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 nondomestic 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 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*).

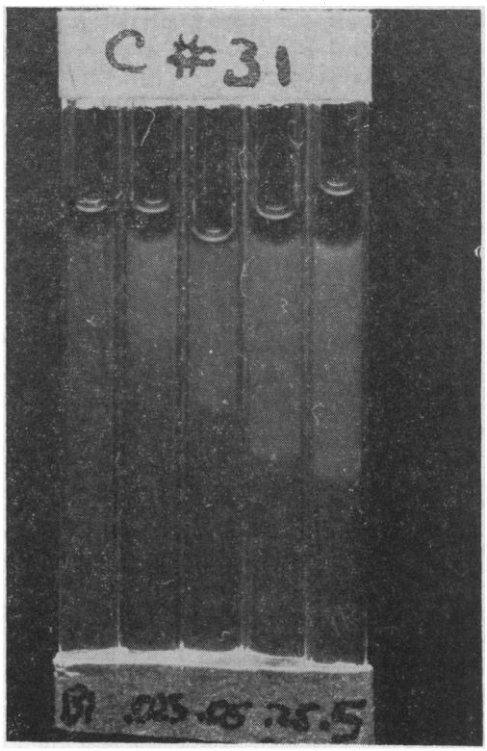

FIG. 1. Agar tube assay for riboflavin illustrating the turbid appearance of the seeded agar medium where the organism (*L. casei*) has grown in response to the supernatant riboflavin solution. From left to right, the tubes contain 0.0 (blank), 0.025, 0.05, 0.25, and 0.5 μg riboflavin/ml, respectively;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

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 B_6 group.

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

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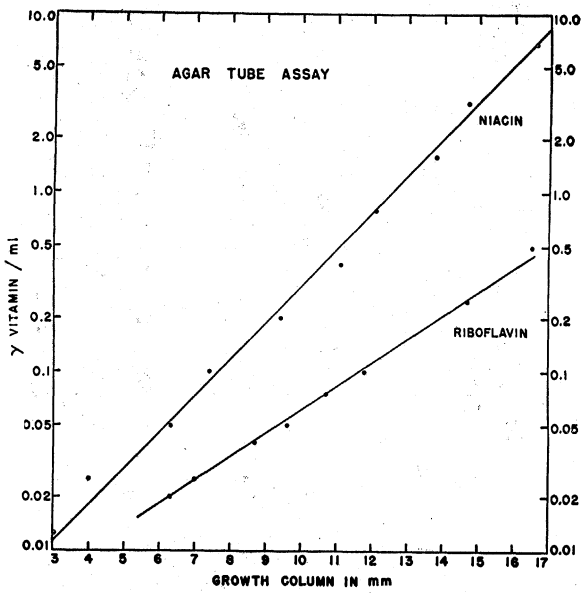

FIG. 2. Agar tube assay for riboflavin and niacin illustrating linear relation between vitamin concentration (logarithmic) and length of growth column. Each point is the average of 3 readings, 0.030 ml solution/tube, incubation for 19 hr at 37° C.

of agar, types and concentrations of basal media and of organisms, sealed vs unsealed tubes, age of bacteria, pH of added solution, methods of filling tubes, and others. The procedure currently used is as follows: The bacterial cultures are handled in the usual fashion for microbiological assay and, after inoculation, 18-hr incubation, and two saline washings, the bacterial suspension is brought to a suitable turbidity (Table 1). Basal medium of the proper strength (Table 1) containing 0.6% agar is autoclaved and allowed to cool to 42°–45° C. Five ml of the bacterial suspension is then added per 100 ml of agar-basal medium, and the tubes are filled. The glass tubes are 8 cm long, 3.5 mm OD and 2.0 mm ID[2] and have the lower end partially flame-sealed to prevent the agar from slipping through.[3] The tubes are filled for approximately 5 cm of their length by partial immersion in the liquified seeded agar and are then left horizontal until the agar has hardened.

