a soluble factor has been confirmed by the data presented.

We wish to acknowledge our indebtedness to Merck and Co., Rahway, N. J., for the crystalline vitamins; to Lederle Laboratories, Pearl River, N. Y., for the pteroylglutamic acid.

Received July 24, 1950. P.S.E.B.M., 1950, v75.

Utilization of L-, D- and DL-Forms of Asparagine and Aspartic Acid by *Leuconostoc mesenteroides* P-60.* (18105)

MERRILL N. CAMIEN† AND MAX S. DUNN

From the Chemical Laboratory, University of California, Los Angeles.

A microbiological method for the determination of L-aspartic acid with *Leuconostoc mesenteroides* P-60 was reported by Hac and Snell(1). These authors showed that L-asparagine had only a fraction of the activity of L-aspartic acid for this organism, but although their method has been widely used it does not appear that the activities of D-aspartic acid and D-asparagine for *Leuconostoc mesenteroides* have been reported previously.‡ It seemed desirable, therefore, to determine the response of this organism to the optically active and racemic forms of aspartic acid and asparagine.

Experimental. The culture of *Leuconostoc mesenteroides* P-60 (American Type Culture Collection catalogue No. 8042) was the same as that described previously(2). The inoculum was prepared as described in Paper 47(3). The experimental medium and assay technics were the same as those described previously for experiments with phenylalanine(4) except that 1 g of DL-phenylalanine was included in the amino acid mixture of the basal medium and the L-asparagine was omitted. In some of the experiments the medium was modified by additions of suboptimal amounts of L-asparagine or D-aspartic acid. Response to L-, DL-, and D-asparagines and aspartic acids§ was determined in the various modifications of the basal medium with incubation periods from 24 to 72 hours.

Discussion. Experiments with L-asparagine gave results which closely resembled those obtained previously by Hac and Snell(1). D-asparagine was entirely inactive in the present experiments (tested at final concentrations as high as 20 mg %), and DL-asparagine had 50% of the activity of L-asparagine.

Experiments with DL-aspartic acid gave essentially the same results (Fig. 1) as those obtained previously by Steele, *et al.*(5), however D-aspartic acid (Fig. 1) elicited a unique response. At concentrations up to about

* Paper 74. For Paper 73, see Eiduson, *et al.*(4). This work was aided by grants from the American Cancer Society through the Committee on Growth of the National Research Council. The authors are indebted to Samuel Eiduson, Gene Molene, and Ruth B. Malin for technical assistance.

† Present address: Université de Liége, Laboratoire de Chimie Physiologique, Institut Léon Fredericq, 17 Place Delcour, Liége, Belgium.

1. Hac, L. R., and Snell, E. E., *J. Biol. Chem.*, 1945, v159, 291.

‡ Steele, *et al.*(5) found that the activity of DL-aspartic acid was essentially the same as that of L-aspartic acid for *Leuconostoc mesenteroides*, but apparently these authors did not test D-aspartic acid.

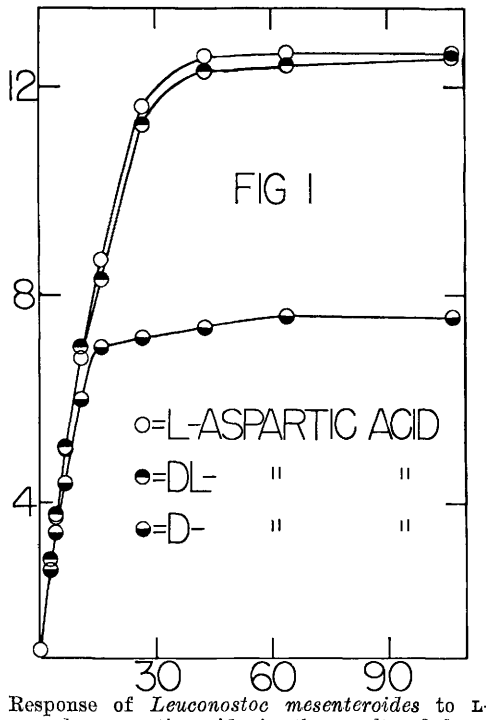
2. Dunn, M. S., Shankman, S., Camien, M. N., and Block, H., *J. Biol. Chem.*, 1947, v168, 1.

