Effects of Inhibition and Repression on the Utilization of Substrates by Heterogeneous Bacterial Communities

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ABSTRACT

STUMM-ZOLLINGER, ELISABETH (Harvard University, Cambridge, Mass.). Effects of inhibition and repression on the utilization of substrates by heterogeneous bacterial communities. Appl. Microbiol. 14:654-664. 1966.—This investigation attempts to evaluate to what extent enzyme inhibition and repression by metabolites, indigenous to the cell, are significant phenomena in natural microbial communities. Three case histories of the kinetics of substrate utilization and growth in multi-substrate media by heterogeneous bacterial populations are presented: (i) concurrent substrate utilization and growth on both substrates simultaneously (glucose plus benzoate); (ii) sequential substrate elimination accompanied by diauxic growth as a result of inhibition of enzyme activity (glucose plus galactose); (iii) sequential substrate utilization accompanied by diauxic growth caused by repression of enzyme formation (glucose plus L-phenylalanine, benzoate plus L-phenylalanine). It is shown that enzyme inhibition was observed in two-substrate media as well as in multisubstrate media and was maintained at low substrate concentrations (few milligrams per liter). A special attempt has been made to maintain the diversity of the experimental microbial population during the adaptation and enrichment period. All substrates were determined with sensitive analytical methods specific for the individual substrates. The results obtained confirm that catabolite repression and the resulting sequential substrate utilization are observed in heterogeneous bacterial populations.

The objective of the study presented here was to investigate how far extent enzyme inhibition and repression by catabolites is a significant phenomenon in natural microbial communities.

A bacterium has the genetic capability to produce a specific spectrum of catabolic enzymes. The realization of this basic capability is governed by inherent regulatory mechanisms responding to internal and external variables. An important determining factor is the nutritional condition.

The effects of the nutritional condition on enzyme synthesis and activity in microorganisms have been investigated in pure cultures of various microbial species. The phenomena that have been studied in greatest detail are enzyme induction, end-product repression, and repression by catabolites (glucose effect). Of these, catabolite repression is the mechanism that specifically regulates the enzymatic response of a microorganism to a multi-substrate environment. Physiologically, two different responses can be distinguished. Either enzymes necessary for the utilization of various substrates are not synthesized by the microbial cell in the presence of a repressing substrate (enzyme repression) or these catabolic enzymes are formed but remain inactive (enzyme inhibition). [The terms repression and inhibition are used in concordance with the definitions proposed by Davis (3).] Catabolite repression has been observed more frequently than catabolite inhibition (5, 7, 8). Both responses lead to diauxic growth and sequential substrate utilization. The most widely accepted hypothesis concerning the mechanism of catabolite repression postulates that compounds produced during metabolism of one substrate act as repressors on the formation of enzymes that lead to the further production of the same catabolites (12).

The enzymatic response of a natural mixed microbial community to the nutritional condition is more complex. The kinetics of substrate utilization is governed not only by regulatory mechanisms inherent to the individual organisms, but also by interactions between various microbial
species and by shifts in the species structure of the population.

This complexity could be expected to blur the substrate-utilization pattern of individual microbial groups in natural mixed populations and thus lead to an unspecific concurrent overall elimination of various substrates from the medium. Gaudy (6), however, has presented evidence that the inhibitive and repressive effects of glucose accompanied by diauxic growth are observed with mixed microbial populations.

This study attempted to confirm the relevance of Gaudy's findings to the processes occurring in natural habitats by extending the range of experimental conditions. Bacterial activities at low temperature and at low substrate concentrations, as well as the effect of enrichment of the cultural medium with a great variety of additional nutrients, were studied. The variety of substrate pairs investigated was extended to noncarbohydrate compounds.

An analysis of bacterial activities in a natural habitat involves inherent difficulties. The maintenance of an experimental mixed population at steady state has been possible only in a few cases where a physiological interdependence between the coexistent organisms was found. The quantitative chemical determination of many nutrients may be difficult when the nutrients occur at growth-limiting concentrations in a complex medium. Substrate-utilization rates in natural habitats are frequently extremely slow.

This study was therefore started with an investigation of relatively simple systems where the variables were known or kept under control. The substrate investigated could be determined with sensitive specific analytical methods. The culture media contained in most cases two carbon sources only. The substrate concentrations chosen were sufficiently large to permit concentration-independent rates of growth. The experimental populations were enriched with microorganisms able to grow fast on the particular substrate. The results, therefore, are particularly relevant to cases where one or a few substrates are predominant in the culture medium. Such conditions are often encountered in the biological treatment of industrial wastes.

