An Interrelationship between Calcium Ion and Serine in the Nutrition of *Lactobacillus casei.** (19941)

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The recent finding that D(-)-lactic acid is an important growth stimulant for *Lactobacillus casei*(1) prompted an investigation of the effect of calcium DL-lactate on the response of this organism to amino acids. A marked stimulation in response to serine was obtained with the addition of this compound to the test medium, but improvements in response to nine other essential (for *L. casei*) amino acids were only slight or negligible. Further investigation revealed that an elevated calcium ion concentration was essential for *L. casei* in media containing relatively low serine levels and that responses to serine and/or calcium were unaffected by additions of sodium DL-lactate.

Experimental. The basal test medium was the same as that used previously(1) except that the casein digests were replaced by 20 mg % each of DL-alanine, L-arginine monohydrochloride, L-aspartic acid, glycine, L-glutamic acid, L-histidine monohydrochloride monohydrate, DL-isoleucine, L-leucine, DL-lysine monohydrochloride, DL-methionine, L-proline, DL-phenylalanine, DL-serine, DL-threonine, L-tyrosine and DL-valine. The response of *L. casei*† to each of the amino acids found previously to be required by this organism(2) was determined in the basal medium (omitting in each case the test amino acid) alone and with additions of calcium chloride and sodium DL-lactate. The experimental procedures were the same as those described previously for amino acid determinations(3).

Results and discussion. Responses to arginine, leucine, phenylalanine, tyrosine and valine were not significantly affected by the presence or absence of calcium chloride and/or sodium DL-lactate at concentrations of 0.001 molar in the basal medium, and only slight stimulatory effects were noted in the responses to cystine, glutamic acid, isoleucine, and tryptophan when the supplements were used. The response to serine was unique, however, in that a marked stimulation was obtained by adding calcium chloride to the basal medium (Fig. 1). Sodium DL-lactate had no effect on response to serine in the basal medium alone and caused no further stimulation in the calcium chloride supplemented medium.

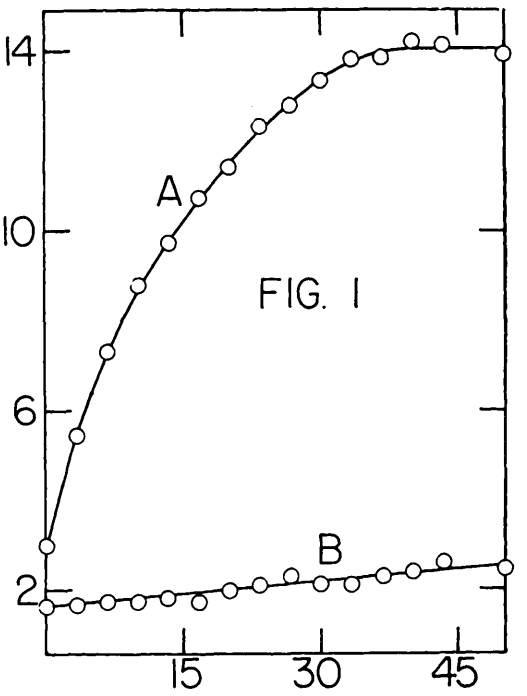

FIG. 1. Response of *L. casei* to DL-serine with 3 days incubation in A) the basal medium supplemented with .001 mM $CaCl_2$/ml, and B) the unsupplemented basal medium. The values on the vertical scale are the ml of .01 *N* NaOH required to titrate one ml of culture, and those on the horizontal scale are the concentrations of DL-serine in μg/ml.

Response to calcium was determined in the basal medium modified to contain a reduced concentration (5 mg %) of DL-serine.

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† American Type Culture Collection No. 7469. This culture was maintained as described previously (3).

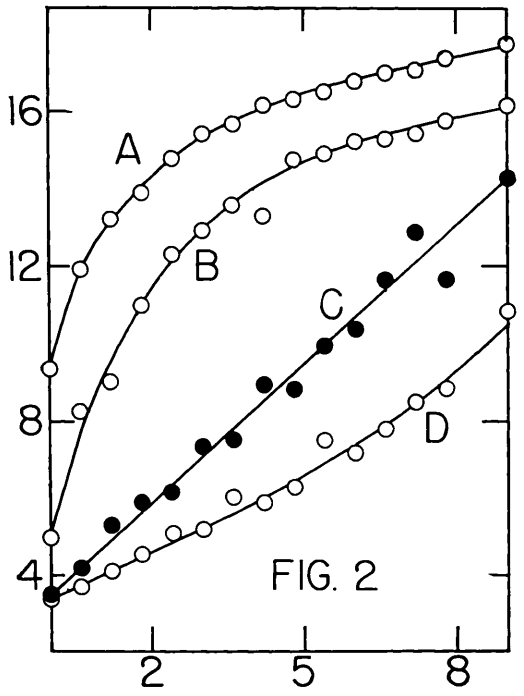

FIG. 2. Response of *L. casei* to $CaCl_2$ with 3 days incubation in the basal medium modified to contain 5 mg % of DL-serine and the following concentrations of sodium (in mM %): A) 7.1, B) 13, C) 25, and D) 36. The vertical scale is the same as in Fig. 1. The values on the horizontal scale are the concentrations of $CaCl_2$ calculated as μg of Ca/ml.

