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Enzymatic assay of D-mannose in serum

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We describe a new and improved enzymatic assay for determining the concentration of D-mannose in sera. Serum D-glucose is selectively converted to glucose-6 phosphate with the highly specific thermostable glucokinase (EC 2.7.1.2) from *Bacillus stearothermophilus*. The anionic reaction products and excess substrates are removed by a rapid and simple anion-exchange chromatography step in microcentrifuge spin columns. D-Mannose in the glucose-depleted sample is then assayed spectrophotometrically by using coupled enzymatic reactions. The quantitative elimination of glucose from the serum samples allowed the accurate and reproducible assay of serum mannose in the 0–200 μmol/L range. Recovery of mannose added to serum (5–200 μmol/L) was 94% ± 4.4%. The intraassay CV was 6.7% at 40 μmol/L mannose (n = 5; 39.6 ± 1.6 μmol/L) and 4.4% at 80 μmol/L (n = 11; 75.0 ± 1.8 μmol/L); the interassay CV at these concentrations was 12.2% (n = 7; 36.9 ± 2.1 μmol/L) and 9.8% (n = 7; 74.2 ± 2.7 μmol/L), respectively. Sera from 11 healthy human volunteers contained an average of 54.1 ± 11.9 μmol/L mannose (range 36–81 μmol/L).

INDEXING TERMS: glucose oxidase • glucokinase • carbohydrate-deficient glycoprotein syndrome • phosphomannose isomerase • phosphoglucose isomerase

In recent years several studies have focused on methods to quantify the concentration of D-mannose in human serum and other body fluids. These measurements are complicated by the presence of a ~100-fold excess of D-glucose, the 2-epimer of mannose. Jolley et al. *[1]* analyzed sera by high-resolution liquid chromatography and reported values of 11.5 ± 0.8 μg/mL (63.9 ± 4.4 μmol/L) D-mannose. Aloia *[2]* analyzed sera by gas–liquid chromatography after treatment with glucose oxidase (GOD) to reduce the amount of D-glucose present and reported values of 7.0 ± 0.02 μg/mL (38.9 ± 0.1 μmol/L).[1] Both these studies revealed technical problems associated with the assay of mannose in the presence of 100-fold excess glucose and did not address the question of recovery (see *Discussion* in ref. *4*).

Soyama *[3]* and Akazawa et al. *[4]* presented enzymatic methods for analysis of D-mannose based on first lowering D-glucose concentrations by oxidation with GOD. The results of these studies indicated that mannose could be accurately measured in sera if the amount of GOD added were proportional to the initial glucose concentration and the glucose concentration were carefully monitored during the reaction until the glucose was reduced to 2–3 times that of mannose. Akazawa et al. *[4]* noted that GOD preferentially oxidized glucose but that loss of mannose increased when the glucose concentration was less than two or three times that of the mannose present. Soyama *[3]* reported the concentration of D-mannose in normal sera to be 21 ± 16 μmol/L (range 0–58 μmol/L; n = 60). Akazawa et al. *[4]* found values of 54.4 ± 2 μmol/L (n = 22) for fasting pregnant women; significantly higher values were associated with diabetes mellitus.

More recently, Pitkanen and Kanninen *[5]* carefully measured the D-mannose concentrations in human serum with more sophisticated techniques involving selective ion monitoring by gas chromatography–mass spectrometry (GC-MS) after sodium borodeuteride reductive labeling of serum monosaccharides. Upon analysis of six normal human sera, they found mannose concentrations of 55.1 ± 10.6 μmol/L, and demonstrated quantitative recovery of mannose added to the sera.

