

product $Nt\Delta n$, where N is the number of layers in a step, t the thickness per layer, and Δn the difference in refractive index for the two polarized rays. Since many different plastics having a wide variety of birefringence and thickness are commercially available, a variety of stepgauges can be made having different "steepness" for the flight of steps. Ordinary cellophane was found to have more birefringence than was desirable for a stepgauge. A flight of steps made of cellophane had too great optical steepness to be generally useful as a measuring instrument.

The steps of a birefringent stepgauge are commonly cut out of a sheet of plastic with all the steps in the same direction of the sheet. In this case the thicknesses add. If a step is cut out of the sheet in a direction at right angles to the direction of the rest of the steps, its thickness will subtract from that of the series.

The birefringent stepgauge is not yet commercially available.

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An Observation on the Infrared Absorption Spectrum of Dextran

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In an extensive study of the physical characterization of dextran, we have examined the infrared absorption spectra of dried films of a wide variety of undegraded and degraded dextrans produced by different organisms under different conditions. The instrument used was a Perkin-Elmer Model 21 infrared spectrophotometer.² Throughout most of the spectral region $3\text{ }\mu\text{--}15\text{ }\mu$, these spectra resemble each other quite closely, but some significant differences have been found. The largest variations occur in the spectral neighborhood of $12.6\text{ }\mu$, and a few examples of particular interest are shown in Fig. 1. It will be noted that, of the commercial dextrans, the nondomestic show marked differences from the domestic in the amount of absorption at $12.6\text{ }\mu$. Samples of dextran produced by *Leuconostoc mesenteroides* NRRL B-512 do not show appreciable absorption at $12.6\text{ }\mu$; NRRL B-512 and the organism that produces the domestic dextran stem from the same original culture. Dextrans produced by other organisms in the NRRL culture collection show varying amounts of absorption at $12.6\text{ }\mu$. One dextran we have studied in particular is produced by *L. mesenteroides* NRRL B-742. Undegraded dextran from this latter source has been fractionated by alcohol precipitation from water. Two fractions, labeled in Fig. 1 as B-742, Type I, and B-742, Type II, have been obtained. The physical

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² Mention of the instrument used does not constitute an endorsement by the U. S. Department of Agriculture.

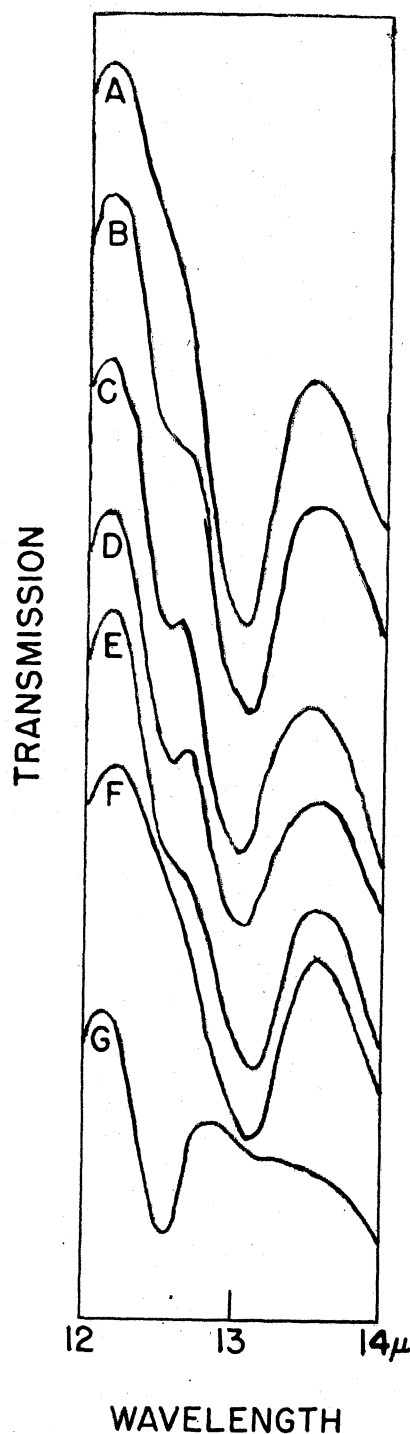


FIG. 1. Infrared absorption spectra of dried films of different dextrans between $12\text{ }\mu$ and $14\text{ }\mu$. A, commercial, domestic; B, C, and D, commercial, nondomestic; E, unfractionated B-742; F, B-742, Type I; G, B-742, Type II.

characterization of these materials will be discussed later by others, but we can say that the molecular weights of the two fractions as determined by light scattering are approximately the same. The spectra of

these and other dextrans that we have studied are unchanged in the low molecular weight (about 30,000) material isolated by alcohol precipitation after acid hydrolysis.