Three representative case histories of the kinetics of substrate utilization and growth in two or multisubstrate media by a mixed bacterial population are illustrated in this paper: (i) concurrent substrate utilization and simultaneous growth on both substrates; (ii) sequential substrate elimination accompanied by diauxic growth caused by inhibition of enzyme activity; and (iii) sequential substrate utilization accompanied by diauxic growth caused by repression of enzyme formation.

**Materials and Methods**

All experiments were performed under aerobic conditions with batch cultures. The culture media used were nutritionally balanced by a supplement of inorganic nutrients including \( \text{NH}_4^+ \) and phosphate.

The experiments were performed at 20 ± 1°C unless specified otherwise.

A synthetic multisubstrate medium consisted of glucose (230 mg per liter), sodium oleate (37 mg per liter), and nutrient broth (575 mg per liter) in tap water.

Complete medium consisted of casein hydrolysate (200 mg per liter) and yeast extract (300 mg per liter; Difco) in tap water.

Microbial concentrations were estimated both by measuring the light absorbance at 600 nm and by dry-weight (110°C) determinations of the suspended solids after separation from the solution by centrifugation.

Glucose was determined according to the Glucostat method (Worthington Biochemical Corp., Freehold, N.J.). A modified Glucostat procedure was used to determine glucose at low concentrations (0.2 to 2 mg per liter). To 1 volume of sample, an equal volume of reagent, at two times the recommended concentration, was added. The reaction was allowed to proceed at 45°C for 30 min, and was then stopped with 60% \( \text{H}_2\text{SO}_4 \) as recommended by Salomon and Johnson (19) and by Washko and Rice (23). The light absorbance was measured at 540 nm.

Galactose was determined according to the Galactostat method (Worthington Biochemical Corp.). A modified Galactostat method was used to determine galactose at low concentrations (1 to 20 mg per liter). To a 10-ml sample, 1.5 ml of reagent, at four times the recommended concentration, was added. The reaction was allowed to proceed at 37°C for 60 min, and was quenched by the addition of 6 ml of 1.5 M glycine buffer.

It was found that some organic substances (e.g., complete medium) in the culture medium partially reduced the peroxide formed during the reaction of glucose with Glucostat reagent (9), thus resulting in a decreased formation of oxidized chromogen. When glucose in complete medium was determined, two or three standards containing various amounts of glucose in complete medium were employed to establish the absorbance–glucose concentration relationship.

Benzoate was determined by measuring the light absorbance at 230 nm at a pH below 3.

\( \text{L-Phenylalanine was determined from its absorbance at 257 nm. In the presence of interfering substances (phenylacetic acid, phenylpyruvic acid, and benzoic acid), L-phenylalanine was determined according to the ninhydrin method (2); in this case NO}_2^- \) was substituted for \( \text{NH}_4^+ \) in the culture medium.}

**Experimental microbial community.** The type of food in a microbial ecosystem is an important factor determining the kind of community which develops.
A natural community growing in a wide variety of substrates consists of many diversified types of organisms. A laboratory community growing in a defined synthetic medium containing one or a few carbon sources is enriched in organisms growing fast on the particular substrates. Bacterial species growing more slowly under the experimental conditions will be fewer in number and eventually will be absent from the culture. The complexity of the community will decrease and its physiological uniformity will increase with prolonged growth in the uniform medium. Prakash and Dondero (18) showed that adaptation of settled sewage to sorbitol as sole carbon source for 72 hr resulted in an increase of the frequency of coliform bacteria in the culture from 23 to 26%, to a frequency of 33 to 77%. In later experiments by the same authors, all the organisms present in the culture after the adaptation period were of the coliform type.

