Leukocyte Phosphomannomutase Activity in Diagnosis of Congenital Disorder of Glycosylation Ia, Anne Barnier, Thierry Dupré, Maryvonne Cuer, Sandrine Vuillaimier-Barrot, Geneviève Durand, and Nathalie Seta* (Laboratoire de Biochimie A, Hôpital Bichat, AP-HP, 75877 Paris Cedex 18, France; *author for correspondence: fax 33-1-40-25-88-21, e-mail nathalie.seta@bch.ap-hop-paris.fr)

Phosphomannomutase (PMM; EC 5.4.2.8) is a cytosolic enzyme that catalyzes the reversible conversion of mannose-6-phosphate to mannose-1-phosphate, a substrate for the synthesis of GDP-mannose, which is a nucleotide sugar required in glycosylation.

Previously known in yeast (1, 2), Van Schaftingen and Jaeken (3) described the enzyme in 1995 in humans and its deficiency in patients with congenital disorders of glycosylation (CDG) Ia, an autosomal recessive disorder characterized by psychomotor retardation and multisystemic involvement (4). PMM deficiency is related to the presence of mutations in the corresponding PMM2 gene (5). Most patients bear the very common R141H mutation, which is never found in the homozygous state because it is probably lethal (6).

Diagnosis of CDG Ia, screening for which involves evidence of abnormally glycosylated serum N-glycoproteins (7), is completed by measurement of cellular PMM activity. Authors who have published PMM activity results (3, 8–13) have worked primarily on cultured fibroblasts and lymphoblasts or peripheral blood mononucleated cells (PBMCs). However, in most studies, the analytical conditions are described rather succinctly, no indication is given of the preanalytical conditions, and well-defined reference values are lacking. The aim of this study was to determine preanalytical conditions for the PMM assay measured in PBMCs from healthy individuals and the corresponding reference values, and to compare them with the results obtained for CDG Ia patients and their relatives.

Deidentified blood samples were obtained (from January 1999 to January 2001) from 414 individuals without CDG I (control group; age range, 1 month to 87 years), 25 CDG Ia patients for whom diagnosis was confirmed by the identification of PMM2 mutations, and 35 of their parents or relatives bearing one mutation on the PMM2 gene.

PBMCs were first isolated on a Ficoll gradient (14). Cell pellets were resuspended in the homogenization buffer [20 mmol/L HEPES, 10 mmol/L KCl, 1.5 mmol/L MgCl₂, 1 mmol/L dithiothreitol, 0.25 mol/L sucrose, and antiproteases (10 mg/L each of Trasylol, leupeptin, and phenylmethylsulfonyl fluoride)] and were lysed mechanically by numerous passes through a syringe needle (25-gauge, 5/8-inch) and then centrifuged at 500g for 10 min. The resulting extract was used for the PMM enzymatic assay, as described elsewhere (3), and protein determination (15). PMM results are expressed as mean ± SD U/g of total protein content (TP).

Mutations on the PMM2 gene were identified by a combination of direct sequencing and restriction analysis, as described elsewhere (16).

The means of the three groups were compared by ANOVA, followed by a post hoc test for pairwise comparison. Receiver operator characteristic (ROC) curves were calculated using the logistic procedure of SAS (SAS Institute).

We first established preanalytical conditions to standardize the PMM assay conditions for samples. Dipotassium EDTA was chosen as anticoagulant because values obtained for dipotassium EDTA-blood samples were statistically higher than those obtained for paired lithium heparin samples (n = 6; P = 0.032).

We studied the stability of PMM activity in whole blood samples. PMM activity was measured once a day for 6 days in samples stored at 4 °C and room temperature (n = 5) and compared with the activity measured directly after withdrawal on day 0. No statistical difference was found between days 0 and 1, whatever the storage temperature. In contrast, results were lower from day 2 at both temperatures (P = 0.028). The time between blood withdrawal and isolation of PBMCs should be at most 24 h at room temperature. PMM activity in cell extracts was altered after day 4 in extracts stored without glycerol at 4 °C (n = 6; day 0, 4.3 ± 0.07 U/g TP; day 4, 2.3 ± 0.04 U/g TP; P < 0.001), whereas no alteration was observed when the cell extract was stored with 150 mL/L glycerol at −80 °C for at least 1 month.

The within-run imprecision (CV), estimated with four successive assays of two control samples (5.3 ± 0.2 and 4.5 ± 0.3 U/g TP) on the same day, was <7%. The mean between-run CV was 4%, as determined by assay ing the same cell extract (4.2 ± 0.2 U/g TP) for 14 consecutive days.