In recent studies *[6]*, we found that addition of mannose to the culture medium of fibroblasts from patients with carbohydrate-deficient glycoprotein syndrome (CDGS) type I corrected the metabolic deficiency that leads to underglycosylation of glycoproteins in vitro. Schaftingen and Jaeken *[7]* have recently shown that the metabolic defect in CDGS type I is associated with re-

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[1] Nonstandard abbreviations: GOD, glucose oxidase; CDGS, carbohydrate-deficient glycoprotein syndrome; GK, glucokinase; G6PDH, glucose-6-phosphate dehydrogenase; HK, hexokinase; PGI, phosphoglucose isomerase; PMI, phosphomannose isomerase; CAT, catalase; NAM, NADP-ATP-$MgCl_2$ mixture; HPAEC-PAD, high-pH anion-exchange chromatography with pulsed amperometric detection; and GC-MS, gas chromatography–mass spectromety.

duced concentrations of phophomannomutase activity. Consequently, we sought to assay D-mannose concentrations in sera from patients with type I CDGS. We have found the previous methods for the enzymatic assay of D-mannose to be unsatisfactory because serum contains an inhibitor of the GOD/catalase (CAT) reactions and GOD uses mannose as a substrate at low glucose concentrations. To circumvent this problem, we developed a new method based on the use of the highly specific glucokinase (GK) from *Bacillus stearothermophilus [8]* to selectively remove glucose from sera before the enzymatic assay of D-mannose.

Materials and Methods

Reagents. All chemical reagents were of analytical reagent grade and unless otherwise noted were obtained from Sigma Chemical Co. (St. Louis, MO). GK (EC 2.7.1.2, cat. no. G-8887), glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49, cat. no. G-8404), hexokinase (HK; EC 2.7.1.1, cat. no. H-5625), phosphoglucose isomerase (PGI; EC 5.3.1.9, cat. no. P-5381), phosphomannose isomerase (PMI; EC 5.3.1.8, P-5153), GOD (EC 1.1.3.4, G-2133), CAT (EC 1.11.1.6, C-40), ATP (cat. no. A-7699), and NADP (cat. no. N-0505) were obtained from Sigma Chemical Co. DE-53 cellulose anion-exchange resin was obtained from Whatman (Fairfield, NJ; cat. no. 4058F050). MicroSpin filters (cat. no. 8502–00), manufactured by Lida Manufacturing Corp. (Kenosha, WI), were purchased from A. Daigger and Co. (Wheeling, IL).

GK was dissolved at 50 kU/L in 0.025 mol/L Tris-HCl, pH 7.8, and stored in an ice bath (GK reagent). G6PDH was dissolved at 100 kU/L in 0.025 mol/L Tris-HCl, pH 7.8, and stored on ice (G6PDH reagent). HK, G6PDH, and PGI (50 U each) were combined and microcentrifuged at 12 000*g* for 10 min at 4 °C. The ammonium sulfate supernatant was carefully removed, and the pellet was dissolved in 1 mL of 0.025 mol/L Tris-HCl, pH 7.8, and stored on ice (HK/G6PDH/PGI reagent). PMI (100 U) was centrifuged at 12 000*g* for 10 min at 4 °C, the ammonium sulfate supernatant was carefully removed, and the pellet was dissolved as described above (PMI reagent). Stock solutions of 100 mmol/L ATP and 100 mmol/L NADP were prepared, adjusted to pH 6.5 with 2.5 mol/L $KHCO_3$, and stored frozen at −20 °C. NAM-A reagent cocktail was prepared by mixing 0.8 mL of 100 mmol/L NADP, 0.8 mL of 100 mmol/L ATP, 0.08 mL of 1 mol/L $MgCl_2$, and 0.32 mL of H_2O. NAM-B reagent cocktail was prepared by mixing 0.067 mL of 100 mmol/L NADP, 0.067 mL of 100 mmol/L ATP, 0.067 mL of 1 mol/L $MgCl_2$, and 1.8 mL of H_2O.

Preparation of DE-53 spin columns. DE-53 anion-exchange cellulose (25 g) was suspended in 300–400 mL of H_2O, allowed to settle, and the fines decanted two or three times. The resin was resuspended in 0.25 mol/L Tris-HCl, pH 8.5, collected on a sintered glass funnel, and washed with 200 mL of 0.25 mol/L Tris-HCl, pH 8.5, followed by three or four washes with H_2O. The resin was gently resuspended in a minimal amount of H_2O, transferred to a 150-mL reagent bottle, and allowed to settle overnight. The total volume was adjusted to 1.6–1.8 times the volume of settled resin, and 0.5 mmol/L sodium azide was added as a preservative.