In this investigation, an attempt was made to minimize the loss of diversity of the microbial population during the enrichment and adaptation procedure prior to the experiment. Growth in the simple synthetic medium was limited to 6 to 12 doubling times. The experimental populations were grown in batch cultures, where there is less selective pressure than in continuous-flow cultures in favor of the organism growing fastest under the experimental conditions. The diversity of organisms originating from a biological sewage-treatment plant was maintained by growing them on synthetic multisubstrate medium in the laboratory. It has been shown that many different bacterial species can be isolated from laboratory activated sludge growing on synthetic dextrose broth medium (11). Before an experiment, the laboratory microbial community was transferred into a synthetic medium containing the specific carbon sources. Before exhaustion of the carbon sources, the organisms were harvested by centrifugation and resuspended in the experimental culture medium. In most cases, the cultures immediately resumed logarithmic growth at a constant rate after resuspension.

 Cultures so prepared differ from the parental culture by containing a predominant number of organisms growing fast on the substrates offered. It is inferred, however, that because of the short duration of the enrichment period the majority of the slower growing organisms also participating in the utilization of either one or both of the carbon sources studied are still present in these experimental populations.

Kinetics of substrate utilization as related to growth. For every set of substrates, two kinds of experiments on substrate utilization have been carried out: (i) substrate elimination was measured as a function of time in cultures of constant bacterial and enzymatic concentration (enzyme activity); (ii) growth and substrate elimination were measured as a function of time in cultures of logarithmically increasing bacterial and enzymatic concentration (enzyme formation).

A combination of the exponential growth equation, \( dB/dt = \mu B \), and of the equation describing the relationship between bacterial growth and substrate utilization, \( -dB/dS = y \), gives an expression that describes the rate of substrate utilization as related to growth.

\[
-dS/dt = (\mu/y)B
\]

where \( B \) is the concentration of organisms (dry weight cells per unit of volume) or a measure of enzymatic activity at time, \( t \); \( y \) is the yield, i.e., the fraction of the substrate converted into bacterial mass; \( \mu \) is the specific growth rate constant (time\(^{-1}\)); and \( S \) is the concentration of substrate.

The specific growth rate constant, \( \mu \), can be kept constant, usually at a maximum, if during the experiment the substrate is maintained at concentrations higher than the growth-rate limiting concentration. If \( y \) is also assumed to be constant (e.g., \( y \sim 0.5 \)), equation 1 predicts that the rate of substrate elimination is proportional to \( B \). The substrate utilization rate is constant, if \( B \) is kept constant experimentally by making it large in comparison to the substrate. In this case, the substrate elimination is measured during a fraction of the generation time only (Fig. 1 and 2a).

If the initial bacterial concentration, \( B_0 \), is small in comparison with the substrate concentration, the rate of substrate elimination increases logarithmically with time in accordance with the logarithmic increase of \( B \):

\[
-dS/dt = (\mu/y)B_0 e^{\mu t}
\]

Integration of equation 2 leads to:

\[
-\Delta S = (B_0/y)(1 + e^{\mu t})
\]

where \( -\Delta S \) is the substrate utilized at any time. Accordingly, a plot of \( \log (-\Delta S) \) versus time gives a linear relationship for \( t \gg 0 \). The slope of the curve is related to the specific growth rate constant (2.3 \( \mu \)), and corresponds to the slope of the semilogarithmic bacterial growth curve (Fig. 2b, c and 3c, d). In experiments of this type, the rate of bacterial growth, as an approximate measure of the rate of enzyme formation, is determined over a period of several generation times.

**Results**

A brief survey of the systems investigated and a characterization of the results obtained is given in Table 1.

