Technical Papers

Determination of Vitamin B_{12} with a Mutant Strain of *Escherichia coli*

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The determination of vitamin B_{12} activity in various natural materials has generally been accomplished by the use of microbiological assays, employing lactic acid bacteria (*1, 2*) or green flagellates (*3–5*). The complex growth requirements and lack of specificity of response in some B_{12}-utilizing microorganisms have made assays with them difficult (*6, 7*) or unreliable, and the precise determinations that can be obtained with *Euglena* require about 7 days for the period of incubation. In our studies on the binding of vitamin B_{12} to various natural materials, it seemed desirable to develop a new method of assay using a mutant strain of *E. coli*. Essential information concerning the assay is reported briefly here.

The mutant strains of bacteria were obtained from Bernard D. Davis (*8*), who produced them by selection from ultraviolet-irradiated cultures of *E. coli* W (ATCC 9637). One strain, 113-3, showed marked responses to both methionine and vitamin B_{12}, whereas another strain, 26-18, was stimulated by methionine or homocystine but not by vitamin B_{12}. The strain 113-3 was employed in establishing dosage-response curves for different levels of the vitamin and for methionine. It was found that the great sensitivity of the organism to relatively low concentrations of vitamin B_{12} as compared with the requirement for much higher amounts of methionine permitted the development of specific vitamin B_{12} assays of many natural materials by appropriate dilution of samples. Proof of the response of strain 113-3 to vitamin B_{12} in complex materials was obtained by making parallel determinations at different levels for the vitamin with strain 113-3 and for methionine with strain 26-18. The possibility of using strain 113-3 for specific assay of vitamin B_{12} was pointed out in the paper of Davis and Mingioli (*8*), who observed that growth of these bacteria in the absence of the vitamin could not be satisfied by a variety of compounds, including certain amino acids, vitamins, purines, pyrimidines, ribonucleosides and ribonucleotides, thymidine, and intermediates of the B_{12} molecule.

The basal medium which we have used is modified after the formula employed by Davis and Mingioli, with the addition of organic sources of nitrogen in accordance with results obtained in tests made with supplements of single amino acids. The composition of the assay medium made double strength is as follows: H_2O, 500 ml; K_2HPO_4, 7.0 g; KH_2PO_4, 3.0 g; Na_3 citrate $\cdot 3H_2O$, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $(NH_4)_2SO_4 \cdot 1.0$ g; dextrose, 10.0 g; asparagin, 4.0 g; arginine, 100 mg; glutamic acid, 100 mg; glycine, 100 mg; histidine, 100 mg; proline, 100 mg; tryptophan, 100 mg; and sodium thioglycollate, 100 mg. The amino acids were added to the medium in order to obtain maximum growth. Incorporation of thioglycollate appears to protect vitamin B_{12} from destruction in the presence of natural substances during autoclaving (*2, 7*). The pH was adjusted to 6.8. Two and one-half ml of the double-strength medium was added to each tube containing a solution of the standard or unknown sample and sufficient water to make a total volume of 5 ml. The tubes were plugged with cotton and sterilized by autoclaving for 5 min at about 115° C. Following rapid cooling in cold water, each tube was inoculated with a drop of freshly prepared suspension of *E. coli* strain 113-3. Stock cultures of the assay bacteria were carried on Difco nutrient agar. The inoculum was grown in Difco nutrient broth for about 10 hr at 37° C prior to use, and the bacteria were prepared by washing, centrifuging, and resuspending in saline in the usual fashion.

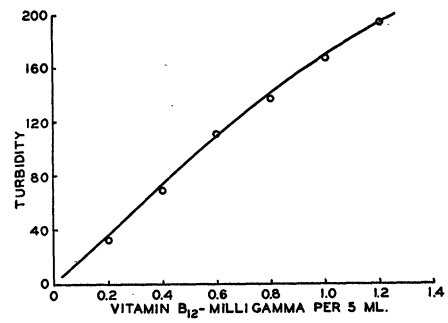

FIG. 1. Dosage response curve of a mutant strain of *E. coli* in relation to different levels of vitamin B_{12}.

It was found that adequate growth could be obtained after 15–18 hr of incubation on a shaking machine with the temperature at 30° C. Doubtless certain other conditions would also be found satisfactory. Growth was estimated with a Klett colorimeter.