Using the above-mentioned assay conditions, we measured PMM activity in PBMC extracts in the different groups (Fig. 1).

PMM activity in the PBMC extract from the control groups is reported in Table 1. PMM values were higher in individuals younger than 2 years compared with older individuals (P < 0.001). For individuals older than 2 years, the mean PMM value was similar to those reported in other studies (8–13). The PMM mean value was lower in women (n = 209; 4.8 ± 1.3 U/g TP) than in men (n = 206; 5.1 ± 1.6 U/g TP; P = 0.029). However, no difference was found in individuals younger than 2 years.

To check that the assay measured PMM activity and not phosphoglucomutase (PGM), we tested the effect of arsenate (5 mmol/L) added to the assay mixture on PBMC extracts from the control groups. The PMM activity was slightly inhibited by arsenate, regardless of the sex or age of the individual (Table 1).

The mean PMM activity for CDG Ia patients was significantly lower compared with the control group (P < 0.0001; Table 1), although individual values varied from undetectable or residual activity up to 1.9 U/g TP. When detectable PMM activity was determined in the presence of arsenate, partial inhibition was also observed.

Heterozygotes had intermediate PMM activity values,
with a large range of variation, from 1.1 to 3.9 U/g TP, which was also partially inhibited by arsenate (Table 1). We also compared the PMM activity in individuals carrying the severe R141H mutation (n = 15) with the activity in other heterozygotes (n = 20). PMM activity in individuals carrying the severe R141H mutation was significantly decreased (2.0 ± 0.6 U/g TP) compared with the activity in other heterozygotes (2.5 ± 0.6 U/g TP; P = 0.017).

Finally, because of a small overlap in PMM values between the control group and the heterozygotes and between the latter and the PMM-deficient patients, ROC plots were used to obtain the following thresholds: 2.1 U/g TP (both sensitivity and specificity = 1) for CDG Ia vs the control group; 3.1 U/g TP (sensitivity and specificity >0.970) for heterozygotes vs the control group; and 1.5 U/g TP (sensitivity and specificity >0.88) for CDG Ia patients vs heterozygotes.

### Table 1. Comparison of PMM activity (with and without arsenate) in PBMCs from CDG Ia patients, heterozygotes, and control groups.

<table>
<thead>
<tr>
<th></th>
<th>PMM activity (mean ± SD), U/g TP</th>
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<tbody>
<tr>
<td></td>
<td>Without arsenate</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
</tr>
<tr>
<td>(&lt;2 years of age)</td>
<td>6.0 ± 1.7</td>
</tr>
<tr>
<td>Control group</td>
<td>4.8 ± 1.4</td>
</tr>
<tr>
<td>(≥2 years of age)</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>6.0 ± 1.5</td>
</tr>
<tr>
<td>CDG Ia patients</td>
<td>0.6 ± 0.6</td>
</tr>
<tr>
<td>Heterozygotes</td>
<td>35.6 ± 0.6</td>
</tr>
</tbody>
</table>

*P <0.0001 vs control group.

CDG Ia occurs worldwide, and patients can present with a wide range of symptoms (17). CDG Ia must thus be biologically confirmed with a well-defined enzymatic assay. Because PMM is measured only in specialized laboratories dealing with metabolic impairment, the assay conditions we have chosen take into account optimal transport and working conditions.

The reference values determined are the first ones published that include a large number of samples and that take into account the gender and the age of the individuals. The 2-year cutoff needs to be refined by adding more samples from younger individuals. The observed increased PMM activity could be related to the high metabolic activity related to growth in the first months of life.

We also characterized the assay by testing the effect of arsenate, which inhibits PGM >80% (9). The mild observed effect of arsenate on the PMM assay suggested that the measured activity was essentially PMM and not PGM. This result was found regardless of the age of the controls, indicating that the increased activity seen in younger controls corresponded to actual PMM activity and not to increased or interfering PGM activity.

PMM has until now been measured primarily in cultured fibroblasts (8–13). The use of cultured cells has several advantages compared with PBMCs, such as the availability of adequate protein content. However, the drawbacks are numerous, the major one being possible pitfalls in the diagnosis of CDG Ia. Indeed, according to Grunewald et al. (13), when PMM was measured in fibroblasts from CDG Ia patients and controls, there was a wide scatter of values in both groups, with overlap. In contrast, overlap was never observed when PMM activities were measured in the corresponding leukocytes (13).

