Enzymatic Determination of d-Mannose in Serum

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A new and simple enzymatic assay for measuring d-mannose in serum is described. Endogenous glucose is eliminated from serum by use of glucose oxidase (EC 1.1.3.4) and catalase (EC 1.11.1.6). d-Mannose concentration is calculated from the increase in NADH formation after mannosephosphate isomerase (EC 5.3.1.8) is added. This increase is a result of coupling the following series of enzymes: hexokinase (EC 2.7.1.1), glucosephosphate isomerase (EC 5.3.1.9), and glucose-6-phosphate dehydrogenase (EC 1.1.1.49, NAD+ dependent). The study included subjects who were healthy volunteers and patients with suspected or proven fungal infections.

Additional Keyphrases: candidiasis · disorders of carbohydrate metabolism · reference interval

In recent years there has been great interest in the concentrations of free and bound monosaccharides in body fluids, particularly in certain enzymatic disorders of carbohydrate metabolism such as galactosemia, fructose intolerance, and mannosidosis. Moreover, evidence has accumulated that d-mannose concentrations in serum are higher in cases of invasive candidiasis than in healthy adults and children (1-5). These studies involved gas-liquid chromatography. Immunological investigations involving detection of antibodies against Candida albicans have also been evaluated as diagnostic aids (6-9), but results and interpretations are conflicting (10, 11). By use of gas-liquid chromatography or immunological techniques, mannann or d-arabinitol has also been detected in patients with invasive candidiasis (12-15).

In an attempt to confirm the association of increased serum d-mannose concentration with invasive candidiasis, I devised a new enzymatic method for measuring the concentration of d-mannose in control populations and in patients with invasive candidiasis. d-Mannose concentration is calculated from the increase in NADH after mannosephosphate isomerase is added. The increase results from the following series of reactions:

\[ \text{d-Mannose} + \text{Mg} \cdot \text{ATP} \xrightarrow{\text{hexokinase}} \text{d-mannose 6-phosphate} + \text{Mg} \cdot \text{ADP} \]
\[ \text{d-Mannose 6-phosphate} \xrightarrow{\text{mannosephosphate isomerase}} \text{d-fructose 6-phosphate} \]
\[ \text{d-Fructose 6-phosphate} \xrightarrow{\text{glucosephosphate isomerase}} \text{d-glucose 6-phosphate} \]
\[ \text{d-Glucose 6-phosphate} + \text{NAD}^+ \xrightarrow{\text{glucose-6-phosphate dehydrogenase}} \text{d-glucose-6-lactone 6-phosphate} + \text{NADH} + \text{H}^+ \]

Materials and Methods

**Serum specimens.** I obtained control sera from 43 healthy volunteers (20 men and 23 women), ages 19 to 54. Sera to be tested were drawn from patients with suspected or proven fungal infections, and from patients with diabetes mellitus. Sera were stored at \(-20^\circ\text{C}\) until analysis.

**Reagents.** The enzymes used were obtained commercially. Mannosephosphate isomerase from yeast (spec. act, 300 kU/L) was obtained from Boehringer, Mannheim, F.R.G. (cat. no. 1182412). Glucosephosphate isomerase from yeast, Type III, 1000 U per vial, was from Sigma Chemical Co., St. Louis, MO (85C-90025). Before use, these enzymes were dialyzed against buffer containing 0.5 mmol of cobaltous acetate per liter according to a reported study (16). Hexokinase (176 kU/g), glucose-6-phosphate dehydrogenase (NAD+ dependent, Grade III, 709 kU/g), and glucose oxidase (II, 140 kU/g) were purchased from Toyobo, Japan. Catalase of bovine liver, twice crystallized, was from Sigma Chemical Co. d-Mannose was from Merck, Darmstadt, F.R.G. ATP and NAD+ were from Kojin, Japan.

**Procedure.** To test cuvettes containing 1.0 mL of a 100 mmol/L solution of sodium 1,4-piperazine diethanesulfonate buffer, pH 7.1, add 200 μL of serum, 10 μL (140 U) of glucose oxidase, and 10 μL (213 U) of catalase. Preincubate the mixture at 37°C for 10 min. Make sure that the cuvette absorbance is no greater than 0.100 at 340 nm due to residual glucose in the sample. When there is no further increase in absorbance at 340 nm for 2 min at 37°C, take the reading (A1) and add 20 μL (1.5 U) of glucose-6-phosphate dehydrogenase. After mixing, measure the absorbance again at 340 nm (A2) in a spectrophotometer that has the cuvette compartment maintained at 37°C. About a 10-min incubation is required to reach maximum absorbance. Derive the concentration of d-mannose in the sample, in micromoles per liter, as follows:

\[ (A_2 - 0.99 A_1) \times 1608 \]

Results and Discussion

A standard curve was prepared from data on mixtures obtained by adding d-mannose to give concentrations ranging from 25 to 1000 mmol/L to serum obtained from healthy adults (intrinsinc glucose and d-mannose values were 5 mmol/L and 40 mmol/L). The mean analytical recovery of authentic d-mannose was 98.0 ± 5.1% (± SD) as shown in Figure 1. This was obtained when the contents of endogenous glucose in the reaction mixture were between 2 mmol and 200 mmol. Longer preincubation than that described resulted in poor recovery. I saw no influence of fructose, galactose, or saccharose in concentrations of 1000 μmol/L on the determination of d-mannose.

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Day-to-day (n = 20) reproducibility (CV) was 5%; the within-run (n = 20) CV was 4% for a mean of 25 μmol/L.

The mean concentration of α-mannose in the 60 control sera was 21 μmol/L (range 0 to 58 μmol/L, SD 16 μmol/L). These values agree well with those obtained by gas–liquid chromatography (1, 17).

Four patients with proven invasive candidiasis (diagnosis confirmed at autopsy) and three patients with suspected invasive candidiasis (oral mucous membrane colonization) were studied. The first proven patient showed α-mannose increasing from 110 to 1315 μmol/L; the second had values increasing from 115 to 605 μmol/L; the third patient had a value of 780 μmol/L; the fourth patient had a value of 123 μmol/L. Three leukemic patients with suspected invasive candidiasis had values that increased from 98 to 980 μmol/L. Two leukemic patients with pulmonary aspergillosis (autopsy-proven) had values of 288 and 900 μmol/L. Five patients with controlled diabetes mellitus showed 0, 18, 24, 58, and 61 μmol/L, and four uncontrolled diabetic patients who had serum glucose >15 mmol/L showed α-mannose concentrations of 38, 62, 105, and 128 μmol/L. In diabetic patients with uncontrolled hyperglycemia, α-mannose concentrations overlapped values found in the patients with invasive fungal infections.

The present study, then, strongly suggests that a prospective study of serum α-mannose concentrations in leukemias is warranted. With more rapid diagnosis and early use of antifungal chemotherapy, morbidity and mortality of fungal infection in this population may be improved.

References