3. Dunn, M. S., Camien, M. N., Malin, R. B., Murphy, E. A., and Reiner, P. J., *Univ. Calif. Publ. Physiol.*, 1949, v8, 293.

4. Eiduson, S., Camien, M. N., and Dunn, M. S., Paper 73, *Archiv. Biochem.*, in press.

§ The L- and DL-asparagines and aspartic acids were A.P. and C.P. products of Amino Acid Manufacturers. The D-asparagine was a gift from M. Palmer Stoddard, Gerber, Calif. and D-aspartic acid ($[\alpha]_D^{25.0} = -25.45°$ in 1.008 *N* HCl) was obtained by acid hydrolysis of this product.

5. Steele, B. F., Sauberlich, H. E., Reynolds, M. S., and Baumann, C. A., *J. Biol. Chem.*, 1949, v177, 533.



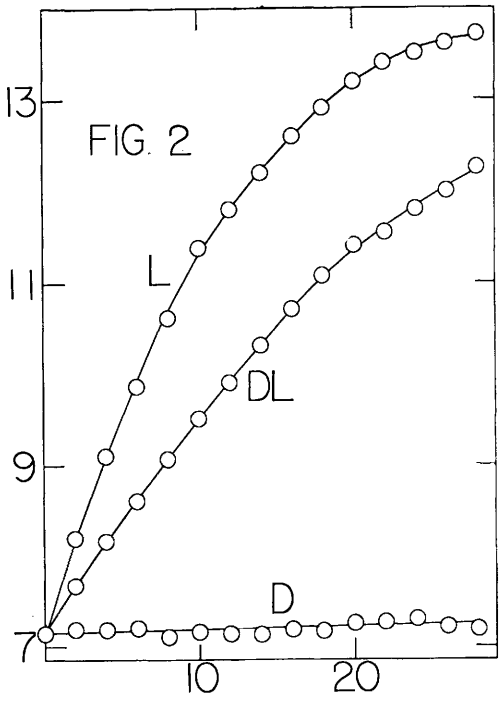
Response of *Leuconostoc mesenteroides* to L-, DL-, and D-aspartic acids in the unaltered basal medium with 65 hours incubation. The values on the horizontal scale represent the concentration of aspartic acid in γ per ml. The values on the vertical scale are calculated as ml of 0.01 *N* NaOH required to titrate 1 ml of culture.

10 γ per ml (Fig. 1) the activity of D-*aspartic* acid appeared to be nearly equal to that of L- or DL-aspartic acid. At higher concentrations of D-aspartic acid only a slightly increased response could be obtained. The response was not significantly greater than that shown in Fig. 1 at final concentrations of D-aspartic acid up to 20 mg %. It appears that a similar type of response to a D-amino acid by a lactic acid bacterium has not been reported previously.

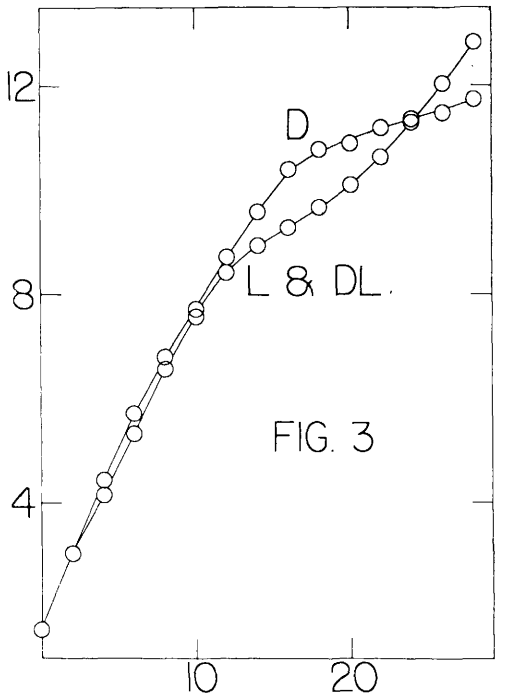
It was found in further experiments that the relative activity of D-aspartic acid became progressively less with shorter incubation, and that with incubation periods as short as 24 hours D-aspartic acid activity was nearly negligible. DL-aspartic acid was only slightly less active than L-aspartic acid, even at short incubation periods.