Dependence of the rate of substrate utilization and the rate of growth on substrate concentration. It appeared necessary to elucidate whether the substrate concentration range used in this study allowed maximal and, therefore, constant enzymatic activity. The rate of growth declines and becomes concentration-dependent, presumably due to unsaturation of a specific enzyme system involved, when the concentration of the substrate becomes very small. This critical concentration is specific for each substrate. Figure 1 shows substrate utilization rates of glucose, galactose, benzoate, and L-phenylalanine, respectively, in three different concentration ranges.
<table>
<thead>
<tr>
<th>Substrates tested for interaction by repressing or inhibiting effects</th>
<th>Mode of effect</th>
<th>Conc range of substrates tested</th>
<th>Quality of culture medium</th>
<th>Remarks</th>
<th>Temp during expt</th>
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</thead>
<tbody>
<tr>
<td>Glucose, galactose</td>
<td>Inhibition, but no repression</td>
<td>200–&lt;1 mg/liter</td>
<td>Nutritionally balanced with inorganic nutrients</td>
<td>Glucose inhibits the activity of the galactose-catabolizing enzymes</td>
<td>20</td>
</tr>
<tr>
<td>Glucose, galactose</td>
<td>No interaction</td>
<td>5–150</td>
<td>Inorganic nutrients</td>
<td>Glucose does not affect activity of galactose enzymes</td>
<td>7</td>
</tr>
<tr>
<td>Glucose, galactose</td>
<td>Inhibition, but no repression</td>
<td>5–150</td>
<td>Culture medium enriched with 0.2 g/liter of casein hydrolysate and 0.3 g per liter of yeast extract</td>
<td>Glucose inhibits the activity of the galactose enzymes</td>
<td>20</td>
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<tr>
<td>Glucose, L-phenylalanine</td>
<td>Repression but no inhibition</td>
<td>5–150</td>
<td>Inorganic nutrients</td>
<td>Glucose represses the formation of L-phenylalanine enzymes</td>
<td>20</td>
</tr>
<tr>
<td>Glucose, L-phenylalanine</td>
<td>Repression but no inhibition</td>
<td>5–150</td>
<td>Inorganic nutrient, but no inorganic N</td>
<td>The repression of the L-phenylalanine-deaminating enzyme is released while enzymes attacking the carbon skeleton of L-phenylalanine are still repressed</td>
<td>20</td>
</tr>
<tr>
<td>Benzoic acid, L-phenylalanine</td>
<td>Repression</td>
<td>5–150</td>
<td>Inorganic nutrients</td>
<td>Benzoic acid represses the formation of the L-phenylalanine enzymes</td>
<td>20</td>
</tr>
<tr>
<td>Benzoic acid, glucose</td>
<td>No interaction</td>
<td>5–150</td>
<td>Inorganic nutrients</td>
<td>Concurrent utilization of both substrates; resulting growth rate larger than growth rate on each individual substrate</td>
<td>20</td>
</tr>
<tr>
<td>Casein hydrolysate and yeast extract, glucose</td>
<td>No effect on enzyme activity</td>
<td>5–500</td>
<td>Substrates in tap water</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Casein hydrolysate and yeast extract, galactose</td>
<td>No effect on galactose enzyme activity</td>
<td>5–500</td>
<td>Substrates in tap water</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Casein hydrolysate and yeast extract, benzoic acid</td>
<td>No effect on benzoic acid enzyme activity</td>
<td>5–500</td>
<td>Substrates in tap water</td>
<td></td>
<td>20</td>
</tr>
</tbody>
</table>

For each experiment, B remained relatively constant. The scales used for the ordinate values have been made in inverse proportion to the bacterial concentrations employed, so that the slopes for each set of experimental points can be directly compared. It is obvious from these results that the critical growth-limiting concentrations for glucose, galactose, L-phenylalanine, and benzoate are below 1, 1, 1, and 20 mg per liter, respectively. Correspondingly, the following experiments were carried out in a concentration range where substrates did not limit the rates of growth.

*Concurrent substrate utilization accompanied*
by simultaneous growth on both substrates. The patterns of utilization of benzoic acid and glucose, respectively, and of both substrates combined are given in Fig. 2. Figure 2a shows the decrease of substrate concentration with time, the concentration of organisms being approximately constant (510 mg per liter, dry weight). The microorganisms had been pregrown on benzoic acid; they contained the glucose-catabolizing enzymes as well as the benzoic acid-catabolizing enzymes at the start of the experiment.

Figure 2b shows microbial growth on the individual substrates and on the two substrates combined, accompanied by concurrent elimination of glucose and benzoate from the culture medium (Fig. 2c). Ordinate values in Fig. 2c are plotted logarithmically to show linear correspondence to logarithmic increase of bacterial concentration (Fig. 2b). The data shown in Fig. 2a give information on the activity of the benzoic acid and the glucose enzymes in the presence of both substrates as compared with enzyme activity in the presence of either one of the two substrates; the data shown in Fig. 2c also give information on the formation of benzoic acid and glucose enzymes during growth in the presence of both substrates.

The following observations can be made from the data given in Fig. 2: (i) neither substrate affects the formation or the activity of the enzymes necessary for the utilization of the second substrate, (ii) the growth rate constant $\mu$ observed in the substrate mixture is larger than the growth rate constants observed in media containing one substrate component only, (iii) the additive rate of growth is a manifestation of the similarly additive and concurrent substrate elimination.

