both of the glycolytic intermediate products was responsible for the inhibition of malate utilization during glucose dissimilation.

From the evidence at hand, it appeared that essentially all regulation of malate metabolism was effected at the level of enzyme function. However, the organism is capable of exerting yet another form of control over malate utilization. A description of that regulatory mechanism is the subject of this report.

## MATERIALS AND METHODS

**Maintenance and growth.** The organism used in these and previous studies, a typical strain of *S. faecalis* recently designated MR, was a gift from M. Rogosa. Procedures for maintenance of the organism

and the composition of the complex malate-containing medium used for its cultivation have been described previously (5). Unless specified otherwise, this medium, containing 0.3% sodium or ammonium L(+)-malate, was used in all growth experiments. Protocol for growth experiments, sampling of cultures and processing of samples were also described earlier (5). Where heavy cell suspensions were used to inoculate cultures, 250-ml seed cultures were harvested aseptically after 14 hr of growth and were resuspended in 5 ml of basal medium (without substrate). The cell density of the growth medium was adjusted to the desired concentration with the aseptically prepared cell suspension. To measure rate and extent of growth accurately in cultures attaining optical densities of 1.5 or greater, as measured with a Gilford model 300 spectrophotometer at 600 nm, dilutions with basal medium were necessary. One optical density (OD) unit is equivalent to 0.16 mg of protein per ml of culture.

**Measurement of malate permease activity.** Cultures (1.5 liter) grown on 0.3% ammonium-L(+)-malate or 0.3% ammonium-L(+)-malate and various concentrations of glucose (generally between 0.1 and 0.3%) were harvested at the end of exponential growth and washed once with 30 ml of 0.05 M potassium phosphate buffer, pH 7.4. The washed cells were adjusted to a density of 3 to 5 mg of protein per ml with the same buffer and stored at 4°C until used. A 5-ml amount of cell suspension was added to a 50-ml Erlenmeyer flask containing a magnetic bar, and the contents were aerated on a magnetic stirrer. After equilibration to ambient temperature, 5 ml of a solution containing 0.044 M sodium-L(+)-malate and 1  $\mu$ C of uniformly-labeled sodium-L(+)-<sup>14</sup>C-malate in 0.05 M potassium phosphate buffer (pH 7.4) was

added to the cell suspension. The final concentration of malate was equal to that amount added to the growth medium (0.3%). In some experiments, glucose was added to the radioactive malate solution to give a final concentration of 0.2% in the reaction mixture.

Samples (1 ml) were removed at 3-min intervals over a 15- to 20-min period and were rapidly filtered through cellulose membrane filters (Millipore Corp., Bedford, Mass.) with an average pore size of 0.45  $\mu$ m. Cells retained on the filter pad were washed twice with 5 ml of chilled (4 C) 0.05 M potassium phosphate buffer (pH 7.4) or once with 5 ml of chilled 5% trichloroacetic acid followed by a wash with the buffer. The filters were placed in glass vials and solubilized in a xylene-containing scintillation fluid (3). Radioactivity retained by the cells was measured in a Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.).

**Enzyme assays and chemical determinations.** The methods used for the preparation of cell-free extracts by ultrasonic treatment and estimation of malic enzyme activity in cell-free extracts or resting cell suspensions were described previously (5). Glucose was measured by the glucostat method (Worthington Biochemical Corp., Freehold, N.J.). Protein was determined by the La Riviere modification of the biuret method (J. W. M. La Riviere, Ph.D. Thesis, Technische Hogeschool, Delft, 1958) or by the method of Lowry et al. (7) with crystalline bovine serum albumin as standard.