The de-fined, equilibrated DE-53 resin was gently resuspended and 0.6 mL pipetted into the filter basket of a MicroSpin centrifuge filter. The column was centrifuged at 3000 rpm for 2 min in a microcentrifuge with swinging tube holders. The resin was washed twice with H_2O and the effluents discarded.

ASSAY PROTOCOL

Preincubation to remove glucose. A 200-μL sample containing serum or mannose calibration solutions is combined with 100 μL of NAM-A reagent cocktail; 80 μL of 0.25 mol/L Tris-HCl, pH 8.5; 10 μL of GK reagent; and 10 μL of G6PDH reagent. Final concentrations were 10 mmol/L ATP, 10 mmol/L NADP, 10 mmol/L $MgCl_2$, 50 mmol/L Tris-HCl pH 8.5, 1.25 kU/L GK, and 2.5 kU/L G6PDH. The mixture was incubated at 37 °C for 20 min, allowed to cool to room temperature, and centrifuged to collect condensation.

DE-53 spin column to remove anionic products and substrates. A DE-53 spin column was washed with H_2O and microcentrifuged at 3000 rpm for 2 min as described above. A 200-μL portion of the glucose-depleted preincubation mixture was pipetted onto the resin bed and centrifuged at 5000 rpm for 2 min. The remaining 200 μL was pipetted onto the resin bed and the centrifugation repeated. The combined effluents were transferred to a clean tube. The resin bed was washed twice with 200 μL of H_2O and transferred to the reaction tube (total volume = 800 μL). To the tube was added 200 μL of 0.5 mol/L Tris-HCl, pH 7.8; 180 μL of NAM-B reagent cocktail; and 40 μL of HK/G6PDH/PGI enzyme reagent cocktail. After mixing, 610 μL was transferred to a new reaction tube (tube B). To the original reaction tube (tube A), 10 μL of H_2O was added and to tube B, 10 μL of PMI enzyme reagent (1 U). After incubating at 37 °C for 15 min, the absorbance at 340 nm was measured to quantify NADPH production. Care should be taken to avoid contamination of samples lacking PMI by samples containing PMI during the pipetting and spectrophotometric readings. Special micropipette tips designed for 1–20-μL volumes should be used as appropriate. Final concentrations of added reagents were 0.08 mol/L Tris-HCl, pH 7.8; 500 μmol/L ATP; 500 μmol/L NADP; 5 mmol/L $MgCl_2$; 1.6 kU/L HK, G6PDH, and PGI; and 3.2 kU/L PMI. The absorbance at 340 nm (A_{340}) of each tube is measured with H_2O as a blank. The difference in A_{340} (ΔA_{340}) between tube A and tube B is due to mannose in the original 200-μL sample (100 μL in tube A and 100 μL in tube B). Since the sample was diluted 6.2-fold during the assay and the μmol/L absorptivity of NADPH is 6.2×10^{-3}, the concentration of

mannose (μmol/L) in the original sample is theoretically 1000 × ΔA_{340}. However, we have found that when the sample contains only 200 μL of H_2O, there is a ΔA_{340} of 0.002–0.012, due possibly to contaminants in the PMI and other reaction components. Therefore, it is necessary to measure a reagent blank with each set of determinations and subtract the ΔA_{340} of the reagent blank from the ΔA_{340} of the samples.