Inhibition of enzyme activity causing sequential substrate elimination accompanied by diauxic growth. The enzymatic and growth responses of a heterogeneous microbial community to the presence of glucose and galactose in the culture medium are given in Fig. 3. Figure 3a shows the decrease of substrate concentration with time when substrates are given individually or combined. The microorganisms were kept constant at a concentration of ca. 360 mg per liter (dry weight); they had been pregrown on galactose, and thus were basically capable of growing fast on galactose without adaptation. Figure 3b gives the results of a similar experiment, but here the microorganisms used (360 mg per liter, dry weight) had been pregrown on glucose. Consequently, the organisms contained no galactose enzymes at the start of the experiment. Figure 3c gives microbial growth on glucose and diauxic growth on glucose and galactose, respectively;
The activation (ii) takes place during the further growth on galactose, indicating that all the organisms present in the culture at the time of glucose exhaustion take part in the further growth on galactose. Thus, the sequential substrate utilization cannot be interpreted as a result of ecological succession.

The experiments reported above were repeated with much smaller concentrations of substrates. The activity of galactose enzymes was measured in the presence of 0.5 to 5 mg per liter of glucose.

Figure 4 shows the concentration decrease of galactose with time in the presence and in the absence of glucose. The concentration of organisms pregrown on galactose was approximately constant (8.8 mg per liter). The results show that fractions of a milligram per liter of glucose in the culture medium still have a marked influence on the galactose enzymes, reducing their activity by approximately 50% in comparison with that observed in the absence of glucose.

Some experiments were also carried out at temperatures other than 20°C. At low temperatures (7°C), the elimination of glucose and galactose by microorganisms pregrown at 7°C occurs concurrently (Fig. 5). The activity of the galactose enzymes in a psychrophilic microbial community, therefore, is not affected by the presence of glucose in the culture medium at low temperatures, whereas it is inhibited in mesophilic microbial communities at 20°C.

A different temperature dependence of diauxie has been shown for Pseudomonas species growing on succinate and hydroxybenzoate. These substrates were utilized concurrently at 35°C and sequentially at 20°C (Sistrom, personal communication).

Enzyme inhibition in a nutritionally rich culture medium. Several hypotheses have been advanced to explain the mode of the glucose effect. According to one hypothesis, the enzymes of glucose metabolism remove nutrients which are necessary for the formation of the repressed enzyme (20). According to a second hypothesis, surplus glucose catabolites repress the formation of enzymes which would lead to their further accumulation (12). While inhibition of enzyme activity has not been separately considered by these authors, similar arguments could be brought forward in an attempt to explain catabolite inhibition.

In a nutritionally rich medium, an excess of
nutrients will be present. Furthermore, many building blocks needed for growth can be taken up from the culture medium by the microbial cell, and do not have to be synthesized. The resulting increased growth rate may lead to a faster utilization and lesser accumulation of glucose catabolites. It was thought that both these effects might relieve the inactivation of the galactose enzymes by glucose.

The decrease in concentration of galactose alone (initial concentration = 150 mg per liter) and galactose (150 mg per liter) in the presence of glucose (150 mg per liter) was measured as a function of time in a culture of microorganisms grown on galactose (constant bacterial concen-
Repression of enzyme formation causing sequential substrate elimination accompanied by diauxic growth. Diauxic growth has also been observed in media that contained L-phenylalanine and glucose as carbon and energy sources (Fig. 6). In this case, however, glucose represses the formation of phenylalanine enzymes.

Figure 6a shows the decrease of substrate concentration when substrates were given individually or combined. The microbial mass (approximately constant concentration of 391 mg per liter) had been pregrown on glucose and phenylalanine. It contained both glucose- and phenylalanine-catabolizing enzymes. In Fig. 6b, microbial growth on individual substrates and diauxic growth on the two substrates combined are shown, and Fig. 6c illustrates the resulting elimination of glucose and phenylalanine from the medium. The organisms, pregrown on glucose and phenylalanine, were capable of growth.

Concurrent utilization of L-phenylalanine and glucose in short-term experiment show concurrent activity of the enzyme systems necessary for the utilization of glucose and L-phenylalanine. (b) Diauxic growth and (c) sequential utilization of substrates are caused by repression of the formation of L-phenylalanine-catabolizing enzymes in the presence of glucose.