## RESULTS

**Effect of glucose concentration on malic enzyme production.** A series of cultures containing increasing amounts of glucose and 0.3% L-malate was analyzed for malic enzyme activity at the end of exponential growth. No significant decrease in the specific activity of malic enzyme in cell extracts was noted at glucose concentrations below 0.1%; addition of 0.1% glucose resulted in a slight decrease in specific activity. However, at a level of 0.2% or greater a marked decrease in malic enzyme specific activity was noted (Table 1, section A). Mixed extract experiments suggested that the lower levels of malic enzyme activity observed in the latter extracts resulted from a reduction in the quantity of enzyme synthesized and not from a soluble inhibitor present in the extract. Since the decrease in specific activity of malic enzyme was accompanied by a decrease in the pH of the culture, it was necessary to determine whether the two events were related. To dissociate the effect of pH change from inhibition of malic enzyme synthesis, the buffering capacity of the medium was increased by the addition of  $K_2HPO_4 \cdot 3H_2O$  to a final concentration of 2.0% (complex medium B). This concentration of phosphate salts did not alter the growth characteristics of the organism and maintained the pH of the culture at 6.7 at the highest

TABLE 1. *Effect of glucose concentration and conditions of growth on the synthesis of malic enzyme<sup>a</sup>*

Glucose concn <sup>b</sup>	A. Complex medium		B. High-phosphate complex medium		C. 2X Complex medium	
	Specific activity <sup>c</sup>	Final pH	Specific activity	Final pH	Specific activity	Final pH
0	0.400	8.4	0.320	8.0	0.320	7.1
0.1	0.310	8.2	0.200	7.6	0.260	7.1
0.2	0.067	6.3	0.073	7.1	0.150	6.9
0.3	0.006	5.4	0.018	6.9	0.02	5.8
0.4			0.007	6.7	0.006	5.5

<sup>a</sup> Growth experiments were carried out with (A) conventional complex malate medium or complex medium modified as follows. (B) The concentration of  $K_2HPO_4 \cdot 3H_2O$  was increased from 0.5 to 2.0%. (C) All components of the medium were increased twofold. After inoculation with an aerobically grown malate culture to give an initial  $OD_{600}$  of approximately 0.10, the flasks were incubated at 30 C on a reciprocating shaker.

<sup>b</sup> Expressed as grams per 100 ml of medium.

<sup>c</sup> Expressed as  $\mu$ moles NAD reduced/min/mg protein.

glucose concentration tested. The results did not differ significantly from those obtained in the former experiment (Table 1, section B). However, only relatively small increases in the final cell yield were observed at concentrations of glucose greater than 0.2%, indicating that one or more growth-limiting nutrients in the medium had been exhausted. Doubling the concentration of tryptone and yeast extract in the medium (complex medium C) had two effects; the final cell yield of the culture was increased twofold and a substantial amount of malic enzyme was synthesized in the presence of 0.2% glucose (Table 1, section C). These results suggested that synthesis of malic enzyme and malate utilization occurred after the limiting quantity of glucose had been metabolized.

**Kinetics of induction of malic enzyme during growth on malate and glucose.** The preceding experiments suggested that glucose inhibited the formation of malic enzyme. However, by the method of examination employed, the inhibition was not apparent at concentrations below 0.1%. To estimate the lowest concentration of hexose capable of inhibiting the formation of malic enzyme, the period of induction and rate of malic enzyme synthesis were observed during aerobic growth in complex malate medium supplemented with 0.025, 0.050, 0.075 or 0.1% glucose. The rate of enzyme formation of a control culture grown on malate alone is shown in Fig. 1A. With the onset of exponential growth, a relatively rapid

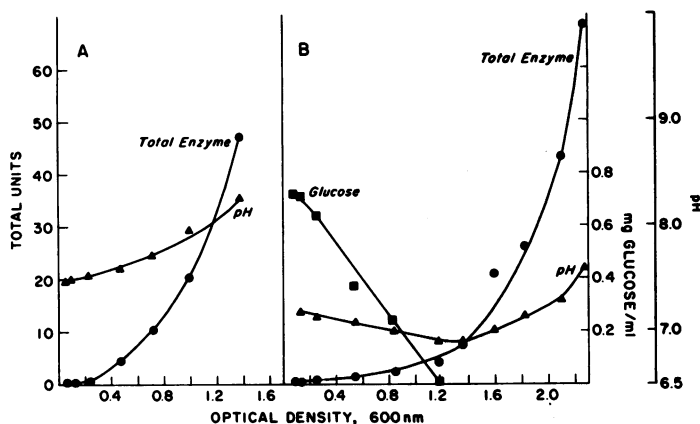


FIG. 1. Effect of glucose on the induction of the malic enzyme; (A) 0.3% *L*-malate control; (B) 0.3% *L*-malate medium supplemented with 0.075% glucose. Total enzyme units expressed as  $\mu$ moles of NAD reduced per min per 600 ml of culture.