High-pH anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) of D*-mannose in serum samples.* HPAEC-PAD analyses were performed by the Glycobiology Core Facility at the Univ. of California, San Diego, Cancer Center. Serum samples were preincubated with GK/G6PDH and the anionic substrates and products were removed with DE-53 spin columns exactly as described in the above protocol. Portions (40 μL of 800 μL total) of the spin column effluent were analyzed by HPAEC-PAD on a CarboPac PA-10 column. The column was eluted with 18 mmol/L NaOH for 22 min for the analytical separation, regenerated for 10 min with 200 mmol/L NaOH for 10 min, and reequilibrated with 18 mmol/L NaOH for 16 min before injection of the next sample. Controls included serum incubated without GK, a reagent blank (200 μL water in lieu of serum), and a mannose-only sample (200 μL of 50 μmol/L mannose). To verify peak identity, a portion of the spin column effluent was supplemented with mannose before analysis (equivalent to an additional 200 μmol/L mannose in the original sera). Mannose peak areas were integrated and nanomoles of mannose were calculated on the basis of a calibration curve generated with mannose calibrators not subjected to the enzymatic and chromatographic steps above.

Results

Removal of D*-glucose from sera with GK vs GOD.* The kinetics of depletion of D-glucose from incubations containing 1000 nmol of D-glucose (0.4-mL reaction; 2.5 mmol/L) are shown in Fig. 1 (curves with negative slope). Depletion of glucose from the sample is >99% complete after a 10-min incubation with 1.25 kU/L GK/G6PDH. G6PDH is included in the reaction to oxidize the product of the GK reaction (glucose-6 phosphate), thus "pulling" the reaction to completion. The depletion of glucose is much slower, even with 25 kU/L GOD and 100 kU/L CAT; ~3% remains after a 60-min incubation. Depletion of glucose from a serum sample (measured by production of glucose-6 phosphate) is also complete within 10 to 20 min with the same concentration of GK/G6PDH (Fig. 1; curve with positive slope). By contrast, we were unable to completely remove glucose from serum samples with 100–200 kU/L GOD and 400 kU/L CAT; 10–30% of the glucose remained, even after a 90-min incubation. However, the rate of glucose removal by 25 kU/L GOD from dialyzed serum reconstituted with 5 mmol/L glucose was the same as that of the glucose calibration solution. We

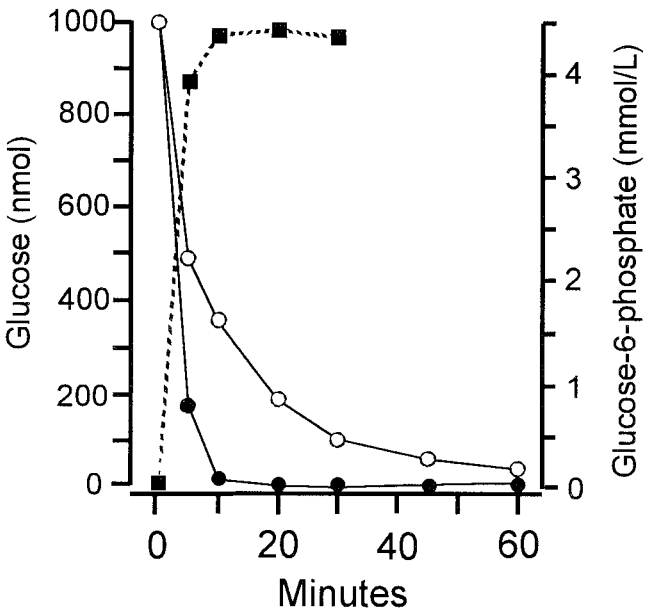

Fig. 1. Comparison of rate of removal of glucose from samples with GOD (○——○) vs GK (●——●).

Samples containing 1000 nmol of D-glucose were incubated either with 25 kU/L of GOD plus 100 kU/L CAT *[3]* or with 1.25 kU/L GK plus 1.25 kU/L G6PDH in a final volume of 0.4 mL as described in *Materials and Methods*. Portions (20-μL) were removed at indicated time points and reducing sugar remaining was assayed by the BCA method *[14]*. A serum sample containing 4.4 mmol/L glucose was incubated with GK plus G6PDH as above. Portions (20-μL) were removed at indicated time points, diluted to 1 mL, and the absorbance at 340 nm determined. The glucose-6 phosphate production was determined by calculating the amount of NAPDH produced after oxidation with G6PDH (■——■).

concluded that sera contain a dialyzable inhibitor of the GOD/CAT reactions. This may explain why previously published methods involving GOD required unusually high concentrations of enzyme (e.g., 1–5 μg of purified enzyme per microgram of glucose) *[3, 4]*.