Besides glucose and galactose, 0.3 g per liter of yeast extract and 0.2 g per liter of casein hydrolysate had been added to the culture medium. This addition increased the growth rate from 0.50 to 0.66 hr⁻¹, but had little effect on the inhibition of the utilization of galactose. In this rich medium, the presence of glucose was still capable of reducing the activity of galactose enzymes by 80% in comparison with that observed in the absence of glucose. These results show that the hypotheses presented above cannot be generally interpreted in the sense that inhibitions cannot be observed in rich multi-substrate media.

Fig. 6. (a) Concurrent utilization of L-phenylalanine and glucose in short-term experiment show concurrent activity of the enzyme systems necessary for the utilization of glucose and L-phenylalanine. (b) Diauxic growth and (c) sequential utilization of substrates are caused by repression of the formation of L-phenylalanine-catabolizing enzymes in the presence of glucose.

Fig. 7. (a) Concurrent utilization of substrates in short-term experiment shows concurrent activity of the enzyme systems mediating the catabolism of benzoate and L-phenylalanine. (b) Diauxic growth is caused by repression of the synthesis of L-phenylalanine-catabolizing enzymes in the presence of benzoic acid.
on phenylalanine and contained the phenylalanine enzymes.

These data convey the following information.
(i) The activity of the L-phenylalanine-catalyzing enzymes is not affected by the presence of glucose in the culture medium or by its metabolism.
(ii) The growth pattern on glucose and phenylalanine combined is diauxic. While some growth on phenylalanine takes place, it is not proportional to the increasing bacterial concentration and soon becomes negligible. (iii) The concentration of phenylalanine enzymes in a culture growing exponentially in a medium containing glucose and phenylalanine does not increase. No new enzyme is formed. The rate of phenylalanine elimination from the medium, therefore, is constant, and thus proportional to the concentration of phenylalanine enzymes formed before the addition of glucose. Glucose represses the formation of new phenylalanine-catalyzing enzymes. A similar enzymatic response by microorganisms was observed when benzoate and L-phenylalanine were given as substrates (Fig. 7).

Repression of L-phenylalanine enzymes in the absence of inorganic nitrogen. In a culture medium containing no inorganic nitrogen source, and containing glucose and phenylalanine as carbon sources, growth on glucose is impossible unless the phenylalanine-deaminating enzymes are formed. The deamination of amino acids is considered to be the first step of the amino acid catabolism.

**Fig. 8.** Repression of L-phenylalanine-catalyzing enzymes by glucose in the absence of inorganic nitrogen. (a) Diauxic growth. (b) Concurrent substrate elimination (deamination of L-phenylalanine).

**Fig. 9.** Ultraviolet light absorption spectra of culture medium containing L-phenylalanine and glucose as substrates. The medium contains no inorganic nitrogen source. (a) Prior to growth, (b) after growth had proceeded for 11 hr, and (c) after growth had proceeded for 16 hr. Absorption spectra of L-phenylalanine, phenylacetic acid, and phenylpyruvic acid, respectively, are given for comparison.
In Fig. 8, the growth and substrate utilization patterns of a microbial culture growing on glucose (initial concentration = 1.8 g per liter) and phenylalanine (2.5 g per liter) are shown. No inorganic nitrogen source was present in the culture medium. The initial microbial concentration was 3.4 mg per liter. It was experimentally verified that, in the absence of phenylalanine, no growth on glucose occurred because of the lack of nitrogen (Fig. 8a). In the presence of glucose and phenylalanine, growth did occur and glucose and phenylalanine concentrations decreased until all the glucose was utilized. This growth phase was followed by a short lag, after which growth was resumed; then phenylalanine was further utilized.

The growth lag which is observed after the exhaustion of glucose from the culture medium would be expected if phenylalanine is utilized in the presence of glucose as nitrogen source but not as carbon source. During the lag period, the enzymes necessary for the breakdown of the carbon skeleton of phenylalanine are formed. The deamination that precedes the lag phase has been verified analytically. Pronounced changes in the ultraviolet spectra occur during this period, and suggest the subsequent transition of 1-phenylalanine into phenylacetic acid and into phenylpyruvic acid (Fig. 9).