rate of enzyme synthesis was observed. The release of ammonium ions during malate dissimilation and subsequent formation of  $\text{NH}_4\text{OH}$  was probably responsible for the pH increase. In the presence of glucose (0.075%), formation of the malic enzyme was repressed until the exogenous supply of sugar was utilized (Fig. 1B). The hydrogen ion concentration of the medium, which had increased during glucose dissimilation, decreased as malate was metabolized. From Fig. 1A and 1B, it can be seen that the rate of malic enzyme synthesis was not linear with the increase in cell mass. A semilogarithmic plot of the data (not shown) established that the rate of enzyme synthesis was actually a logarithmic function of increase of cell mass.

Replotting the data on a semilogarithmic scale, the transition from glucose to malate-mediated growth became readily apparent (Fig. 2). Distinct biphasic slopes can be seen for increase in optical density and total enzyme units. Initiation of growth on malate was accompanied by a 30-fold increase in the rate of malic enzyme synthesis per unit time and a decrease of the specific growth rate. Except for the length of time during which the repression was in effect, the results obtained with 0.025, 0.050, and 0.1% glucose, respectively, were essentially the same as those described above. In all four experiments, a low level of malic enzyme synthesis similar to that shown in Fig. 1B was observed during growth on glucose. The quantity of enzyme formed per milligram of cell protein was nearly identical in all instances and appeared to be independent of glucose concentration in the range tested.

**Characterization of the malate permease.** The results of the growth experiments described here appeared to contradict those of earlier experi-

ments (5) in which the addition of 0.025 to 0.075% glucose to cultures growing at the expense of malate did not produce a marked reduction of malic enzyme synthesis. Two subsequent experimental observations were made which appeared to have a direct bearing on this apparent anomaly. First, the dye reduction assay used to measure malic enzyme activity in malate-grown cells was found to be unreliable when glucose was added to the medium. The linear relationship between dye reduction and the direct determination of malic enzyme in extracts by NAD reduction which was typical of growth on malate no longer existed when glucose was included in the complex malate medium. Evidence will be presented later which suggests that in the presence of glucose the dye reduction assay probably measures malate transport activity rather than malic enzyme activity. The second experiment demonstrated that synthesis of the dye-reducing system was not inhibited during growth on any level of glucose. Cells harvested from complex malate medium supplemented with either 0.1% (induced) or 0.3% glucose (noninduced) oxidized the hexose with concomitant dye reduction; however, only the cell suspension grown at the lower glucose concentration oxidized malate rapidly using the dye as a terminal electron acceptor (Table 2). The presence of a diaphorase-like enzyme and significant levels of malic enzyme in cells harvested from malate medium containing 0.3% glucose suggested that some other component of the malate oxidation system was inoperative or absent. For this reason, a study of the malate entry system was initiated.

Resting cell suspensions of malate-grown cells rapidly accumulated  $^{14}\text{C}$ -malate (Fig. 3). A single wash with cold 5% trichloroacetic acid removed

