NOTES

Improvement of the Microbiological Assay for Calcium Leucovorin

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The calcium leucovorin assay was improved by adding THORAL germicide which resuspended the cells so that an accurate turbidity reading could be obtained.

_Pediococcus cerevisiae_ (ATCC 8081) is used to assay calcium leucovorin by the technique given in U.S. Pharmacopoeia (USP), XVI, p. 868. As seed, I prefer to use a frozen culture (1). The broth [single-strength CF Assay Medium (Difco) plus 10 mg of yeast extract per 10-ml tube] is inoculated with 1.5 to 2.0 ml of thawed culture and incubated for 5 to 6 hr at 37 C on a rotary shaker. After incubation the culture is centrifuged, washed once with sterile single-strength CF Assay Medium, resuspended to original volume, and diluted 2:100 (v/v) in the same medium. For the assay, one drop of inoculum per tube is used. Maximal growth is reached in 18 to 20 hr at 37 C.

Regardless of the state of the inoculum, fresh slant (USP XVI) or frozen culture, the response of _P. cerevisiae_ to calcium leucovorin was frequently erratic and unpredictable. It was noticed that the growth often tended to be stringy and the cells formed a film on the walls of the tubes. Since a quaternary germicidal agent (THORAL, Turco Products, Inc., New York, N.Y.) is used in the vitamin B6 assay (2) to prevent the settling of cells, its effect on the Citrovorum assay was determined. One drop of this germicide was added to each tube just before reading the assay. This addition increased the homogeneity of the cell suspension and an accurate turbidity reading was obtained.

As noted in Fig. 1, this germicide had little effect on the control (leucovorin-free) tube. However, in the tubes containing 0.0008 μg. of calcium leucovorin, a decrease of about 20% in transmittance (Lumetron colorimeter) was recorded in the tubes treated with the germicide, and erratic responses were largely eliminated. One drop of Antifoam AF emulsion (Dow Corning Corp., Midland, Mich.) diluted 1:100 (w/v) was also added to each tube to counteract the foaming produced when the tubes were shaken. This Antifoam agent has been found to be useful in other vitamin assays, because it quickly eliminates air bubbles, which interfere with turbidity readings.

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LITERATURE CITED