Our results indicate that there are pitfalls in the diagnosis, even when leukocytes are used, when dealing with heterozygotes or CDG Ia patients with mild PMM2 mutations. Indeed, for some CDG Ia patients with confirmed PMM2 mutations, there was an overlap in their values and the range for the heterozygotes. When we applied the 1.5 U/g TP threshold, 2 (with a PMM value of 1.9 U/g TP and bearing the E139K and R141H mutations) of the 25 CDG Ia patients could not have been correctly diagnosed and considered as heterozygotes. For the heterozygotes compared with the control group, an overlap was also observed, with one value (3.9 U/g TP, bearing the E139K mutation) above the 3.1 U/g TP threshold. Although not as crucial as with the fibroblasts (13), PMM activity from PBMCs must be considered with care when looking for heterozygous status, and sequencing may be needed to resolve the uncertainties.

Considering the PMM activity of heterozygotes bearing the R141H mutation, overall values lower than the values corresponding to the other mutations are in accordance with the severely reduced activity of the R141H mutated protein produced in *Escherichia coli* (16) compared with the other mutated PMM proteins.

In conclusion, we present a clearly defined method and reference values that should help biochemical genetics laboratories in the diagnosis of CDG Ia.
Comparability of Blood Glucose Concentrations Measured in Different Sample Systems for Detecting Glucose Intolerance, Rainer Haackel, Ute Brinck, Dubravka Colic, Hans-Uwe Janka, Isabel Püntmann, Jörg Schneider, and Carsten Viebrock

Glucose concentrations are usually measured in whole blood or plasma. Plasma values are influenced by the concentration of proteins, especially those with large volumes, such as lipoproteins. Blood values additionally depend on the total volume of the various blood cells, which is usually expressed as the hematocrit (1, 2).

The interconversion of glucose values for venous and capillary blood is further complicated by the arteriovenous difference. In the fasting state, the glucose concentrations in arterial, capillary, and (forearm) venous blood are supposed to be almost indistinguishable. In contrast, arterial blood glucose values may differ by 20% or as much as 70% in the postprandial state (3, 4). The mean arteriovenous differences are largest in lean nondiabetic individuals, smallest in diabetic individuals, and larger in deep veins than in superficial vessels (1, 5). Other factors can influence the differences in glucose concentrations among the various samples (6–9). Thus, the conversion of concentration values from one system (or sample type) to another is subject to unpredictable errors.

Several authors have already rejected the practice of converting glucose concentrations and have recommended that plasma be used for all glucose determinations (2, 10, 11). In a recent editorial, glucose measurement in whole blood was considered anachronistic (12), but only whole blood is used by home monitoring and near-patient monitoring devices. Many laboratories measure the glucose concentration in whole blood, especially in capillary whole blood, for therapeutic monitoring and for diagnosing hypo-, normo-, and hyperglycemia. However, the applicability of whole blood for determining glucose intolerance is still a matter of debate. Many practitioners tend to use capillary blood (CB) for diagnostic purposes (13, 14). The decision limits usually applied for whole blood are those recommended by WHO (15–17) and the American Diabetes Association (18), which are based on epidemiologic studies with venous plasma (VP).

In practice, either measured values or decision limits are converted from one sample system to another. The present study was undertaken to reinvestigate the comparability of glucose determinations in venous blood (VB), VP, and CB.

The study group consisted of 147 individuals from outpatient departments (internal medicine and dermatology) who were able to walk to the laboratory for blood collection (age range, 25–76 years). Using values recommended by WHO (15, 16) for the classification of plasma glucose concentrations, we separated the individuals into three groups according to whether they displayed a “healthy” (n = 74), impaired (n = 36), or diabetic glucose tolerance (n = 37). Oral glucose tolerance tests (GTTs) were performed according to WHO recommendations (15, 16). Participants ingested 75 g of glucose as Dextro O.G.T. (Roche Diagnostics).

VB samples were drawn into 2.7-mL monovettes containing lithium heparinate (cat. no. 05.1553; Sarstedt AG). Capillary and VB samples were collected within 2 min of each other by separate medical staff. Plasma was prepared within 10 min of blood sampling.

Glucose concentrations were determined in 500 μL of hemolyzing reagent plus 10 μL of blood or plasma (collected in heparinized end-to-end glass capillaries; cat. Tech Briefs