The test material is added at the top of the tube with a constriction micropipette (*1*) of 0.015 ml or greater volume and incubated in a well-humidified chamber for 16–20 hr at the optimum temperature for the test organism. The tubes need not be sealed. The length of the growth zones is measured with an inexpensive vernier caliper allowing readings to 0.1 mm; a black background and fluorescent light assist in noting the boundary. Provided complete evaporation of the test solution does not occur during incubation, appreciable variation in the volume added does not appear to influence the length of growth column significantly. Standard nutrient solutions are run in triplicate at suitable concentrations. Sterile technique is necessary only in the handling of the cultures and basal media.

The seeded tubes may be wrapped in batches in vaporproof material and stored in the refrigerator until ready for use. We have tested agar tubes seeded with *Lactobacillus casei* and stored for varying periods; good readings with riboflavin have been obtained following 3–4 days' storage. Column lengths at a given vitamin concentration increase with storage time.

It has been found that the effective (readable) range of the agar tube assay is much greater than for the standard tube assay (Table 1). With pure riboflavin solutions serving as "unknowns," the accuracy of the agar tube assay approximates that of the Snell and Strong assay procedure (*11*).

The simplicity, speed, and extensive range of the agar tube assay commend it for trial in control work with potent vitamin sources and in establishing the range of vitamins in solutions of unknown strength preliminary to measurement by established chemical and microbiological procedures. Further investigation

TABLE 1

DATA ON AGAR TUBE MICROBIOLOGICAL ASSAY

Vitamin	Organism and ATCC No.		Medium and strength		T*	Effective range (μg)	
						Agar tube†	Liquid tube
Thiamin	*L. fermenti*	9338	D-B‡	1X	90–95	0.02 –10.0	0.01–0.04
Riboflavin	*L. casei*	7469	D-B; (*11*) modified	1X	81–83	.02 – 1.0	.05–0.3
Niacin	*L. arabinosus*	8014	D-B	1X	81–83	.05 –12.5	.01–0.4
Pantothenic acid	" "	"	D-X	½X	81–83	0.025–12.5	0.01–0.08

* T = optical density of suspension of culture used for seeding, using Evelyn colorimeter, 540 filter.

† Using 0.03 ml of vitamin solution. The upper limits of effective range and the linearity of response have not yet been thoroughly investigated above these levels.

‡ D-B = Difco-Bacto assay medium for the particular assay.

[2] Similar results have been obtained using tubes of 0.9 mm ID; the degree to which diameter may be reduced would appear to be limited only by practical considerations of methods of introducing liquid and of measuring growth column.

[3] Glass tubing, cleaned with acid and washed until rinsings are no longer acid, must be treated with a detergent such as Alconox in order to prevent changes in the agar that result in imbibition of the supernatant liquid, with drying and fragmentation of the agar surface and variable results.

will determine whether it will stand on its own merits as an independent assay procedure with biological material.

References

1. BESSEY, O. A. In P. Gyorgy (Ed.), *Vitamin Methods*, Vol. I. New York: Academic Press, 289 (1950).
2. SNELL, E. E. *Ibid.*, 328.
3. BACHARACH, A. L. *Nature*, **160**, 640 (1947).
4. TORII, T., KAWAKAMI, Y., and KOZIMA, H. *J. Penicillin (Japan)*, **1**, 281 (1947).
5. MITCHISON, D. A., and SPICER, C. C. *J. Gen. Microbiol.*, **3**, 184 (1949).
6. DAVIS, W. W., PARKE, T. V., and DAILY, W. A. *Science*, **109**, 545 (1949).
7. DAVIS, W. W., *et al. J. Am. Pharm. Assoc.*, Sci. Ed., **39**, 331 (1950).
8. BACHARACH, A. L., and CUTHBERTSON, W. F. J. *Analyst*, **73**, 334 (1948).
9. GENGHOF, D. S., PARTRIDGE, C. W. H., and CARPENTER, F. H. *Arch. Biochem.*, **17**, 413 (1948).
10. CUTHBERTSON, W. F. J., PEGLER, H. F., and LLOYD, J. T. *Analyst*, **76**, 133 (1951).
11. SNELL, E. E., and STRONG, F. M. *Ind. Eng. Chem.*, Anal. Ed., **11**, 346 (1939).