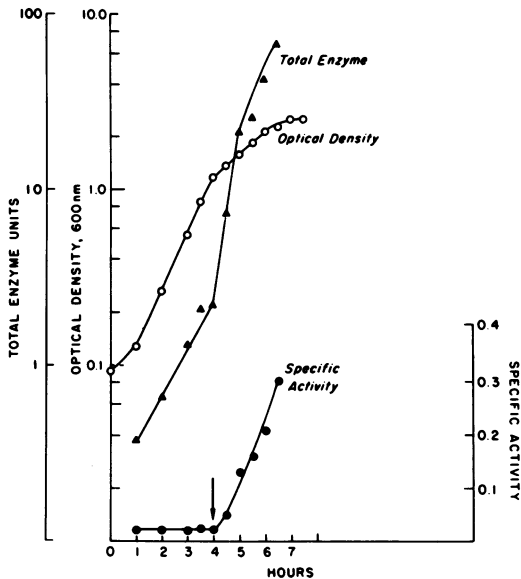


FIG. 2. Biphasic growth of *S. faecalis* on glucose and malate. Arrow indicates the point at which exogenously supplied glucose disappeared from the medium. Total enzyme units expressed as  $\mu$ moles of NAD reduced per min per 600 ml of culture. Specific activity is expressed as micromoles of NAD reduced per minute per milligram of protein.

TABLE 2. Relative rates of dye reduction by resting-cell suspensions oxidizing malate or glucose

Concn of substrates added to the medium <sup>a</sup>	Specific activities <sup>b</sup>	
	Malate	Glucose
0.1% Glucose + 0.3% malate	0.410	0.085
0.3% Glucose + 0.3% malate	0.009	0.215

<sup>a</sup> Growth experiments carried out in complex malate medium supplemented with glucose. Cultures were grown aerobically at 30 C on a reciprocating shaker, and cells were harvested from the stationary-phase of growth.

<sup>b</sup> Expressed as micromoles of dye (dichlorophenolindophenol) reduced per minute per milligram of cell protein.

approximately 80% of the radioactivity from cells trapped on the filter pad, suggesting little incorporation of malate carbon into cell material had occurred during the course of the experiment. Cells harvested from a malate control culture were compared with cells grown in malate medium supplemented with 0.2% glucose for permease activity (Fig. 4). Accumulation of <sup>14</sup>C-malate by cells grown in 0.2% glucose-malate medium was barely detectable. Addition of 0.2% glucose had no adverse effect on the entry system of the mal-

ate-grown control; in fact, the extent of malate accumulation was markedly increased. In another experiment not shown here, the rate of <sup>14</sup>C-malate uptake by cells harvested from complex malate medium containing 0.1% glucose was comparable to the rate observed with the malate control

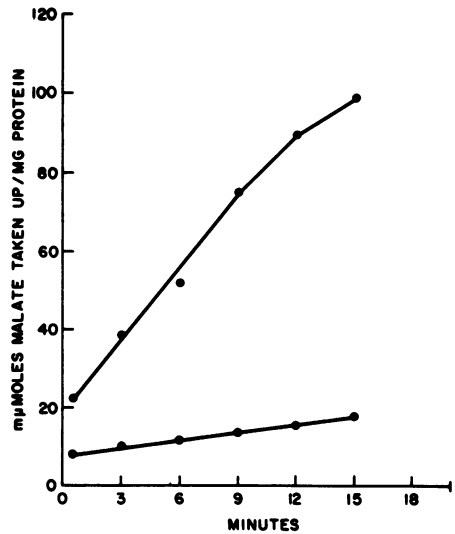


FIG. 3. Accumulation of <sup>14</sup>C-malate by malate-induced resting cells. Cells washed twice with phosphate buffer (●); cells washed once each with 5% trichloroacetic acid and phosphate buffer (○).

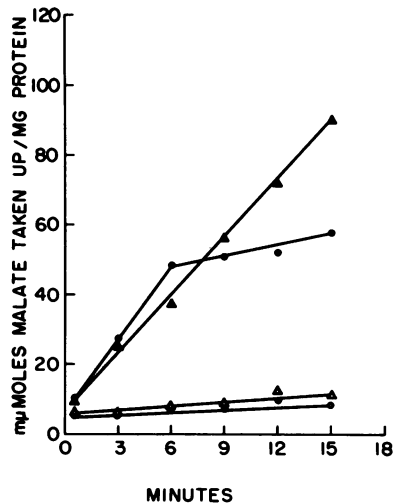


FIG. 4. Repression of malate permease by addition of saturating levels of glucose to complex malate medium. Cells grown on malate medium (closed symbols); <sup>14</sup>C-malate (●); <sup>14</sup>C-malate plus 0.2% glucose (▲). Cells grown on malate medium supplemented with glucose (open symbols); <sup>14</sup>C-malate (○); <sup>14</sup>C-malate plus 0.2% glucose (Δ).

culture, whereas cells grown in 0.2% glucose again failed to take up radioactive malate. The cumulative results of these experiments and those summarized in Table 1 suggest that the synthesis of malate permease and malic enzyme are inter-related events.

