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2,3-Dihydroxybenzoic Acid, a New Growth Factor for Multiple Aromatic Auxotrophs

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Strains of *Escherichia coli* with metabolic blocks affecting early (common) reactions in the pathway of biosynthesis of aromatic compounds may require for optimal growth in a glucose-mineral salts medium the addition of phenylalanine, tyrosine, tryptophan, *p*-aminobenzoate, *p*-hydroxybenzoate, and 3,4-dihydroxybenzaldehyde (B. D. Davis, J. Bacteriol. **64**:729, 1952; B. D. Davis, Congr. Intern. Biochim., 2nd, Paris, Symp. Metabolisme Microbien, p. 32, 1952). Some strains with blocks in the common pathway (multiple aromatic auxotrophs) grow only poorly, or not at all, in the medium supplemented as above unless a small amount of yeast extract is added (e.g., J. Pittard and B. J. Wallace, J. Bacteriol. **91**:1494, 1966).

It has now been found that 2,3-dihydroxybenzoate will replace the requirement for yeast extract. Figure 1 shows a dose-response curve with 2,3-dihydroxybenzoate and *E. coli* 83-1 as the test organism in shaken cultures. This organism was derived from *E. coli* W, but the *E. coli* K-12 strains described by Pittard and Wallace as growing poorly also respond to 2,3-dihydroxybenzoate. A number of factors influence the 2,3-dihydroxybenzoate requirement, including aeration, the presence of certain metal ions, and the basal medium used. For example, *E. coli* 83-1 showed no marked 2,3-dihydroxybenzoate requirement when tested in the medium 56 described by J. Monod, G. Cohen-Bazire, and M. Cohn (Biochim. Biophys. Acta **7**:585, 1951), in contrast to the requirement (Fig. 1) in medium E of H. J. Vogel and D. M. Bonner (J. Biol. Chem. **218**:97, 1956). Conversely, the *E. coli* K-12 strains grow well in medium E without 2,3-dihydroxybenzoate but show a marked requirement for the growth factor in medium 56. Preliminary experiments show that important differences between the two media used are probably the presence of citrate in medium E and the presence of iron in medium 56. The addition of shikimate to a culture of *E. coli* 83-1 in medium E supplemented as for Fig. 1, in addition to 10^{-5} M 2,3-dihydroxybenzoate, gave no further stimulation of growth rate. Therefore, under the conditions used, all the aromatic requirements of the cell were satisfied.

2,3-Dihydroxybenzoate is not replacing one of the other trace growth factor requirements, since

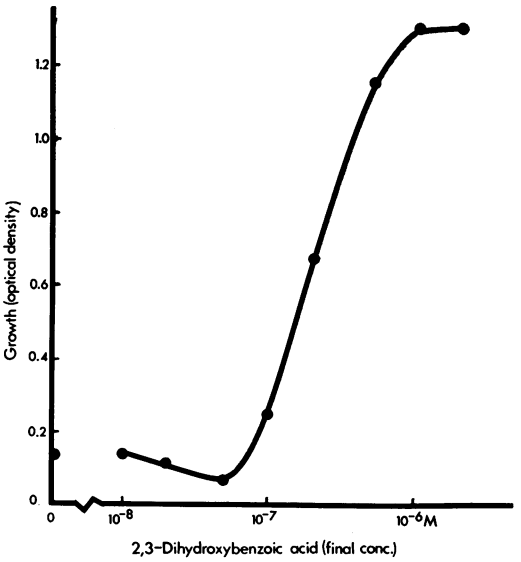

FIG. 1. *Response of Escherichia coli 83-1 to 2,3-dihydroxybenzoic acid. The growth tests were carried out in shaken tubes for 22 hr at 37 C. The medium consisted of a glucose-citrate-mineral salts medium E supplemented with the aromatic amino acids (L-tryptophan, L-tyrosine, and L-phenylalanine; 10^{-4} M) and aromatic vitamins (p-aminobenzoic acid, p-hydroxybenzoic acid, and 3,4-dihydroxybenzaldehyde; 10^{-6} M). The inoculum, from a 24-hr nutrient agar slope and washed once in distilled water, contained an initial population of 6×10^5 cells per milliliter. Growth was measured in a Spekker colorimeter with a neutral density filter.*

it has been observed that in either of the media used there is still a requirement for *p*-aminobenzoate in the presence of 2,3-dihydroxybenzoate. Further, growth can be obtained with 2,3-dihydroxybenzoate in the absence of either 3,4-dihydroxybenzaldehyde or *p*-hydroxybenzoate, the latter two compounds being required for

vitamin K and ubiquinone biosynthesis, respectively (G. B. Cox and F. Gibson, Biochem. J. **100**:1, 1966). Cells grown under such conditions do not form either vitamin K or ubiquinone, indicating that 2,3-dihydroxybenzoate is not serving as a precursor of these quinones.

2,3-Dihydroxybenzoate therefore is, under certain conditions, an essential bacterial growth factor for multiple aromatic auxotrophs with complete metabolic blocks, and should be included in synthetic media to give optimal growth.

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