One way of accounting for the spectra of the non-domestic dextrans would be to assume that they are mixtures of Types I and II in varying quantities. If we further assume (1) that the spectrum of dextran is independent of type except from 12 μ to 14 μ and (2) that the Type II dextran, the spectrum of which is shown in Fig. 1, contains a negligible amount of impurities, we can assign percentages of Type II dextran to the various dextrans whose spectra are shown in Fig. 1. The percentages so obtained follow:

A	5
B	25
C	35
D	40
E	35

A more extensive discussion of the infrared absorption spectra of dextrans will be published elsewhere.

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An Ultramicronutritional Bio-Assay Technique Employing Seeded Agar Tubes

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The need for estimating nutrients present in foods and biological materials has resulted in the development of valuable microtechniques (chemical, microbiological, and chromatographic). However, there is a continuing requirement for simple and sensitive procedures for use in clinical work, especially with children, for field work in nutrition surveys, and for small-animal experimentation to avoid sacrificing the organism; the procedures should be capable of measuring nutrients in very small amounts of biological fluids and tissues. Available microchemical procedures for the routine determinations of thiamin, riboflavin, ascorbic acid, vitamin A, and carotene on a few drops of blood (1) require expensive and delicate equipment, presenting a serious problem in field work and in modestly equipped laboratories. In addition, ultramicrochemical methods are not available for many of the B vitamins. Microbiological assay techniques of either the test-tube or agar-plate type as commonly employed (2) are not sensitive enough for the purposes indicated, but they have a simplicity and range that make adaptation of their principle desirable at ultramicro levels.

We report here initial observations on a simple and rapid microbiological assay procedure, suitable for vitamins and amino acids, which employs a small-bore

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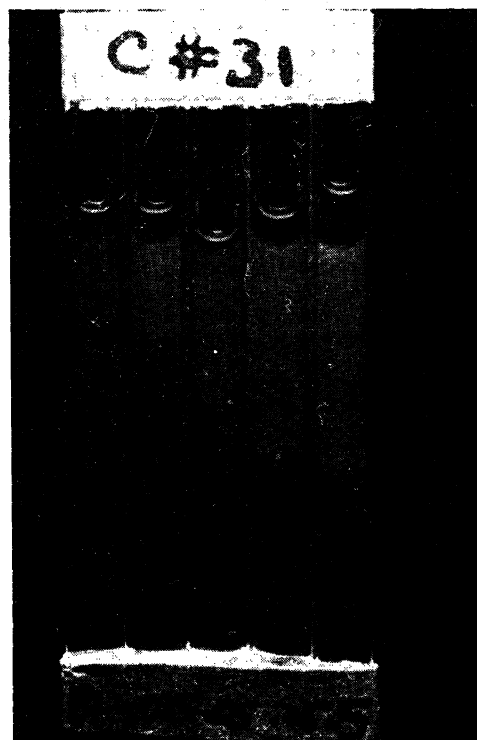


FIG. 1. Agar tube assay for riboflavin illustrating the turbid appearance of the seeded agar medium where the organism (*L. casei*) has grown in response to the supernatant riboflavin solution. From left to right, the tubes contain 0.0 (blank), 0.025, 0.05, 0.25, and 0.5 μ g riboflavin/ml, respectively; in each instance 0.035 ml of solution was added. Incubation for 18 hr at 30° C.

glass tube containing agar-basal medium seeded with the test organism. The basal medium is deficient only in the nutrient to be assayed, and the organism employed requires the nutrient for growth. The test solution is added above the agar column, and after suitable incubation the length of the column of growth is measured (Fig 1). The procedure is an adaptation of the agar-plate assay technique (3) but with many advantages in sensitivity and simplicity. Seeded agar tubes in various modifications have been used for antibiotic assay (4-7).

The graded response (length of growth column) has been found to bear a linear relation to the logarithm of the concentration of the added nutrient over most or all of the ranges tested (Fig. 2). A similar relation has been found in various agar-plate assays (8-10). Four vitamins and three organisms thus far tested have shown the graded response (Table 1), and it is believed that this response will obtain wherever an organism will grow in a low oxygen tension without gas formation upon the addition of a missing essential nutrient. *Saccharomyces carlsbergensis* ATCC No. 9080, for example, has not yielded satisfactory results to date in testing for the vitamin B₆ group.

A number of variables have been and are being tested for optimum results, including concentrations