To establish that the radioactivity in the cells represented an accumulation of malate, a 10-fold excess of unlabeled malate was added to a cell suspension which had been incubated in the presence of radioactive substrate for 25 min. Within 3 min, 65% of the cell-associated radioactivity was lost to the filtrate supernatant fluid. Although no attempt was made to identify the radioactive substance, the rapidity with which the exchange occurred suggests that the major portion of radioactive material in the cells was indeed malate.

In the absence of glucose, the rate of  $^{14}\text{C}$ -malate accumulation decreased markedly 5 to 8 min after initiation of the reaction. The decrease in the rate of uptake was attributed to a competitive interaction between accumulation of malate by the permease and the conversion of intracellular malate to acetate and  $\text{CO}_2$  (5) by the dissimilatory system. The extensive accumulation of radioactive malate observed in the presence of glucose was not entirely unexpected. Product inhibition of malic enzyme by two intermediate products of glycolysis, FDP and 3-PGA (6), was probably responsible for the enlargement of the intracellular pool of malate. Advantage was taken of this inhibition by glucose to approximate the maximum pool size of malate. In the presence of 0.2% glucose,  $^{14}\text{C}$ -malate was accumulated to a concentration of 200 to 250 nmoles per mg of cell protein.

In other experiments not reported here, we also demonstrated that the rate of malate entry was concentration and pH dependent. The optimal pH for malate uptake was 6.7; however, the rate changed little within the pH range of 6.2 to 7.4.

**Relationship of malate permease to the induction of malic enzyme.** It was tentatively concluded that glucose or an intermediate product of glucose catabolism repressed the synthesis of the malate entry system. However, since addition of glucose to a culture growing at the expense of malate did not immediately inhibit malic enzyme formation (5), it occurred to us that inhibition of malic enzyme synthesis may not be coordinated with the repression of permease synthesis. If this were the case, it should be possible to induce for malic enzyme synthesis while concomitantly inhibiting production of malate permease.

A flask of malate medium supplemented with 0.2% glucose was inoculated with a sufficiently

heavy suspension of malate-adapted cells to give an initial OD of 0.400. Upon attaining an OD of 1.420, the culture was harvested and the cells were used to prepare a suspension approximately equal in density to that used as the inoculum. At this time, glucose was still present in the medium. A comparison of transport, malic enzyme, and dye reduction activities was made between a portion of the malate-grown cell suspension used as the inoculum and the cell suspension prepared from the glucose-malate culture. Since the culture had undergone approximately two cell doublings, a fourfold decrease in the specific activity of the entry system was expected. Experimental measurements indicated that a fivefold decrease occurred (Table 3). Although a similar loss in dye-reducing specific activity was noted, malic enzyme specific activity only decreased by a factor of 2.5. Comparing the total units of the various activities present at the beginning and end of growth, a loss of 34 and 35% was observed for transport and dye-reducing activity, respectively, whereas the total units of malic enzyme increased by 30%. The proportionality between the decrease in dye-reducing activity and the loss of permease activity can be explained by assuming that the transport of substrate had become rate limiting. If this is the case, then the dye-reduction assay actually measured the level of permease activity, not the rate of malate metabolism.

In another experiment, a culture growing in malate medium containing 0.1% glucose was harvested shortly before making the transition from glucose to malate utilization. The activities of the malate entry system and malic enzyme of the partially induced cells were compared with fully induced cells (Table 4). Whereas the partially induced culture possessed only 6% of the fully induced level of permease activity, nearly 40% of the fully induced malic enzyme level was already present.

