Diauxie Used as a Microbiological Assay for
L-Isoleucine or L-Valine in Escherichia coli

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Table 1 shows the growth data for cultures used to assay L-isoleucine. Cells of E. coli K-12 was grown exponentially on glucose, were harvested and resuspended in fresh medium (Fig. 1, legend). After 20 min of incubation without a carbon source, lactate was added; the culture was then divided into several cultures, and

L-valine (156.3 μg/ml) and various concentrations of L-isoleucine were added to pairs of these cultures (Fig. 1, legend). The lag due to the valine alone was 222 min, in addition to the normal 1-hr diauxic lag in the control cultures (no amino acid added). When glucose, instead of lactate, was added to similarly treated cells, no lag was observed, whereas a 20-min lag resulted when 156.3 μg/ml of L-valine per ml was also present.

Figure 2 shows the interval of time (from Fig.
Fig. 2. Shortening of diauxic lag versus L-isoleucine concentration. Straight line fitted by method of least squares.

1) by which this lag extension was shortened, plotted against the concentration of isoleucine producing the shortening. Thus, 5.2 mg/ml was detected. Duplicate cultures gave intervals within 5 min of their average interval in the worst cases, corresponding to an error of ±0.7 mg/ml.

The level of valine used here was found convenient. Concentrations of valine 33% higher or lower gave similar results with little change in sensitivity to isoleucine or in accuracy.

As the concentration of isoleucine was increased beyond a level where maximal shortening occurred, the shortening again decreased, probably owing to competition between valine and isoleucine for entry into the cell, as suggested by H. E. Umbarger and B. Brown (J. Bacteriol. 70:241, 1955).

Owing to the nature of the assay, isoleucine cannot be measured in mixtures containing valine. Similarly, we have found that leucine interferes with the isoleucine assay, probably because of the competition of valine, leucine, and isoleucine for entry into the cell (G. S. Cohen and H. V. Rickenberg, Ann. Inst. Pasteur 91:693, 1956). D-Isoleucine (Sigma Chemical Corp., St. Louis, Mo.), with d-allo, at 39 or 468 mg/ml did not shorten the extended lag due to 234 mg of valine per ml nor affect the shortened lag produced by 39 mg of L-isoleucine per ml on the same level of valine.

By adding various concentrations of L-valine to the culture at the time of transfer from glucose to lactose and measuring the resultant lag extensions, an assay for L-valine has been obtained (Fig. 3). In this way, about 20 mg of L-valine per ml can be detected. d-Valine, at 0.1 mg/ml or 1.0 mg/ml, did not produce an extended diauxic lag nor did it affect the extended lag obtained with 39 mg of L-valine per ml.

Fig. 3. Lengthening of diauxic lag versus L-valine concentrations. Straight line fitted by method of least squares.

It is possible that this system could be used to assay other metabolites which inhibit growth or reverse such inhibition. In particular, leucine might be assayed in a system containing fixed concentrations of valine and isoleucine, since it changes the length of the lag in this case. Preliminary data indicate that threonine is capable of reversing the extended lag due to valine. Thus, it may become possible to assay, in a manner analogous to that for isoleucine, any precursor of isoleucine, in spite of the fact that threonine (10 mg/ml) does not reverse the growth inhibition during exponential growth on glucose due to valine (3 mg/ml). D. Rowley (J. Gen. Microbiol. 9:37, 1953) has reported other inhibitor-antagonist sets of amino acids which might be assayed by this method, with only an inducible enzyme necessary for growth in the organism used. Sensitivity would depend in these cases on the properties of each system.

The assay described here for valine and isoleucine is more sensitive than the usual microbiological assays which are dependent on increases in culture turbidity. It is easy to apply and requires no specialized media. It cannot be used to assay in certain mixtures of amino acids, but can resolve the L form in the presence of D.

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