Inhibition by EDTA of Growth of *Lactobacillus casei* In the Folate Microbiological Assay and Its Reversal by Added Manganese or Iron, T. Tamura, L. E. Freeberg, and P. E. Cornwell (Dept. of Nutrition Sci., Univ. of Alabama at Birmingham, Birmingham, AL 35294)

When we used plasma containing EDTA as an anticoagulant, the growth of *Lactobacillus casei* was inhibited in the folate microbiological assay involving use of 300 μL of assay mixture in a 96-well microtiter plate (1–3). We postulated that this inhibition was caused by EDTA chelation of manganese, iron, and magnesium ions, all of which are essential for growth of *L. casei* (4, 5). The single-strength medium manufactured by Difco Laboratories (Detroit, MI) contains manganese, iron, and magnesium in concentrations of 25, 20, and 450 μmol/L, respectively. Therefore, we tested the effect of adding either manganese, iron, or magnesium to the medium to reverse the growth inhibition of *L. casei* caused by EDTA.

Figure 1 (top) shows *L. casei* response curves to (6S)-5-HCO-H₄PteGlu with various concentrations of EDTA. Inhibition of *L. casei* growth became obvious at an EDTA concentration of 0.65 mmol/L. We considered that 2.6 mmol of EDTA per liter would be the maximum concentration possible if we used 50 μL of plasma per assay from 4.0 mL of whole blood drawn into a tube containing 10.5 mg of disodium EDTA. Addition of manganese or iron to the assay mixture restored the full growth of *L. casei* in the presence of an equimolar amount of EDTA, whereas added magnesium failed to restore full growth (Figure 1, bottom). Addition of these ions in the absence of EDTA did not affect the growth of *L. casei*.

Our observation of the inhibitory effect of EDTA on *L. casei* growth was similar to the findings of MacLeod and Snell (5), who demonstrated that the addition of citrate to the medium increased the requirement for manganese and magnesium for *L. casei* growth. Addition of ferrous sulfate produced precipitates in the assay mixture in the absence of EDTA, so we recommend the addition of manganese sulfate (final concentration 2.6 mmol/L) in the assay mixture for routine microbiological assay of folate in plasma.

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References


Unbound and Transthyretin-Bound Retinol-Binding Protein in Serum and Urine from Diabetic Patients with Nephropathy and Proteinuria, Jan Holm and Lars Hemmingsen (Dept. of Clin. Chem., Central Hospital Nyskøbing Falster, DK-4800 Nyskøbing Falster, Denmark)

Retinol-binding protein (RBP) is a low-molecular-mass protein (21 kDa) present in plasma, where 90% of the protein is bound to transthyretin (55 kDa). The free fraction of RBP, i.e., RBP not bound to transthyretin, is filtered freely through the glomerules and catabolized after reabsorption in the proximal tubules (1–4). The urinary excretion of RBP is thus a sensitive index of tubular proteinuria caused by impaired proximal tubular reabsorption of low-molecular-mass proteins (1–4). In contrast to β₂-microglobulin (11 kDa), RBP is stable in acid urines (1). We and other investigators have recently observed an increased urinary excretion of RBP in insulin-dependent diabetics in the absence as well as in the presence of albuminuria (5–7). In examining glomerular proteinuria (albuminuria), however, one could theoretically encounter spurious tubular proteinuria, owing to an increased urinary excretion of transthyretin-bound RBP associated with an increase in the glomerular permeability. We performed the present study to establish whether the urinary excretion of transthyretin-bound RBP is increased in albuminuric diabetic patients.

In healthy persons and in micro/macrolebuminuric diabetic patients, the gel-filtration profiles of RBP in serum contain (Figure 1A) a major fraction at the position of transthyretin (55 kDa) and a minor fraction at myoglobin (17 kDa). Overlap between the two peaks could be due to the presence of dimeric forms of RBP (9). The profile for serum from a hemodialysis patient with reduced glomeru-