thiouracil, and various other iodine containing preparations, the shorter interval is highly desirable.

The reference sample diluted to constant volume is placed in a small saline bottle and counted at the same distance of 17 cm. The percentage uptake in the gland is represented by:

$$Up \frac{(Cg \times 100)}{(Cs)} F_G.$$

Where Up denotes the percentage uptake, Cg is the count rate over the gland, Cs is the count rate over the standard sample, and F_G is the geometrical factor.

This geometrical factor is a function of the ratio of the solid angles presented by the gland and the reference sample. If the reference sample is standardized, this factor reduces to a function of the variation of the solid angle presented by the gland. Consequently this factor is a function of the variation in gland size. Standardizing on the basis of the "average gland", this F_G will be greater than one for all glands larger than "average" and so on. However, reconsidering the distributive picture of the directional characteristics of the tube, it is readily seen that no serious error will be introduced by assuming this factor equal to one.

Summary. It has been found that with the bismuth cathode tube, a 50 uC dose can be used efficiently, and that a tube-to-patient distance of 17 cm will cover a sufficiently large area to make variations in gland size unimportant to the accuracy of the test.

Received May 28, 1951. P.S.E.B.M., 1951, v77.

Mandelic Acid Antagonism to Phenyllactic Acid Utilization in *L. casei*.* (18921)

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In the course of experiments designed to develop microbiological methods for the determination of specific aromatic acids in urine, *L. casei* was found to utilize phenyllactic acid in an otherwise complete but phenylalanine-free medium. This observation has recently been made also in other laboratories(1,2). The present report concerns the growth inhibitory activity of certain structural analogues of phenyllactic acid on this organism.

Methods. Our microbiological assay procedures, media, cultures, inocula, etc., have been described elsewhere(3). DL-Phenyllactic acid was prepared by the methods of Erlenmeyer and Lipp(4), and Fischer and Zemplén(5). Both products when recrystallized twice from carbon tetrachloride melted at 95°C and gave identical growth curves. Melting points of 95-96 and 96-97(6), 97(7), and 97.5°C(2) have been reported for DL-phenyllactic acid.

The growth stimulating effect of phenyllactic acid, phenylalanine, and the various ana-

* This investigation was supported (in part) by a grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service, and a grant-in-aid from the Bressler Alumni Research Fund.

† John F. B. Weaver Fellow in Biological Chemistry.

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TABLE I. Growth Promoting Activity of Phenyllactic Acid Analogues on *L. casei*.

Compound studied	Quantity of analogue (μg/tube) 0	2.5	5	10	20	40
	Titration values as ml 0.02 N NaOH					
Phenylacetic acid*	1	.9	1	1	.8	1.1
trans-Cinnamic acid†	1	.8	1	1	1	.9
Hydrocinnamic acid*	.9	.9	1	1	1.1	1
trans-o-Coumaric acid‡	1	1.1	1	.8	1.1	1
DL-mandelic acid*	1	.8	1.1	1	1	1.1
p-Hydroxyphenylacetic acid*	1	1	1.1	.9	.8	1
p-Hydroxybenzoic acid†	1.1	.9	1	1	.9	1
β-Phenylisoserine§ (m.p. 231°C)	.2	.4	.3	.2	.2	.2
β-Phenylisoserine§ (m.p. 265°C)	.2	.3	.2	.3	.3	.2
DL-Phenylalanine‖	.8	1.7	3	5.3	7.8	—
DL-Phenyllactic acid	.8	3	5.2	7.8	9.2	—

* Bios Laboratories, Inc., New York City.
† Eastman Kodak Co., Rochester, N. Y.
‡ Synthesized (m.p. 207-208.5°C).
§ Two unidentified isomers synthesized by Dr. Robert Ellin of the School of Pharmacy. Although each isomer could consist of a mixture of two structural forms, no attempt at separation was made. Fourneau and Billeter(7) prepared similar isomers melting at 230-232 and 275-285°C, respectively.
‖ Merck and Co., Inc., Rahway, N. J.

TABLE II. Effect of Analogues on Utilization of Phenyllactic Acid by *L. casei*.

Analogue studied	Phenyllactic acid, μg/tube	Quantity of analogue required (μg/tube) For first indication of inhibition	For complete inhibition	Max quantity of analogue tested
Phenylacetic acid	5	5000	*	8000
trans-Cinnamic acid	5	*	*	400
Hydrocinnamic acid	5	*	*	6000
trans-o-Coumaric acid	5	*	*	250
DL-Mandelic acid	5	500	5000	50000
p-Hydroxyphenylacetic acid	5	500	*	20000
p-Hydroxybenzoic acid	5	2000	*	2000
β-Phenylisoserine (m.p. 230-232°C)	5	*	*	10000
β-Phenylisoserine (m.p. 265°C)	5	*	*	10000

* This value could not be ascertained definitely due to the relative inactivity and/or insolubility of the compound under investigation.

logues was determined by adding appropriate quantities to assay tubes containing 1 ml of the phenylalanine-free basal medium. The contents of each tube were adjusted to pH 6.9, diluted to 3 ml, autoclaved at 18 pounds steam pressure for 10 minutes, cooled, inoculated, and incubated at 34°C for about 72 hours. The acidity produced was titrated with 0.02 N sodium hydroxide to pH 6.9. The inhibitory activity of certain analogues on phenyllactic acid utilization was studied in a similar manner.

