Growth Response of *Lactobacillus casei* (ATCC 7469) to Riboflavin, FMN, and FAD.* (31077)

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A method for determination of total tissue flavins (riboflavin, FMN, and FAD) involves extraction with 0.1 N HCl and microbiological assay of the extract using riboflavin as the standard(1). The growth response of *Lactobacillus casei* (ATCC 7469) to these flavin compounds, based on acid production, has been noted to be identical(2).

We present evidence here that shows that the growth response of this organism, measured turbidimetrically, differs significantly between the 3 flavin compounds, riboflavin, FMN, and FAD.

Freshly prepared standard solutions of riboflavin, FMN, and FAD (Sigma Chemical Co., St. Louis, Mo.) (100 $\mu\mu$mol/ml) were used for each assay. Ten separate assays were conducted. Six assays covered the range from 0 to 50 $\mu\mu$mole per tube in 10 $\mu\mu$mole increments and 4 assays covered the range from 0 to 60 $\mu\mu$mole per tube in 20 $\mu\mu$mole increments.

An aliquot of the standard solution was placed in an assay tube and sufficient water was added to give a volume of 1.5 ml. Double strength assay media (Difco Laboratories, Detroit, Mich.) was then added to a final volume of 3 ml. All 3 compounds were assayed concurrently in triplicate. The assay tubes were sterilized by autoclaving, cooled, and inoculated with a freshly prepared inoculum of *L. casei* (ATCC 7469)(3). After incubation at 37°C for a period ranging from 21-22 hours, growth was measured turbidimetrically at 650 mμ. The results are expressed in terms of absorbancy units/$\mu\mu$mole flavin.

Paper chromatographic analysis of the standard compounds was conducted in 3 of the experiments using the solvent systems described by Huennekens and Felton(4).

Statistical analysis was by the "t" test as described by Snedecor(5).

* Abbreviations: FMN = flavin mononucleotide, FAD = flavin adenine dinucleotide.

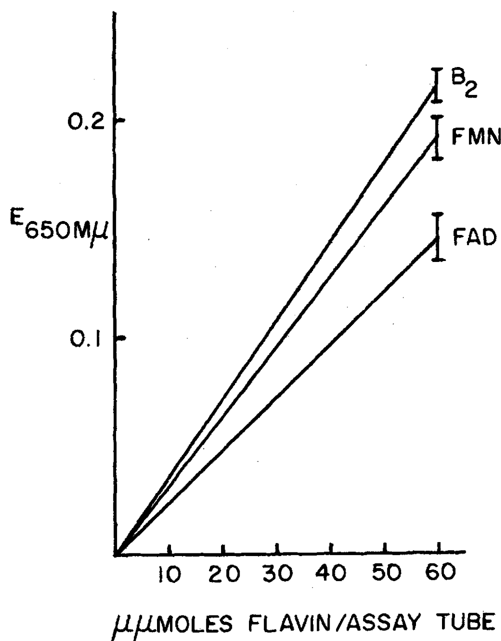

FIG. 1.

The results (Fig. 1) show that there is a significant difference between riboflavin and FMN and a highly significant difference between riboflavin and FAD and between FMN and FAD with regard to the growth response of *L. casei*. A linear response of *L. casei* to all flavins tested was noted in all experiments.

In the acid extraction of tissue flavins there is a degradation of the FAD to FMN but little or no breakdown of the FMN to riboflavin(6-8). Riboflavin *per se* accounts for a quantitatively insignificant amount of the total tissue flavin(8). The results presented here clearly show that the growth response to riboflavin and FMN is not the same when measured as described. The use, therefore, of riboflavin as a standard in the microbiological assay for tissue flavin extracted by acid would lead to quantitatively inaccurate results because very little of the extracted flavin is actually riboflavin.

Paper chromatographic analysis and scanning with ultraviolet light showed trace contamination of the FMN with riboflavin and trace contamination of the FAD with FMN and riboflavin. The amount of the contaminants was so low as not to be of the order that would statistically alter the observed results.

Based upon the observations described herein, it is suggested that in the turbidimetric microbiological determination of tissue flavin, where the flavins are extracted by acid hydrolytic means, FMN should be used as the primary standard.

Summary. The growth responses, measured turbidimetrically, of *Lactobacillus casei* (ATCC 7469) to riboflavin, FMN, and FAD differ significantly from each other. In the assay of acid extracts of tissues for flavin content, the use of FMN, as the standard, is recommended.

1. Adrian, J., Le dosage microbiologique des vitamines du groupe B Cahier Technique 4 du CNERNA Centre National de la Recherche Scientifique, Paris, France, 1959, p57.

2. Snell, E. E., in The Vitamins: Chemistry, Physiology, and Pathology, Sebrell, W. H., Harris, R. S., eds., Academic Press, Inc., New York, 1954, v3.

3. Difco Manual, 9th Ed., Difco Laboratories, Inc., Detroit, Mich., 1953, p214.

4. Huennekens, F. M., Felton, S. P., in Methods in Enzymology, Colowick, S. P., Kaplan, N. O., eds., Academic Press, Inc., New York, 1957, v3, p957.

5. Snedecor, G. W., Statistical Methods, Iowa State College Press, Ames, Iowa, 1956.

6. Abraham, E. P., Biochem. J., 1939, v33, 543.

7. Kuhn, R., Rudy, H., Z. Physiol. Chem., 1936, v349, 47.

8. Bessey, O. A., Lowry, O. H., Love, R. H., J. Biol. Chem., 1949, v180, 755.

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Isolation of a Reaginic Antibody Fraction with Properties of γ_G-Globulin.* (31078)

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Attempts to isolate highly purified and highly active reaginic antibodies have, in general, achieved limited success. Previous attempts to apply DEAE-cellulose ion-exchange chromatography(1-4) to the isolation of reagin from grass and ragweed pollen sensitive patients resulted in a distribution of the reaginic activity into several fractions containing both γ_G- and γ_A-globulins. With the availability of Sephadex G-200, attempts to apply the gel-filtration method in conjunction with ion-exchange chromatography(4-6) have yielded much the same results. This report describes the isolation of a highly active reaginic antibody fraction which has a sedimentation coefficient of $6.7S_{20,w}$, is heat unstable at 56°C, and gives a single line of precipitation against rabbit anti-total human globulins and specific rabbit anti-human γ_G-globulin.

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Materials and methods. Allergens and reagin. Reaginic serum was obtained from an untreated timothy sensitive patient (E.F.). This serum was collected during the pollen season, and was kept at —20°C until used. Preliminary to the isolation of the reaginic antibody fraction described below, this serum gave positive Prausnitz-Kustner (P-K) reactions at a dilution of 1:10,000 in 4 non-allergic human volunteers. The allergens used in passive transfer experiments in both man and rhesus monkeys were a crude water soluble timothy extract (WST) and a partially purified fraction of timothy pollen (>40% ETOH). Methods for preparation of both WST and >40% ETOH fraction have been described(7).

Antisera to human serum proteins. Rabbit