**Dependence of malic enzyme induction on malate permease.** The previous experiments suggested that induction of permease and malic enzyme are not coordinated events; however, under conditions of growth where permease synthesis was blocked, it was apparent that the synthesis of malic enzyme was not proportional to the increase in cell mass (Table 3). Since the entry system must also transport the inducer of malic enzyme into the cell, the failure to observe a fourfold increase in total malic enzyme units during growth on glucose (Table 3) may have resulted from the organism's inability to accumulate and maintain an adequate intracellular level of malate. If this assumption is correct, the quantity of malic enzyme synthesized will depend upon the amount of permease introduced into

TABLE 3. Effect of glucose-mediated growth on the levels of components comprising the malate dissimilatory system

Enzyme system	(A) Initial values	(B) Final values	Ratio A/B
Malate permease specific activity <sup>a</sup> .....	0.009	0.0016	5.4
Total units <sup>b</sup> .....	0.184	0.120	1.53
Malic enzyme specific activity <sup>c</sup> .....	0.340	0.132	2.5
Total units.....	13.2	18.5	0.71
Dye reduction specific activity <sup>d</sup> .....	0.070	0.014	4.9
Total units.....	1.420	1.000	1.44

<sup>a</sup> Expressed as micromoles of <sup>14</sup>C-malate taken up per minute per milligram of cell protein.

<sup>b</sup> Expressed as activity per 600 ml of culture ( $\mu$ moles per min).

<sup>c</sup> Expressed as micromoles of malate oxidized per minute per milligram of protein.

<sup>d</sup> Expressed as micromoles of dye reduced per minute per milligram of cell protein.

the culture as the inoculum and upon the relative increase of cell mass. The following experiments were made to determine the relationship between the size of the inoculum (total permease input) and the rate and extent of synthesis of malic enzyme.

Flasks containing malate medium supplemented with 0.2% glucose were inoculated with a malate-adapted cell suspension adjusted to give an initial OD between 0.08 and 0.8. To minimize the carryover of intracellular pools of inducer, the cells used as inocula were harvested from stationary-phase cultures after exhaustion of malate. The rate of malic enzyme synthesis was estimated with the NAD assay. Examination of the clarified culture fluid showed that glucose was present throughout exponential growth and that it disappeared from the medium only after the culture entered the stationary phase of growth. The dependence of both the rate and extent of malic enzyme synthesis on the size of the inoculum is apparent from the results of four experiments summarized in Fig. 5. Although the rates of malic enzyme synthesis appear as linear slopes in Fig. 5, the same values yield hyperbolic curves when plotted on a semilogarithmic scale (Fig. 5, insert). The kinetics of malic enzyme production were markedly different from those observed during growth on malate (see Fig. 1A). It will be recalled that the rate of malic enzyme synthesis was a logarithmic rather than a linear function of increase in cell mass. The continuously decreasing rate of malic enzyme synthesis observed during growth on glucose (Fig. 5, insert) was probably a

direct result of dilution of permease activity. Further examination of the data revealed a linear dose response relation between total malic enzyme synthesized and the size of the inoculum (Fig. 6).

**Malate permease as the rate-limiting step in malate dissimilation.** The data presented in Tables 3 and 4 point up a large disparity between the specific activities of the entry system and malic enzyme; the latter was present in a 37-fold excess. The rate-limiting step in the conversion of malate to pyruvate and CO<sub>2</sub> appeared to be the transport of substrate into the cell. However, acetate and CO<sub>2</sub>, or acetate, ethyl alcohol, and CO<sub>2</sub>, not pyruvate, are the end products of malate dissimilation (5). Although the possibility that one of the reactions catalyzing the dissimilation of pyruvate was rate limiting can not be completely excluded from consideration, other evidence