Results. The data in Table I show that the various phenyllactic acid analogues were not available to *L. casei*. However, both isomers of DL-phenyllactic acid appeared to be utilized when in the proper concentration. An equal response to D- and to L-phenyllactic acids by *L. casei*(2) and by *L. arabinosis*(1) have been obtained in other laboratories. We found that *L. casei* was not able to convert phenyllactic acid into tyrosine, since growth was not obtained on a medium free of both phenylalanine and tyrosine.

The data in Table II show that DL-mandelic acid is a potent inhibitor of phenyllactic acid utilization as are p-hydroxyphenylacetic acid and p-hydroxybenzoic acid to a lesser degree. Decisive results were not obtained with some of the other analogues due to their relative inactivity and/or insolubility. It is interesting to note that the two mixed isomers of β-phenylisoserine are inert since DL-phenylserine inhibits competitively the func-

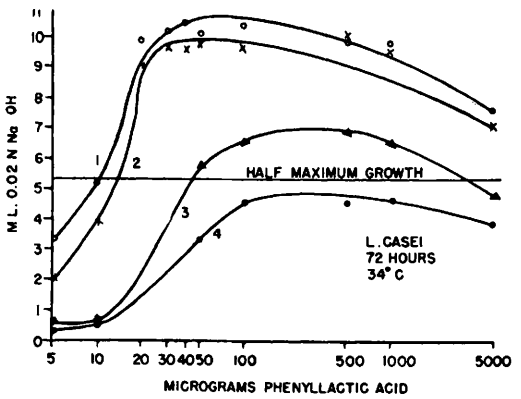

FIG. 1. Effect of DL-mandelic acid on DL-phenyllactic acid utilization. The abscissa is in log scale. Curve 1, 0; curve 2, 1,000; curve 3, 5,000; curve 4, 10,000 μg mandelic acid, respectively.

tioning of phenylalanine in *L. arabinosis*(8).

The good growth response to increments of DL-phenylalanine ranging from 0 to 10,000 μg, even in the presence of 0 to 10,000 μg of mandelic acid per assay tube (data omitted) indicates that mandelic acid *per se* was not toxic to *L. casei* and did not interfere appreciably with the microbial utilization of phenylalanine. The potency of this inhibitor appears to rest primarily on its ability to prevent the enzymatic conversion of phenyllactic acid to phenylalanine.

A more precise examination of the mandelic acid-phenyllactic acid antagonism was made by adding 0, 500, 1,000 5,000, and 10,000 μg of mandelic acid, respectively, to assay tubes each containing 1 ml of phenylalanine-free medium and graded quantities of phenyllactic acid ranging from 0 to 5,000 μg. The assay was carried out in the usual manner and curves were then constructed from the titration data which relate concentration of inhibitor to the amount of growth. A typical response is illustrated by Fig. 1. The data show that phenyllactic acid can reverse the toxicity of mandelic acid to *L. casei*. However, complete reversal was not obtained. While half maximum growth was not achieved in the presence of 10,000 μg of inhibitor, regardless of the amount of phenyllactic acid added, in a second experiment somewhat higher titration values were obtained, namely 5.8, 6.4, and 5.4 ml of 0.02 N sodium hydroxide upon the addition of 100, 500, and 1,000 μg of phenyllactic acid, respectively, to 10,000 μg of mandelic acid. Apparently large quantities of phenyllactic acid are somewhat toxic *per se*.

An examination of the curves in Fig. 1 and other data (omitted) indicates that the inhibitory action of mandelic acid is not competitive over a wide range of nutrilite to inhibitor under our experimental conditions. In a similar but more detailed experiment, the addition of 1,000, 2,000, 3,000, 4,000, and 5,000 μg of mandelic acid to a series of assay tubes containing the basal medium and increments of phenyllactic acid ranging from 0 to 500 μg produced half maximum growth in the presence of 8, 10.5, 15, 20, and 30 μg of phenyllactic acid which yielded inhibition indices of 125, 190, 200, 200, and 167, respectively. Hence, within a narrow range, the inhibitory action of mandelic acid appeared to be competitive.

In view of the widespread use of mandelic acid as a urinary antiseptic, its potency as a metabolic antagonist appears most interesting.

Summary. The growth stimulatory and inhibitory action of several analogues of phenyllactic acid on *L. casei* were studied. DL-mandelic acid was found to be a potent inhibitor of phenyllactic acid utilization by this organism. The action was not competitive, except over a narrow range of inhibitor to nutrilite. Mandelic acid did not interfere with the functioning of phenylalanine.

Received May 28, 1951. P.S.E.B.M., 1951, v77.

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