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Effect of Wind-generated Waves on Migration of the Yukon River in the Yukon Flats, Alaska[1]

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Wind-generated waves influence the migration of the Yukon River in east-central Alaska. At Circle, 125 miles downstream from the Alaska-Canada boundary, the Yukon River enters the eastern end of the Yukon Flats, an alluvial basin 20–75 miles wide and nearly 200 miles long. The Flats comprise a lowland that includes the Yukon Valley and lower parts of tributary valleys. Tributaries entering from the north are Porcupine, Sheenjek, Christian, Chandalar, Hodzana, Hadweenzic, and Dall rivers. Those entering from the south are Birch and Beaver creeks. Seven miles below Stevens, the river leaves the Flats through a narrow canyon.

From Circle to Fort Yukon the course of the Yukon is N 45° W and it is complexly braided. From Fort Yukon to a point downstream from Beaver the river changes its course to S 75° W and flows in a wide main channel, from which bow-shaped sloughs branch and re-enter. Along the north bank the channel is complicated by numerous distributaries of the Chandalar and Porcupine rivers. Near Stevens the Yukon is confined to one broad watercourse that locally branches into as many as three or four smaller channels.

Throughout the Yukon Flats, the riverbanks consist of unconsolidated alluvial gravel, sand, and silt, with minor amounts of organic material. In many places these banks are perennially frozen. Where the north bank of the river is 20′–30′ above summer river level, it is characterized by a well-developed soil profile. The vegetation is more like that found on older surfaces near the margin of the Yukon Flats than like vegetation on the south bank of the river, which is lower and lacks a well-developed soil profile. South of the river islands and bars are more numerous, and abandoned channels are filling with silt deposited during floods. These facts suggest that the alluvial features to the south are more recent than those to the north, and that the south bank has grown northward by deposition as the north bank retreated by erosion.

Russell (*1*), Goodrich (*2*), and Eakin (*3*) were among the first to suggest that the Yukon is migrating northward, basing their conclusion on the fact that the current is swifter along the north bank, where the stream is eroding the older, higher ground. To account for this migration in the Yukon Flats, Goodrich (*4*) applied Ferrel's law of terrestrial rotation, in which horizontally moving bodies are deflected to the right in the Northern Hemisphere. Deflection of a west-flowing river, such as the Yukon, would force migration to the north. Goodrich (*5*) attributed the asymmetry of some of the smaller tributary valleys to the effects of geologic structure and regional tilting. If the tilting theory were applied to the Yukon in the Flats, uplift of the area south of the river might force the river northward.

Field evidence indicates that the course of the Yukon was shifted south of its present position by deposition of gravel fans in the lower valleys of the Christian, Chandalar, and Sheenjek rivers during a period when glaciers, with sources in the Brooks Range, moved down the valleys. Since the last major glacial advance, the Yukon appears to have migrated northward and, in a few places, is eroding the lower part of the gravel fan of the Chandalar River.

At present the strongest summer winds are from the southwest, as observed by the writer and by residents of Beaver, a small village on the north bank of the Yukon. These winds, blowing against the river current, produce choppy waves with a trough-to-crest height up to 3′. The waves attain their maximum height and erosive power along the north bank of the river, especially where the wind blows unobstructed across a wide expanse of water. The south bank, in contrast, is protected from waves generated by summer winds; and in winter the prevailing northeast winds and the strong southwest winds associated with cyclonic storms cannot form waves on the ice-covered river.

Frozen banks are thawed rapidly at water level and below, and at a slower rate by warm air above water level. They thus become prey to undercutting at a rate that depends on the degree of cementation of the alluvium by ice and on the rate of removal of the eroded sediment. Frozen silt, the most cohesive bank material, can be undercut farther than frozen gravel or sand or thawed material. The process is accelerated by wind-generated waves and results in the collapse of large blocks of silt, which temporarily defend the bank against further erosion by waves and current

[1] Publication authorized by the director, U. S. Geological Survey.