However, using GK plus G6PDH to selectively remove the glucose from sera created a new problem. The reaction requires high concentrations of ATP and NADP substrates and generates high concentrations of ADP and NADPH products, which would interfere with the mannose determination using previously published methods *[3, 4]* involving PMI, PGI, and G6PDH. To overcome this, we designed a rapid, quantitative method to selectively remove these anionic substrates and products from the unreacted neutral mannose. We chose anion-exchange chromatography in microcentrifuge spin columns to allow rapid processing of multiple samples and quantitative recovery of sample volumes. Whatman DE-53, a weak anion-exchange resin, was chosen because it has excellent physical properties compatible with the microcentrifuge spin column method and a high exchange capacity (≥2 meq/g dry weight). The use of strong anion-exchange resins leads to base-catalyzed isomerization of the mannose to fructose. The method was verified by showing that >98% of the NADPH (99% ± 0.5%; n = 26; range 96.9–99.4%) produced during the preincubation was removed from the spin column effluent and that >95% of a

sample of radioactive mannose (98.4% ± 4.1%; n = 11; range 94.3–105%) was recovered in the effluent.

Assay of mannose calibrators in the presence of serum or 5 mmol/L glucose. To validate the procedure described in *Materials and Methods*, we assayed a series of mannose calibration solutions (5–200 μmol/L) and the same concentrations in the presence of 5 mmol/L glucose or human serum. The results in Fig. 2 show that the determination of mannose concentration was unaffected by the presence of 5 mmol/L glucose; the calibration curves are superimposed. The linear regression slope was 0.94 in the absence of glucose and 0.92 in its presence. Incubation of increasing concentrations of mannose in the presence of pooled human sera (53 μmol/L mannose) demonstrates the quantitative recovery of mannose from serum samples. The linear regression slope of the mannose calibration curve in the presence of serum was 0.90. (The manufacturer does not state the water content of the mannose used for these calibrators; a 92% recovery is equally compatible with a loss of 8% or the presence of 1 mol of water per mole of mannose.)

From these analyses (in the presence and absence of glucose) the interassay CV was 12.2% at 40 μmol/L (n = 7; 36.9 ± 2.1 μmol/L) and 9.8% at 80 μmol/L (n = 7; 74.2 ± 2.7 μmol/L). The intraassay CV was determined by assaying samples containing 40 μmol/L mannose or containing 80 μmol/L mannose; the intraassay CV at these concentrations was 6.7% (n = 5; 39.6 ± 1.6 μmol/L) and 4.4% (n = 11; 75.0 ± 1.8 μmol/L), respectively. The average recovery of mannose added to the sample of pooled human serum over a range of 5–200 μmol/L was 94% ± 4.4%.

Analysis of serum mannose concentration by GK/G6PDH reaction and HPAEC-PAD. To confirm the values obtained by the enzymatic assays above, a set of samples was treated exactly as described by the assay protocol in *Materials and Methods,* except that the effluents from the DE-53 spin columns were analyzed by HPAEC-PAD instead of with the HK/PGI/G6PDH/±PMI enzymatic assay. The results in Fig. 3A show that the reagent blank