When D-aspartic acid at a final concentration of 20 mg % was included in the basal medium (Fig. 2) the response to DL-aspartic acid was approximately 50% of that to L-aspartic acid, and no significant response was obtained to further additions of D-aspartic acid. An incubation period of 48 hours was found to be nearly optimum for the demonstration of this effect. At longer incubation periods the blank titrations were excessively high, and at shorter incubation times the overall acid production was undesirably low. It seems probable that L-aspartic acid could be determined in samples containing relatively large amounts of D-aspartic acid with *Leuconostoc mesenteroides* under the conditions stipulated for Fig. 2.

Additions of L-asparagine to the basal medium markedly enhanced the response to D-aspartic acid (Fig. 3 and 4). D-asparagine appeared to be entirely inactive in producing this effect. A final L-asparagine concentration of 2.5 mg % appeared to be nearly optimal in producing approximately equal response to L-, DL-, and D-aspartic acids (Fig. 3). At lower L-asparagine concentrations the re-



Same as Fig. 1 except the basal medium was supplemented with D-aspartic acid (final concentration, 20 mg %), and the incubation period was 48 hours.

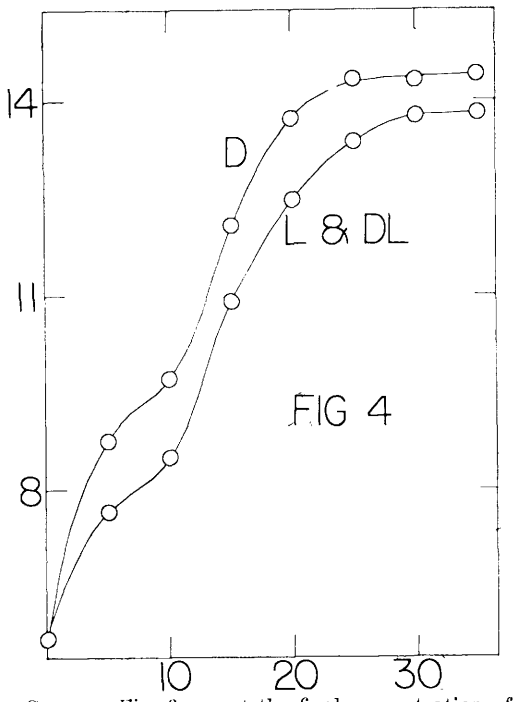


Same as Fig. 1 except the basal medium was supplemented with L-asparagine monohydrate (final concentration, 2.5 mg %), and the incubation period was 72 hours. The L- and DL-aspartic acid curves are superimposed. The deviations did not exceed the radii of the plotted circles.

sponse to D-aspartic acid tended to level off before the maximum acid production was obtained, and at higher concentrations high blank titrations and relatively high response to D-aspartic acid resulted (Fig. 4). It seems likely that total (L- and D-) aspartic acid could be satisfactorily determined with *Leuconostoc mesenteroides* under the conditions stipulated for Fig. 3, if the test range were limited to aspartic acid concentrations below 12 γ per ml. (The activity of D-aspartic acid appears to be significantly greater than that of L-aspartic acid at concentrations between 12 and 24 γ per ml.)

The facts that D-aspartic acid under certain conditions (Fig. 4) is more active than L- and DL-aspartic acids and that L-asparagine seems to have some function, particularly in the utilization of D-aspartic acid, indicate that D-aspartic acid and L-asparagine may be essential metabolites for *Leuconostoc mesenteroides* even though these compounds are less active than L-aspartic acid under the test conditions which are usually employed. The role of D-alanine(6-8) and D-glutamic acid(9) as essential metabolites for lactic acid bacteria has been suggested previously.

Summary. The utilization of the optically active and racemic forms of asparagine and aspartic acid by *Leuconostoc mesenteroides* has been studied under a variety of test conditions. D-asparagine was found to be entirely inactive, and DL-asparagine was found to have 50% the activity of L-asparagine. Conditions were described under which D-aspartic acid was essentially inactive and under which DL-aspartic acid had 50% the activity of L-aspartic acid. Under other con-



Same as Fig. 3 except the final concentration of L-asparagine hydrate in the basal medium was 5.0 mg %.