**Discussion**

Little information is available on growth of heterogeneous populations in multisubstrate media. It has frequently been inferred that total growth is simply the additive result of the growth patterns of the individual species growing on individual substrates. Correspondingly, collective metabolic parameters, such as total organic carbon or oxygen consumption, have been used to describe rates of substrate utilization and growth. The results of this study show, however, that it is not possible to obtain from such parameters conclusive detailed information on the relations of the kinetics of growth and substrate utilization. While monoauxic growth and concurrent substrate utilization by heterogeneous cultures may be observed on many substrate combinations (e.g., glucose plus benzoate), the case histories, presented in this study illustrate that interaction of substrates does occur in heterogeneous populations. The observed growth patterns furthermore show that the diauxie is caused by sequential enzyme formation (glucose plus phenylalanine) or by inhibition of enzyme activity (glucose plus galactose) within the individual microbial cell, and that it is not a consequence of ecological succession during the experiment.

There are many reports on enzyme repression and enzyme inhibition as observed in pure bacterial cultures. These findings, however, are restricted to a few taxonomic groups of microorganisms. The fact that in this study substrate interactions have been observed with heterogeneous populations indicates that repression and inhibition by metabolites may be frequent phenomena, to be observed in a great variety of microorganisms.

It is of interest to compare our results with phenomena observed by others in studies with pure bacterial cultures. In *Aerobacter aerogenes* and in *Escherichia coli*, glucose has been found to repress the formation of a number of amino acid-catabolizing enzymes (5, 16). In an *E. coli* mutant, glucose inhibited the activity of the uptake mechanism for tryptophan (1), whereas in *E. coli* glucose did not affect the formation or the activity of serine deaminase (17). Utilization of glucose, however, was shown to be inhibited by casein digest in *Micrococcus lysodeikticus* (5). Galactose adaptation was repressed by glucose in yeast (4) and in *E. coli* (14). The activity of the galactose-catabolizing enzymes was not affected by glucose in *E. coli* (21).

Inhibition and repression by metabolites have been most frequently observed with carbohydrates. These effects, however, can also be demonstrated with other carbon sources. Neidhardt and Magasanik (16) reported that *myo*inositol represses histidase in *A. aerogenes*. In this study, benzoate repressed the formation of phenylalanine-degrading enzymes.

There are indications that the control of enzyme formation by metabolites in microorganisms is linked to growth requirements. L-Phenylalanine is not utilized in the presence of glucose. The formation of phenylalanine-catabolizing enzyme is repressed. If, however, phenylalanine is the only nitrogen compound in the culture medium, it serves as nitrogen source. Phenylalanine deaminase is formed and is active; the intermediates phenylacetic acid and phenylpyruvic acid are excreted by the microbial cells. Similarly, Neidhardt and Magasanik (16) found in *A. aerogenes* that L-histidine-degrading enzymes were repressed by glucose but were derepressed if no inorganic nitrogen source was added to the culture medium.

In this study, enrichment of the culture medium with casein digest and yeast extract increased the growth rate of the microorganisms, but did not relieve the inhibition of galactose utilization by glucose. Mandelshtam (13) reported an obliteration of the glucose effect in *E. coli* by addition of amino acids to the culture medium.
Neidhardt and Magasanik (15), however, found no such effect of amino acids in *A. aerogenes*.

All experiments reported here have been carried out with substrate concentrations that permitted full enzyme activity. Occasionally, a decrease in the specific substrate utilization rate was observed. It is shown experimentally (Fig. 1) that such a rate decrease must not be interpreted as reflecting a dependence of the substrate-utilization rate on substrate concentration.

Catabolite inhibition was maintained at low substrate concentrations (few milligrams per liter). In natural systems, however, many substrates are present at growth-limiting concentrations. The microbial community growing in a dilute medium may differ markedly from the communities growing in more concentrated media. It therefore appears desirable to expand this type of study to the range of substrate concentrations found in natural habitats. Only when such results are available can definite conclusions on the significance of catabolite repression in natural environments (e.g., natural waters, soils) be drawn. A few results obtained by others with pure bacterial cultures indicate that interaction of substrates at growth-limiting concentrations does occur. Repression of formation of β-galactosidase in *E. coli* by several substrates at growth-limiting concentrations in continuous-flow cultures was reported by Kennell and Magasanik (12). Repression of fructose utilization by growth-limiting concentrations of glucose has been observed in continuous-flow cultures consisting of yeast, *E. coli*, and *Pseudomonas* species (Mateles, personal communication).

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**Literature Cited**