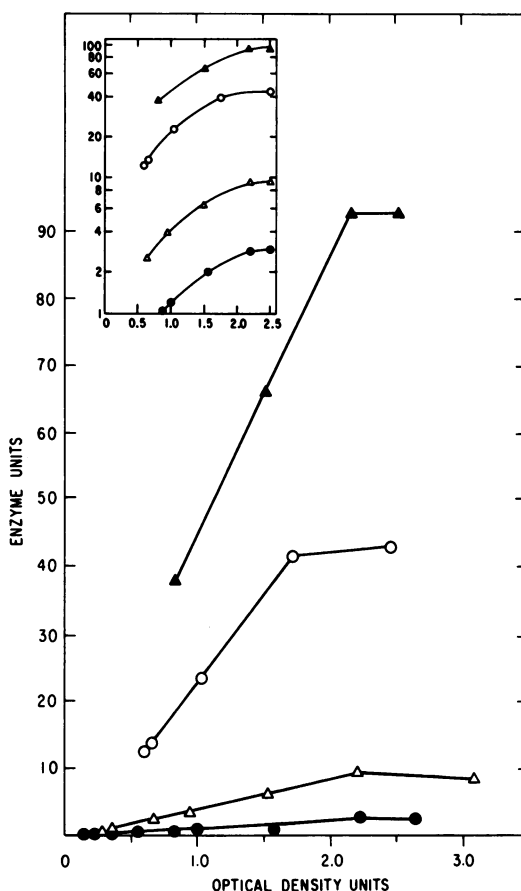


FIG. 5. Effect of inoculum size on rate and extent of malic enzyme synthesis. Initial OD<sub>600nm</sub>: 0.081 (●), 0.270 (△), 0.600 (○), 0.825 (▲). Enzyme units expressed as  $\mu$ moles of NAD reduced per min per 600 ml of culture. Insert: rates of malic enzyme synthesis shown on semilogarithmic scale.

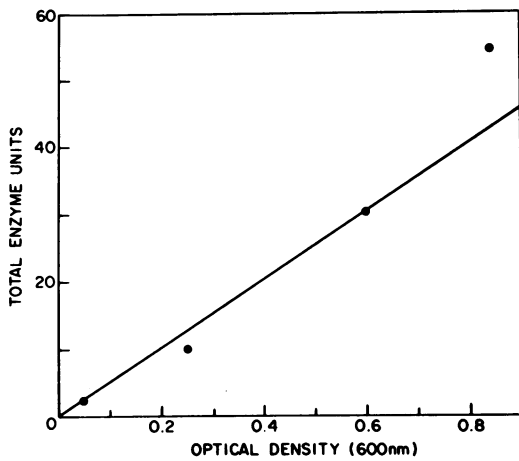


FIG. 6. Relation of total enzyme production to size of inoculum. The values for total enzyme units are corrected for the number of enzyme units introduced with the inoculum. Enzyme units are expressed as  $\mu$ moles of NAD reduced per min per 600 ml of culture.

TABLE 4. Relative specific activities of components of malate dissimilation system in partially and fully induced *S. faecalis* cultures

Enzyme system	Specific activities		
	(A) Fully induced	(B) Partially induced	Ratio A/B
Transport system.....	0.005 <sup>a</sup>	0.00028	17.8
Malic enzyme.....	1.11 <sup>b</sup>	0.433	2.5

<sup>a</sup> Expressed as micromoles of <sup>14</sup>C-malate taken up per minute per milligram of cell protein.

<sup>b</sup> Expressed as micromoles of malate oxidized per minute per milligram of protein.

indicated that this is not the case. The maximal rate of substrate utilization during exponential growth on malate was measured experimentally and the data appear in a previous publication (5). A calculation based on this information and the cellular protein content of the culture gave a value for the rate of substrate disappearance of 9  $\mu$ moles per min per mg of cellular protein. The close agreement between this value and that obtained in the <sup>14</sup>C-malate uptake experiments (Table 3) suggest that the rate-limiting step in malate catabolism is indeed the entry system.