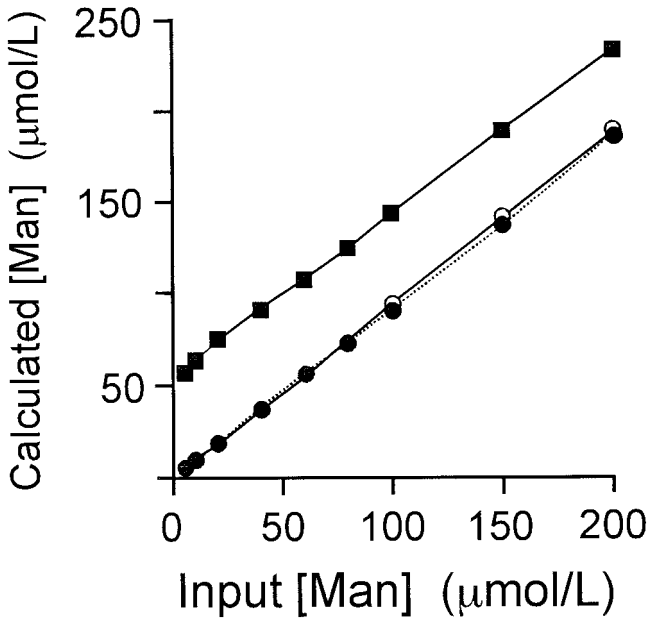

Fig. 2. Mannose calibrators analysis and recovery of mannose from serum.

Calibration solutions of D-mannose (Man) were assayed by the enzymatic protocol described in *Materials and Methods* (○——○). The same concentrations were also assayed in the presence of 5 mmol/L D-glucose (●——●). Portions of a pooled human serum sample were supplemented with the same concentrations of D-mannose and assayed (■——■).

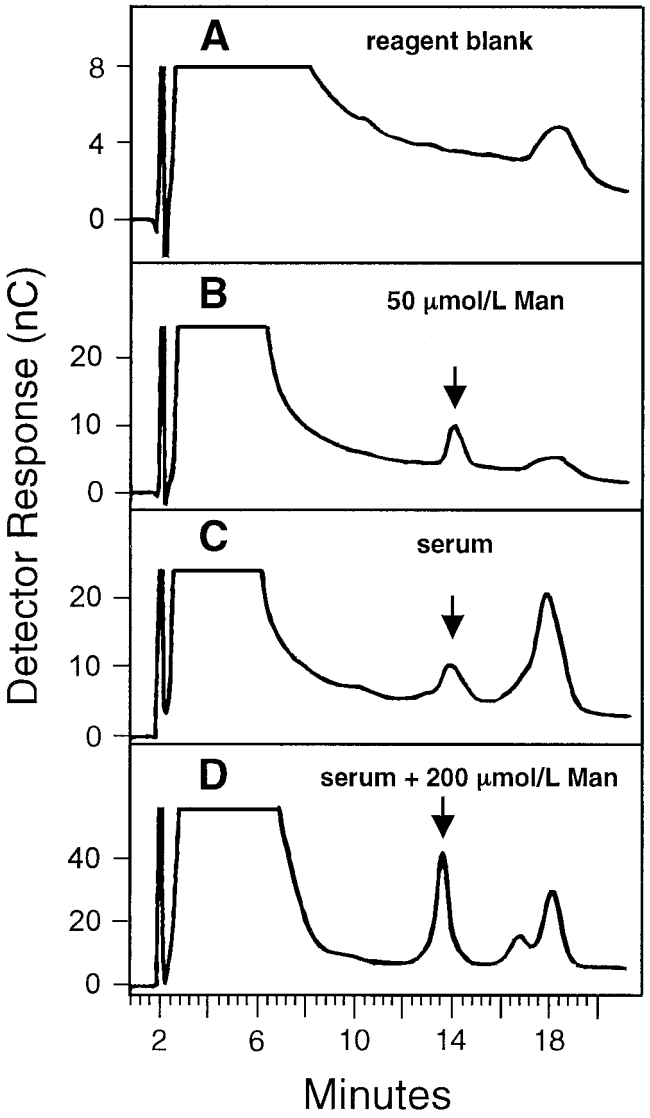

Fig. 3. Analysis of D-mannose by HPAEC-PAD after glucose depletion of samples by GK.

