THE ACTIVITY OF "LACTOBACILLUS CASEI FACTOR," "FOLIC ACID," AND "VITAMIN B," FOR STREPTOCOCCUS FAECALIS AND LACTOBACILLUS CASEI

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(Received for publication, January 24, 1946)

It has been established that the terms vitamin M, vitamin B₁₂, norit eluate factor, Lactobacillus casei factor, factor U, folic acid, and factor SLR all refer to a group of compounds (or a compound) with similar characteristics and similar biological activities. Of these factors seven have been reported in purified form: folic acid (isolated from spinach) (1), three L. casei factors (one from liver, one from yeast, and one from a fermentation residue) (2, 3), synthetic liver L. casei factor (4), vitamin B₁₂ (from liver or yeast) (5), and factor SLR (6).

Pure folic acid was originally (1) assigned a potency of 40,000 (i.e. the pure compound was said to be 40,000 times as active as Wilson's liver fraction B). In the work reported from this laboratory (7) on the excretion of folic acid in sweat and urine, a folic acid concentrate furnished by Dr. R. J. Williams was used as standard, and the results were reported in terms of 40,000 potency. However, other active compounds have become available in pure form and Mitchell, Snell, and Williams (8) have reported a folic acid concentrate with a potency of 137,000. Therefore, in order to obtain a means of comparing data reported on the basis of 40,000 potency folic acid with data based on pure compounds as standards, the relative activities of vitamin B₁₂, folic acid concentrate, synthetic Lactobacillus casei factor, and fermentation L. casei factor for Streptococcus faecalis and L. casei were determined. The activities of several of these materials in terms of requirements for half maximum growth have been reviewed by Luckey, Teply, and Elvehjem (9) and by Peterson and Peterson (10).

EXPERIMENTAL

Streptococcus faecalis Assay—Streptococcus faecalis folic acid assays were carried out by the procedure of Luckey, Briggs, and Elvehjem (11) modified by the inclusion of 5 cc. of Salts B (12) and the replacement of pyridoxine by 100 γ of pyridoxamine per 500 cc. of double strength medium. Turbidities were read at 650 mμ with the Coleman universal photoelectric colorimeter after 16 hours incubation at 30°.

* We wish to acknowledge the technical assistance of Shirley Spaeth.

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The activities of the following materials were compared: (a) a 7.7 per cent folic acid concentrate of potency 3100 (1) kindly supplied by Dr. R. J. Williams of the University of Texas, (b) a solution of crystalline vitamin Bc (5) kindly furnished by Dr. O. D. Bird of Parke, Davis and Company, (c) a solution of crystalline Lactobacillus casei factor (3) and (d) synthetic Lactobacillus casei factor (4) both kindly supplied by Dr. E. L. R. Stokstad of the Lederle Laboratories, Inc.

The dose-response curves for these compounds on the growth of Streptococcus faecalis are given in Fig. 1. The folic acid is plotted both on the basis of 40,000 potency as usual and also on the basis of 137,000 potency.

**Lactobacillus casei Assay**—Two methods of assay were used: the Landy and Dicken method (13) modified by the addition of 10 γ of p-aminobenzoic
acid and the replacement of the pyridoxine by 100 γ of pyridoxal per 500 cc. of double strength medium, and the method of Teply and Elvehjem (14). With both methods the tubes were titrated after 72 hours incubation at 37°.

The same materials were compared as to relative activity for Lactobacillus casei as had been tested with Streptococcus faecalis and the dose-response curves are plotted in Fig. 2. For these assays the medium of Teply and Elvehjem was used. While the relative activities of the four standards were the same by the modified Landy and Dicken medium, the total amount of acid produced was much less. In Fig. 2 the Texas folic acid concentrate is plotted on the basis of 137,000 potency only.

DISCUSSION

From Fig. 1 it is evident that Lederle synthetic Lactobacillus casei factor, Parke-Davis vitamin B₁₂, and Texas folic acid, calculated on the basis of 137,000 potency for the pure material, have equal activities for Streptococcus faecalis. Lederle fermentation Lactobacillus casei factor has only one-twentieth of the potency of these other materials. These data indicate that the first three materials are the same compound.
Some differences in activity are indicated in Fig. 2. However, in view of the lack of difference in response with *Streptococcus faecalis*, these differences may not be significant. These curves were obtained with the Teply and Elvehjem (14) medium with 1 mg. of norit-treated peptone per tube. The differences in the graphs may represent the effect of traces of other materials present rather than different compounds possessing different activities.

The half maximum doses for the compounds calculated from Fig. 2 are vitamin B₁₂ 0.50 millimicrogram per tube, Lederle synthetic *Lactobacillus casei* factor 0.70 millimicrogram per tube, and Texas folic acid (137,000 potency) 0.80 millimicrogram per tube and *L. casei* factor 3.7 millimicrograms per tube. These figures agree favorably with those summarized by Luckey, Teply, and Elvehjem and by Peterson and Peterson (10). It was found that by changing the amount of norit-treated peptone in the Teply and Elvehjem medium (14) these half maximum doses could be changed. Eliminating the peptone increased the half maximum dose for Texas folic acid from 0.80 millimicrogram to 1.0 millimicrogram per tube, and increasing the peptone from 1 mg. to 2 mg. per tube decreased the half maximum dose for vitamin B₁₂ from 0.50 millimicrogram to 0.35 millimicrogram per tube. The inverted dose-response curves to the crystalline *L. casei* factor (from a fermentation residue) was reported previously (7) and has recently been confirmed by Dr. E. L. R. Stokstad.¹ Day et al. (15) have reported that treatment of a similar preparation with enzyme greatly increased its activity toward *Streptococcus faecalis*.

Because three of the standard materials had equal activities for *Streptococcus faecalis* and *Lactobacillus casei*, it was decided to compare the activities of other materials for these two organisms, following enzymatic digestion. The values obtained for Difco yeast extract were 84 γ per gm. for *Lactobacillus casei* and 83.5 γ per gm. for *Streptococcus faecalis*. The enzyme used was desiccated hog kidney prepared by the method of Bird *et al.* (16). Other materials assayed did not give similar values with the two organisms. This may be due either to incomplete enzymatic liberation of the conjugates present in the other materials or merely to different relative activities of related compounds, as has been found for biotin and its vitamers (17), pyridoxine and its metabolites (18), and nicotinic acid and nicotinamide (19).

**SUMMARY**

1. A comparison was made of the activities of four different "folic acid"-active materials: synthetic "*Lactobacillus casei* factor" (Lederle), "*L. casei factor*" (Lederle), "vitamin B₁₂" (Parke-Davis), and "folic acid" concentrate (Texas) as growth factors for *Streptococcus faecalis* and *L. casei*.

¹ Private communication.
2. The synthetic "Lactobacillus casei factor," "vitamin B₉," and "folic acid" calculated on the basis of 137,000 potency for pure "folic acid" were found to be equally active for Streptococcus faecalis and almost equally active for Lactobacillus casei, with "vitamin B₉" exhibiting the greatest activity and "folic acid" the least.

3. Yeast extract alone gave equal assay values with both organisms when various materials were assayed following digestion with "vitamin B₉ conjugase."

BIBLIOGRAPHY
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