#### DISCUSSION

Growth of *S. faecalis* on malate is preceded by the induction of a malate entry system and NAD-dependent malic enzyme. Acquisition of these enzymes allows the organism to channel carbon of malate into the Embden-Meyerhof-Parnas

pathway at the level of pyruvate. Our observations suggest that malate acts as inducer for both the permease and malic enzyme; however, only the former enzyme system appears to be subject to negative control by a glucose-mediated catabolite repression. This latter conclusion is supported by the following experimental results. First, when growth was initiated with a relatively small inoculum (initial OD<sub>600nm</sub> of 0.10 or less) in a medium containing both glucose and malate, a low but constant rate of malic enzyme synthesis was observed during the dissimilation of the former substrate; permease activity was not detected until nearly all exogenously supplied glucose had been utilized. Second, no immediate cessation of malic enzyme synthesis occurred upon addition of glucose to a culture growing on malate. Under identical conditions, permease synthesis stopped and the activity was subsequently diluted at a rate roughly proportional to the increase in cell population. Finally, relatively high rates of malic enzyme synthesis were maintained for approximately one generation in cultures growing at the expense of glucose if a heavy cell suspension was used to inoculate the culture.

The regulation of malate dissimilation is apparently achieved by the combined action of catabolite repression of malate permease and product inhibition of malic enzyme; these two mechanisms provide the organism with a means of coarse and fine control, respectively. Since both types of inhibition are produced by glucose or intermediate products of glycolysis, the two forms of control are probably exercised simultaneously. In contrast, the only negative control for malic enzyme synthesis is apparently exerted indirectly via repression of malate permease, which results in a reduction of the intracellular concentration of inducer of malic enzyme. Although this form of regulation is potentially wasteful, experimental evidence suggests that the organism must maintain a nearly fully induced level of permease to sustain malic enzyme synthesis at the fully induced level. The strict dependence of malic enzyme induction on the level of permease probably aids in conserving energy and preformed biosynthetic material. Moreover, it is doubtful that the wastage resulting from over production of malic enzyme is critical for successful competition or survival of the organism. Since *S. faecalis* is a relatively fastidious organism requiring a variety of amino acids and vitamins, it will be restricted to environments rich in organic material.

Whittenbury (9) reported that *S. faecalis* exhibited diauxic growth in media containing glucose and malate. In this context, a distinction must be made between the biphasic growth pat-

tern observed here with dual substrates and true diauxic growth. Classically, the "glucose effect" has been typified by a cessation of growth during the period of transition from utilization of one substrate to another (8). During this period, the inducible enzyme required for the dissimilation of the second substrate is synthesized, and when sufficient enzyme has been produced growth of the culture resumes. The rapid transition from glucose to malate utilization and the absence of a growth lag suggest that adequate levels of permease and malic enzyme were present in *S. faecalis* cells on depletion of the exogenous supply of glucose. To accomplish this, the repression of permease synthesis must have been relieved, at least partially, prior to the complete disappearance of glucose from the culture. The low but significant levels of malic enzyme synthesized throughout growth on glucose probably sufficed to initiate growth on malate. Finally, the growth rate on malate following glucose utilization is markedly lower than that observed with the hexose.

The incorporation of permeases into specific regulatory systems is apparently common among bacteria. Kepes and Cohen (2) pointed out that transport systems provide many microorganisms with a means of regulating the synthesis of inducible and repressible enzyme systems. In this connection, the authors cited as examples the roles played by the  $\beta$ -galactoside permease and maltose permease of *Escherichia coli* in the induction of  $\beta$ -galactosidase and amyloamylase respectively. Similarly, induction of an L- $\alpha$ -glycerophosphate dehydrogenase in *Aerobacter aerogenes* is dependent upon a specific permease to transport the inducer, L- $\alpha$ -glycerophosphate, into the cell (1,4). A similar relationship appears to exist between malate permease and malic enzyme.

Malate permease and malic enzyme appear to be induced and repressed independently of each other. It is unlikely, therefore, that the genes coding for these two functions are members of an operon under the control of a common operator locus. Until a detailed genetic analysis can be carried out, our provisional conclusion is that the structural genes bearing the information for the transcription of malate permease and malic enzyme function separately as independent entities.

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