Samples were treated with GK and G6PDH and excess substrates, and the anionic products were removed by DEAE chromatography spin columns as described in *Materials and Methods*. Portions of the spin column effluent were analyzed by HPAEC-PAD. (*A*), reagent blank (200 μL of water as the sample); (*B*), calibration solution of 50 μmol/L D-mannose; (*C*), serum from a healthy volunteer; and (*D*), analysis of the same serum sample after supplementing with the equivalent of an additional 200 μmol/L mannose.

has no peak in the area where mannose is expected to elute. Panel B shows a trace for the analysis of a calibrator containing 50 μmol/L mannose. Panel C shows the trace for a serum sample containing 49.7 ± 6.8 μmol/L mannose (determined by enzymatic assay). Panel D shows the same serum sample supplemented with 200 μmol/L mannose.

The results of the quantitative analysis of four serum samples by HPAEC-PAD compared with the analysis by the enzymatic procedure are shown in Table 1. The values determined by both methods are in excellent agreement. Only single determinations by HPAEC-PAD were performed because of expense, availability of instrument time, and concern that the high concentration of protein and other serum components not removed by the DE-53 spin columns might damage the column. Attempts to analyze serum mannose by HPAEC-PAD without pretreatment with GK to remove glucose were not successful; the mannose peak area was obscured by the 100-fold greater glucose peak. Although the HPAEC-PAD method described here is useful as an alternative method for mannose determination, only one sample per hour (50 min) can be analyzed after the preincubation and chromatography step. By contrast, after the preincubation and chromatography step, the enzymatic assay of 12 samples can be completed in <1 h, and the data reduction and tabulation is also simpler and faster.

Also shown in Table 1 is the average value determined for the mannose content of 11 sera from healthy human volunteers. The value of 54.1 ± 11.9 μmol/L agrees quite well with the value reported by Pitkanen and Kanninen *[5]* of 55.1 ± 10.6 μmol/L determined by GC-MS.

Discussion

D-Mannose is an important metabolic intermediate required for biosynthesis of most of the structural and secretory glycoproteins in the body. Mannose-specific transport systems have been identified that may function specifically to retain and utilize mannose *[9, 10]*. Mannose can induce insulin release *[11]*, and increased concentrations of mannose have been found in patients with diabetes mellitus *[2–4]*. Sera from patients with invasive candidiasis or aspergillosis have mannose concentrations 10 to 20 times higher than that found in normal sera *[3, 12]*. CDGS type I has been shown to be associated with a deficiency of mannose utilization *[7]*. These and other studies have led to development of assays to quantify D-mannose concentrations in sera and other body fluids *[1–5]*.

The major obstacle of these analyses is the presence of 50–200-fold higher concentrations of D-glucose, which obscures or impedes the mannose determination by chromatographic methods of analysis. This problem was overcome by Pitkanen and Kanninen by using selected ion monitoring during GC-MS after sodium borodeuteride reduction of serum hexoses *[5]*. While sophisticated and accurate, this method would be difficult for rapid routine analyses. Other assays *[3, 4]* have relied on the use of GOD to reduce the high glucose concentrations before enzymatic analysis of mannose. These methods frequently result in very high backgrounds due to residual glucose remaining after the GOD treatment. We have observed that sera contain dialyzable inhibitors of the GOD system, which results in variable success in depleting sera of glucose. Furthermore, the high K_m of GOD for glucose (≥33 mmol/L) *[13]* leads to the need for unusually high concentrations of enzyme or lengthy incubations to reduce the serum glucose to a suitable concentration (100–200 μmol/L). The method described in the present study circumvents these problems by using the highly specific GK from *B. stearothermophilus* to remove glucose from the sera rapidly and quantitatively. The K_m of this GK for glucose and ATP are 0.1 mmol/L and 0.05 mmol/L, respectively *[8]*. This low K_m for glucose facilitates the rapid and quantitative removal of glucose from sera. This GK shows no activity with mannose or fructose as substrates *[8]*.