Under these conditions growth was poor in the absence of added calcium, but good growth was obtained with moderate additions of this ion,‡ (Curve C, Fig 2). In separate experiments it was shown that further slight stimulations of growth were obtained with still higher calcium concentrations up to those at which salts precipitated from the medium.

Mutual antagonisms between the ions of calcium, sodium and potassium are recognized in the biochemistry of higher organisms, and it seemed possible that the requirement of *L. casei* for calcium in the low-serine medium might be dependent upon the relatively high sodium content (24.6 mM %) of this medium.§ Accordingly the response to calcium was redetermined under the same conditions except that the sodium content was varied form 7.1 - 36 mM % in a series of 6 media.‖

A marked antagonism between sodium and calcium was evidenced by an increased slope of the calcium response curve at the lowest sodium concentrations (Curves A and B, Fig. 2) and by a decreased slope at higher sodium concentrations¶ (Curves C and D, Fig. 2). It appeared, however, that the calcium requirement was not dependent upon an excessively high sodium ion concentration.

No explanation of the unique relationship between serine and calcium is apparent at this time, and further investigations of the calcium metabolism of *L. casei* are in progress.

Summary. A requirement of *L. casei* for calcium was induced by employing a reduced serine concentration in the basal medium. The calcium requirement was not demonstrable with a reduced concentration of any one of the remaining essential (for *L. casei*) amino acids. A marked increase in the sensitivity of response to calcium was obtained with reduced sodium concentrations, but the requirement for calcium in the low-serine medium persisted even at the lowest test levels of sodium.

‡ The basal medium contained about 0.2 μg of calcium (as calcium DL-pantothenate) per ml.

§ This relatively high sodium chloride concentration is routinely used in media for studies in this laboratory which might lead to applications as assay methods. The use of a high basal salt concentration makes it possible to eliminate disturbing salt effects by compensating for the high salt concentrations which often are encountered in test samples(3).

‖ Sodium chloride and ammonium chloride were eliminated from the basal medium, and part of the sodium acetate was replaced by ammonium acetate (retaining the original acetate and ammonium concentrations) to obtain the initial sodium concentration of 7.1 mM %.

¶ An increased scattering of points may be noted in curves C and D of Fig 2. It seems likely that this effect may be due to variations in calcium dependence brought about by differing degrees of adaptation or mutation occurring in the separate assay tubes, since adapted cultures of *L. casei* which are capable of rapid and profuse growth in low-serine media without additions of calcium have been readily obtained under the conditions of curve C, Fig. 2 (unpublished data of D. E. Atkinson with the present authors). If this explanation is correct, it would appear that the adaptations were favored by the higher sodium concentrations.

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The Distribution of C^{14} Labeled Isonicotinic Acid Hydrazide in Normal Mice.* (19942)

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Isonicotinic acid hydrazide and its isopropyl derivative are now being subjected to widespread clinical evaluation throughout this country(10). The promise that these drugs have shown warrants a further study of their pharmacological properties, whether or not these drugs do become accepted generally for use in the treatment of tuberculosis. This paper is the first report of our group on a series of investigations to determine such properties of isonicotinic acid hydrazide. As a basis for interpreting future work, we have studied the absorption, distribution and excretion of the drug in normal mice.

The isotope tracer technic was used in this problem since it is a rapid yet sensitive technic well suited to determining micro quantities of a drug and its metabolite in many tissues. We have been able to obtain isotopically labeled isonicotinic acid hydrazide through the cooperation of the Health Division, Los Alamos Scientific Laboratory, Los Alamos, New Mexico. The drug which was synthesized by Murray and Langham(1) is labeled with C^{14} in the carboxyl position and has an activity of 0.045 mc/mg.†

Materials and methods. A saline solution of the labeled isonicotinic acid hydrazide diluted with crystalline normal drug was made up to contain 400 micrograms per milliliter with an activity of 2.54×10^6 cpm. The refrigerated solution was found by paper chromatography to be stable for longer than one month. Forty-two female mice of the CF_1 strain weighing approximately 20 g each were used for this study. Each mouse received a subcutaneous injection in the right hind leg of 0.5 ml of the above solution (10 mg/kg). The mice were kept in all-glass metabolism chambers(2) throughout the experimental period and the exhaled CO_2 trapped in towers of sodium hydroxide solution. At the end of each experimental period, the mice were sacrificed by cervical fracture, the heart rapidly exposed and 0.25 to 0.5 ml of blood withdrawn by cardiac puncture. The tissues were dissected out, weighed and aliquots plated directly in duplicate(3) on copper discs as 10% water homogenates. Three mice were used for each of the shorter experimental periods and four or more for each of the longer ones. All tissue samples were counted in windowless gas-flow counters(4) with a counter efficiency of 65%. Standard corrections were applied for geometry, resolving time, and self-absorption before the average of the duplicate plates was taken. In view of the high activity of the injected drug, statistically significant counts could still be detected in some tissues as long as one week after injection. Since this represented only a relatively small amount of drug, only values greater than twice the standard deviation of the counting background were considered as significant. Thus counts equivalent to less than 0.0075 μg of drug or metabolite per gram of tissue are

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† The C^{14} used for the synthesis of this compound was obtained on allocation from the Isotope Div., U.S. A.E.C.