to a reaction of the oxidant or reductant with the intermediary products of the oxidation of tyramine-HCl. If the production of tyrosinase is occasioned by the denaturation of an inhibitor, an increase in —SH groups seems probable; this, however, is not the case.

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Inhibition of Utilization of Aspartic Acid for Growth of *Leuconostoc mesenteroides* P-60. (20472)

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γ-L-Glutamylhydrazide was an effective inhibitor of growth of 2 strains of streptococci(1) and of *L. arabinosus*(2) under conditions in which small amounts of glutamine produced a marked acceleration of growth. In both instances it was found that β-aspartylhydrazide was a weaker inhibitor than the glutamyl derivative. α-DL-Methylglutamic acid was also found to be an inhibitor of the utilization of D- and L-glutamic and α-ketoglutaric acids by *L. arabinosus*(2,3), while α-DL-methylaspartic acid and α-DL-methylasparagine were ineffective. It was of interest to determine whether the aspartic acid analogues could inhibit the utilization of aspartic acid under suitable circumstances.

Materials and methods, compounds used. L-Aspartic acid was employed in all experiments. γ-L-glutamylhydrazide (GGH), β-DL-aspartylhydrazide (BAH), α-DL-methylglutamic acid (AMG), α-DL-methylaspartic acid (AMA), and α-DL-methylasparagine (AMAS) were kindly given to us by Dr. Karl Pfister of Merck and Co., Inc. The procedure of Hac and Snell(4) was employed for microbiological assay of L-aspartic acid with *L. mesenteroides* P-60, and growth was determined turbidimetrically and the results expressed in optical density units (O.D.). Solutions of all of the substances tested for inhibition were sterilized by Seitz filtration. The final volume of the assay medium was 1.5 ml per tube. The basal medium and the test samples were added to the assay tubes with the rapid automatic dispenser of Cannon.

Experimental results. In Fig. 1A is seen a typical standard curve obtained after 75 hours of incubation at 37°C, showing the graded response of the bacteria to increasing amounts of L-aspartic acid. An experiment was performed simultaneously in which incubations were made with 60 γ of L-aspartic acid per tube in the presence of 1-5 μM of the ana-

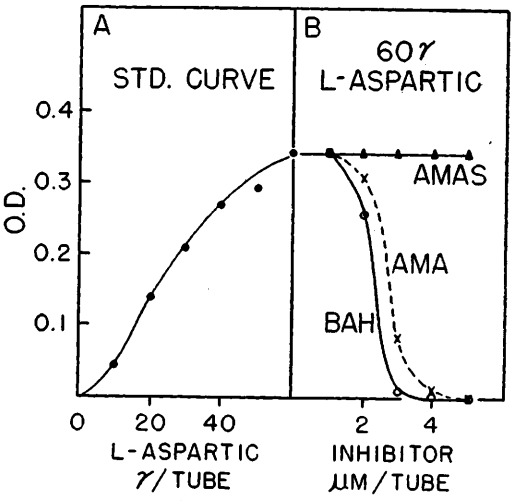

FIG. 1. Influence of varying amounts of aspartic acid analogues on growth on 60 γ of L-aspartic acid. BAH, β-DL-aspartylhydrazide; AMA, α-DL-methylaspartic acid; AMAS, α-DL-methylasparagine.

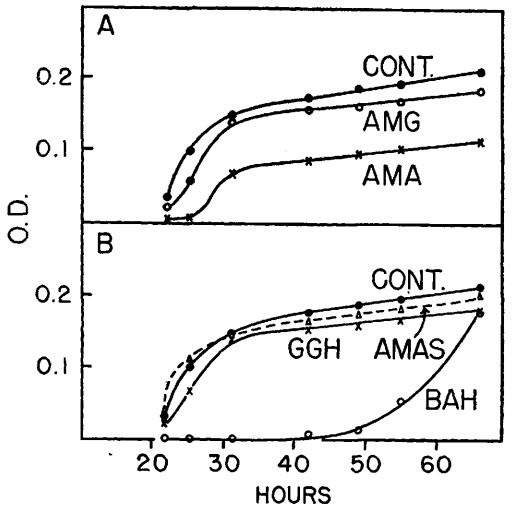

FIG. 2. Comparison of inhibition by glutamic acid and aspartic acid analogues. GGH, γ-L-glutamylhydrazide; AMG, α-DL-methylglutamic acid.

logues (Fig. 1B). Both BAH and AMA were found to be relatively effective inhibitors of growth, giving 50% inhibition at molar ratios of inhibitor to metabolite of 4.9:1 and 5.9:1, respectively, while AMAS was ineffective. In further experiments it was shown that the complete inhibition of growth produced by 5 μM of GGH in the presence of 60 γ of L-aspartic acid was not reversed by the presence of an additional 10-50 γ of L-aspartic acid or 20 γ of asparagine. When L-aspartic acid was added to similarly constituted incubation mixtures containing 5 μM of AMA, no growth occurred until the total content of L-aspartic acid was increased to 90 and 100 γ per tube, under which conditions there occurred 31 and 42%, respectively, of the growth with 60 γ of L-aspartic acid in the absence of inhibitors. Although the degree of inhibition given by a particular concentration of analogue varied somewhat from one experiment to another, the relative efficacy remained the same.

The results in Fig. 2 show the time-course of growth on 60 γ of L-aspartic acid in the absence of inhibitor and in the presence of 2 μM per tube of the 5 test substances. It is seen that the aspartic acid analogues, AMA and BAH, are much more effective than their glutamic acid counterparts, AMG and GGH. The latter 2 substances were, at most, only slightly inhibitory. The presence of asparagine up to 16 γ per tube did not alter the results obtained, in keeping with the relative ineffectiveness of asparagine in this system(4).

Discussion. AMG probably exerts its action on glutamic acid utilization in *L. arabinosus* chiefly by inhibiting the synthesis of glutamine, while GGH acts by inhibiting the utilization of glutamine in subsequent reactions(1-3). The inhibitory action of the comparable aspartic acid analogues, AMA and AGH, is probably different since asparagine is not utilized as well as aspartic acid and appears to play no significant role in growth on aspartic acid under these conditions(4). It is planned to test these substances in bacterial systems in which asparagine is necessary for rapid growth in media containing aspartic acid. Further studies are being made of the mechanism of inhibition of growth by the above compounds.

Substances other than those above which have been shown previously to inhibit utilization of aspartic acid by microorganisms are cysteic acid(5), DL-*para*-hydroxyaspartic acid(6), *meso*-diaminosuccinic acid(6), α-aminolevulinic acid(7), and heated aspartophenone(7).

Summary. β-DL-Aspartylhydrazide and α-DL-methylaspartic acid were found to be good inhibitors of the utilization of L-aspartic acid for the growth of *L. mesenteroides* P-60. The specificity of the inhibition was shown by the finding that the corresponding analogues of glutamic acid were much less active.

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