We used G6PDH in conjunction with the GK to "pull" the GK reaction to completion during a short incubation time. In these studies >99% of the glucose was removed from serum sample assays (>99.2% ± 0.15%; n = 29; range 99.0–99.5%). Because the GK does not phosphorylate mannose or fructose *[8]*, the effluent from the DE-53 spin column may contain both mannose and fructose. Normal sera contain 18.3 ± 7.6 μmol/L fructose *[5]*. The second enzymatic step to determine the mannose concentration in the sample depleted of glucose is done in the absence (tube A) or presence (tube B) of PMI, and the mannose concentration is calculated from the difference of absorption at 340 nm (ΔA_{340}). HK phosphorylates any residual glucose present in the sample as well as the fructose and mannose present. PGI is included in both assay tubes to convert fructose-6 phosphate to glucose-6 phosphate and, thus, to blank out any contribution of fructose to the ΔA_{340}. Although we have not attempted to do so here, the fructose concentration could also be measured with this procedure by carrying out the final

Table 1. Comparison of D-mannose values determined by enzymatic assay and by HPAEC-PAD analysis.

	D-Mannose, μmol/L	
	Enzymatic assay	**HPAEC-PAD**
Serum A	60.5 ± 10.6 (n = 3)	60.0
Serum B	65.0 ± 14.4 (n = 3)	62.2
Serum C	71.0 ± 4.7 (n = 3)	64.9
Serum D	49.7 ± 6.8 (n = 3)	48.5
Normal human sera	54.1 ± 11.9 (n = 11) (range 36–81)	—

Four serum samples from normal human volunteers were assayed for D-mannose concentrations enzymatically and by HPAEC-PAD as described in *Materials and Methods*. Seven additional sera were assayed by the enzymatic method only.

incubation in the presence and absence of PGI. For the mannose determination, PGI is required in both tubes since PMI converts mannose-6 phosphate to fructose-6 phosphate.

A critical step in the protocol presented is the use of microcentrifuge spin-column chromatography to remove the anionic components produced during the GK incubation step. The spin-column technique allows the quantitative and reproducible control of sample volume and recovery required for the assay without further processing for the final enzymatic determination of D-mannose. Several samples (12 or more) can be processed concurrently. Whatman DE-53 resin (DEAE-cellulose) was chosen for these spin columns because of its physical properties and high exchange capacity. Use of other resins with a lower exchange capacity may result in the incomplete removal of NADPH produced during the preincubation and thus a high background. However, careful handling of the resin is required to prevent breakdown of the microgranular fibers, which may affect the chromatographic properties of the resin (e.g., avoid vigorous mixing or shaking during resuspension of resin).

With this protocol, the reagent blank should give an A_{340} of 0.025 to 0.050 vs an H_2O blank and a ΔA_{340} of 0.002 to 0.012. With serum samples the background A_{340} (−PMI; tube A) is variable because of variation in UV-absorbing macromolecules and other components and is normally in the range of 0.150 to 0.350 vs a water blank. This variability in background does not directly affect the ΔA_{340} but the higher background values do result in greater measurement error for low mannose concentrations. Hemolyzed sera give higher background values, which leads to greater measurement error. Lipemic sera tend to clog the DE-53 spin columns and necessitate longer centrifugation times or separate steps to remove lipid before analysis of the mannose concentration (e.g., centrifugation of serum samples at high speed to remove the lipid).

This assay can be used to measure mannose in the sera of CDGS type 1 patients who are defective in mannose utilization. In studies to be reported elsewhere (Panneerselvam et al., manuscript in preparation), we use this assay to show that CDGS type I children have significantly reduced concentrations of serum mannose compared with healthy volunteers and pediatric patient controls. Since addition of mannose to the culture medium of fibroblasts from CDGS type I children corrects the protein glycosylation deficiency that is a hallmark of this disorder, this assay will be especially important for evaluating the success of proposed dietary mannose therapy for CDGS type 1 children. The assay should also be easily adaptable for determining mannose concentrations in the sera of other animals or in tissue culture media.

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