6. Holden, J. T., Furman, C., and Snell, E. E., *J. Biol. Chem.*, 1949, v178, 789.

7. Holden, J. T., and Snell, E. E., *J. Biol. Chem.*, 1949, v178, 799.

8. Camien, M. N., and Dunn, M. S., *J. Biol. Chem.*, 1950, v185, 553.

9. Dunn, M. S., Camien, M. N., Rockland, L. B., Shankman, S., and Goldberg, S. C., *J. Biol. Chem.*, 1944, v155, 591.

ditions L-, DL-, and D-aspartic acids had nearly equal activities. Methods for the separate microbiological determination of L-aspartic acid and total (L- and D-) aspartic acid with *Leuconostoc mesenteroides* were suggested.

Received July 24, 1950. P.S.E.B.M., 1950, v75.

Effect of Phlorhizin on Intestinal Absorption of Glucose, Galactose, Fructose, Mannose, and Sorbose. (18106)

E. M. BOGDANOVE* AND S. B. BARKER

From the Department of Physiology, State University of Iowa.

Although phlorhizin has repeatedly been shown to inhibit the intestinal absorption of glucose(1-8), the extent of inhibition reported has varied from 30%(7) to 90%(1). The effect of this glucoside on the absorption of hexoses other than glucose has not been investigated as thoroughly. Wertheimer(6), using the Cori technic, measured the absorption of glucose, galactose, fructose, and mannose by pairs of unanesthetized rats. One rat of each pair received an alkaline solution of 100 mg of phlorhizin in sugar solution by gastric tube; the control received only the sugar solution. Although he used only 2 pairs of rats each to examine fructose and galactose, and only one pair for mannose, he concluded that the inhibition exerted by phlorhizin upon glucose absorpton applied also to the absorption of galactose, fructose, and mannose. Yoshikawa(8), using 2 consecutive 30 cm segments of rabbit jejunum, introduced 5.4% solutions of various sugars into these loops and removed samples for analysis at regular intervals. By putting into one of the loops a solution of phlorhizin, sugar, and sodium bicarbonate, and into the other loop only the sugar and bicarbonate, he was able to use the same intestine both for control and experiment. He found that the order of absorption for the sugars tested was: galactose, glucose, mannose, arabinose, xylose, and fructose. The greatest phlorhizin inhibition appeared to be upon galactose absorption, the least upon fructose.

Cori(9) and Wilbrandt and Laszt(10) have found glucose and galactose to be rapidly absorbed, mannose and the pentoses to be absorbed quite slowly, and the absorption rate of fructose to be intermediate between that for glucose and that for mannose. Wertheimer's(6) data do not include the various rates of absorption, since he measured the percentage of sugar absorbed. Yoshikawa's (8) finding that fructose was absorbed more slowly than mannose, or even xylose, suggested the need for further experiments along this line.

Experimental. The experiments were performed on albino and hooded rats of both sexes selected from stock raised in this laboratory. Absorption was studied by means of a technic employing the entire small intestine. Prior to the experiment the rats received 6 subcutaneous injections of a 10% suspension

* This investigation was performed in partial fulfillment of the requirements for the degree of Master of Science in the Department of Physiology, State University of Iowa.

1. Althausen, T. L., and Stockholm, M., *Am. J. Physiol.*, 1938, v123, 577.

2. Lundsgaard, E., *Biochem. Z.*, 1933, v264, 221.

3. Nakazawa, F., Tohoku, *J. Exp. Med.*, 1922, v3, 288.

4. Ohnell, R., and Höber, R., *J. Cell. Comp. Physiol.*, 1939, v13, 161.

5. Soulairac, A., *Ann. d'Endocrinologie*, 1947, v8, 377.

6. Wertheimer, E., *Arch. Ges. Physiol.* (flügers), 1933, v233, 514.

7. Wilson, R. H., *J. Biol. Chem.*, 1932, v97, 497.

8. Yoshikawa, T., *Sei-i-kwai Med. J.*, 1935, v54, 75.

9. Cori, C. F., *J. Biol. Chem.*, 1925, v66, 691.

10. Wilbrandt, W. and Laszt, L., *Biochem. Z.*, 1933, v259, 398.