A typical dosage response curve is shown in Fig. 1. The half-maximum level of growth occurs at about .12 mγ of B_{12}/ml. Under the specified conditions of assay, the organism responds in similar fashion to vitamins B_{12} and B_{12a} (*9*).[1, 2] Determinations of Vitamin B_{12} activity in various natural materials were accomplished satisfactorily when the ratio of methionine to vitamin B_{12} was such as to permit avoiding methionine activity by simple dilutions. In duplicated assays with *Euglena* and with *E. coli*, blood serum proteins, animal tissue extracts, and various preparations of bacteria and algae yielded satisfactory results. Whole

[1] Supplied by Merck & Co., Inc., Rahway, N. J.

[2] The half-maximum growth in response to methionine occurs at a level of 6 γ/ml, or about 50,000 times the development of B_{12} required to produce an equivalent amount of growth.

normal blood appeared to have amounts of vitamin B_{12} too low to permit direct assay in the presence of free methionine, and for such materials the *Euglena* assay is recommended. It was found necessary to release bound vitamin B_{12} from tissues, blood serum, etc., by heat or enzyme treatments prior to performing the assays (*4, 10*). In situations where methionine interference can be avoided, as in the determination of potency of vitamin concentrates, etc., this bacterial method may have considerable value because of its simplicity and the short time required for making the determinations.

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The Reversible Depolymerization of Fibrin

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In a previous article the author has shown that a gel formed by the action of thrombin upon purified fibrinogen can be dissolved in 6 *M* urea or 3 *M* guanidine hydrochloride to give a product of the same molecular weight as native fibrinogen under the same circumstances (*1*). A similar result has been obtained by Ferry and Shulman and by Mihalyi (*2, 3*).

Upon dialyzing off the urea against buffer, a gel is regained. It was thought desirable to ascertain whether the fine structure of the gel so produced was equivalent to that of a native fibrin gel. Electron microscopy was employed for this purpose.

A novel technique was utilized for preparing the specimens. A drop of Formvar was spread upon a thin trough of water and the resultant thin film scooped off upon a glass microscope slide. A drop of fibrin in 6 *M* urea was spread evenly over the film, and the latter refloated upon borate buffer-KCl of pH 8.0 and ionic strength .40. The urea quickly dialyzed through the Formvar film, and the gel reformed. The film plus a thin layer of gel was deposited upon wire mesh screens in the usual manner.

The specimen was then dried, washed, and shadowed with gold. The microphotographs obtained under these conditions showed a general resemblance to the published pictures of native fibrin gels under the same conditions (*4*). A network of strands was clearly formed, as is shown in Fig. 1.

[1] The author wishes to acknowledge helpful discussions with K. Laki and George Rozsa, of the National Institutes of Health.

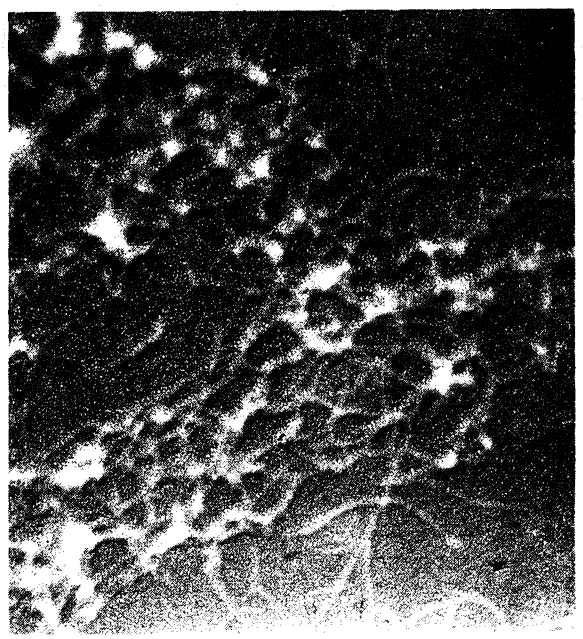

FIG. 1. Gold-shadowed regenerated fibrin clot, pH 8.0, P = .40. × 12,000.

In this manner further evidence is obtained as to the reversibility of the breakdown of fibrin in 6 *M* urea. The depolymerized material is capable of spontaneously reforming a network upon removal of the dispersing agent. It is to this depolymerized material that we may perhaps give the title "profibrin" (*5, 6*).

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The Wave-Frequency Dependence of the Duration of Radar-Type Echoes from Meteor Trails

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A brief discussion of the dependence of the duration of meteor echoes on wave frequency has been given by Lovell (*1, 2*), who concludes that the evidence shows that the duration is approximately proportional to the square of the wavelength. Data collected by the Central Radio Propagation Laboratory of the National Bureau of Standards, consisting of simultaneous measurements of duration at 27.2 Mc and 41.0 Mc, lend support to this conclusion, and in view of its fundamental importance to the theory of