German Red Cross Blood Service
University of Heidelberg
Faculty of Clinical Medicine
Mannheim, Germany

*Address correspondence to this author at: Institute of Transfusion Medicine and Immunology, German Red Cross Blood Service, University of Heidelberg, Faculty of Clinical Medicine, Mannheim, Germany. Fax 49-621-3706851; e-mail p.bugert@blutspende.de.

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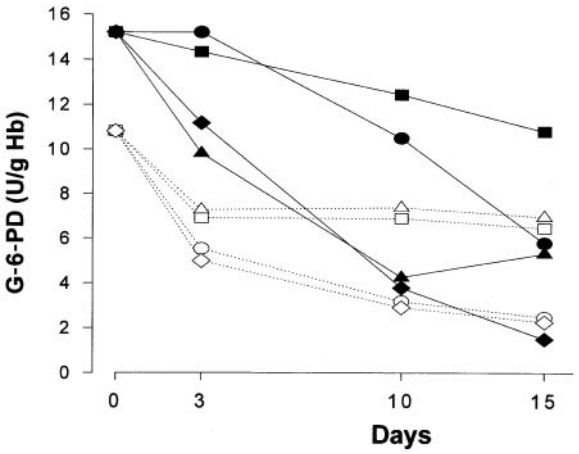

Fig. 1. Mean G-6-PD activity at days 0, 3, 10, and 15.

Results for whole blood stored at room temperature (●), refrigerated (■), frozen (▲), and at 37 °C (♦) and dried blood spots stored at room temperature (○), refrigerated (□), frozen (△), and at 37 °C (◇). *Hb*, hemoglobin.

Evaluation of Glucose-6-Phosphate Dehydrogenase Stability in Blood Samples under Different Collection and Storage Conditions

To the Editor:

Fujimoto et al. *(1)* reported that the stability of galactose 1-phosphate in dried blood spots for neonatal screening was adversely affected by humidity and temperature, especially when low-value samples are evaluated. We extend these findings to glucose-6-phosphate dehydrogenase (G-6-PD; EC 1.1.1.49) activity, deficiency of which is by far the most common genetic disease worldwide *(2)* and accounts for more than one-half of the cases of severe jaundice among male newborns in Greece, China, and in Sephardic Jewish groups *(3)*. Tests for G-6-PD deficiency are thus included in newborn screening programs in some regions.

We collected whole-blood specimens from 20 volunteers and spotted them on filter papers (Schleicher & Schuell no. 903) or placed them in EDTA tubes. G-6-PD activity was measured on the day of collection, and samples were immediately divided into 8 portions. Portions 1–4 were dried on filter paper and stored at room temperature, 2–8 °C, −20 °C, and 37 °C, respectively. Portions 5–8 were stored as whole blood under the same conditions. Subsequent assays were performed on days 3, 10, and 15. Whenever dried blood spots were used, we allowed them to dry at room temperature for 6 h and then placed them in air-tight plastic bags with desiccants, from which we eliminated air by squeezing the bag before closing. Thus, the effect of refrigeration and ambient room humidity was minimized.

The G-6-PD Deficiency Neonatal Screening Test Kit (cat. no. 3570-050; Interscientific Corporation) was used for quantitative measurement of G-6-PD activity *(4)*.

Increased temperature decreased the stability of G-6-PD in both liquid blood and dried blood spots (Fig. 1). The samples responded differently to the same temperature exposure, a finding that needs further investigation because it suggests a multiparametric influence.

Enzyme activity in some dried-blood samples appeared to be more tolerant of high temperatures than the activity in other samples. This could be attributed to several factors, which may include (but are not limited to) the filter-paper matrix, the initial ambient humidity in the card's environment, the presence of enzyme-protective factors in quantities that vary among samples, and the overall status of the erythrocytes. Further work needs to be conducted to elucidate the findings reported here. We conclude, however, that finding low activity in several samples in a batch of specimens delayed in the mail is very likely.

Importantly, this study demonstrates that time lost during the transport of Guthrie cards to screening centers should be kept to a minimum. Rapid delivery of cards and/or the use of heat-insulated envelopes and refrigerated transport may be a solution to prevent heat inactivation *(5)*. The latter approach may be difficult for newborn screening programs because of cost.

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Simone M. Castro[1*]
Raquel Weber[1]
Vivian Dadalt[1]
Vanessa F. Santos[2]
George J. Reclos [3]
Kenneth A. Pass[4]
Roberto Giugliani[5]

[1] Faculdade de Farmácia
Universidade Federal do Rio

Grande do Sul
Porto Alegre, Brazil

[2] *Centro Universitário Feevale*
Novo Hamburgo, Brazil

[3] *R&D DIAGNOSTICS Ltd.*
Papagos, Greece

[4] *New York State*
Department of Health
Wadsworth Center
Albany, NY

[5] *Serviço de Genética Médica*
Hospital de Clínicas de Porto Alegre
Universidade Federal do Rio
Grande do Sul
Porto Alegre, Brazil

*Address correspondence to this author at: Faculdade de Farmácia, UFRGS, Av. Ipiranga, 2752, Porto Alegre, RS, Brazil, 90610-000. Fax 55-51-33165434; e-mail scastro@adufrgs.ufrgs.br.

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Morphologic Changes in Urine Particles during the Menstrual Cycle

To the Editor:

Our previous study demonstrated that the numbers of epithelial cells and erythrocytes in midstream urine vary significantly during the menstrual cycle *(1)*. We now report the influence of the menstrual cycle on the number and morphology of urine particles.

Specimens were obtained from 67 healthy female students (age range, 18–20 years) at the College of Medical Technology. All participants gave written, informed consent; were asymptomatic; and provided information about their menstrual cycles. None had urologic conditions at the time of the study. Participants were instructed to collect midstream urine samples in sterile containers. Particles in midstream urine were analyzed by use of a fully automated urine cell analyzer, the UF-100 (Sysmex Corporation), within 2 h of collection. Specimens were classified into 3 groups according to the number of days after menstruation: menstrual (1–7 days after menstruation; n = 15), proliferative (8–14 days after; n = 24), and secretory (15–34 days; n = 22) phase.

Erythrocytes detected in the urine were classified into 2 groups, isomorphic and dysmorphic, according to the forward scattered-light intensity and distribution width. The UF-100 measures leukocyte size and counts small round epithelial cells by the forward scattered-light intensity.

The morphologic information obtained for urine particles in each phase is shown in Fig. 1. The frequency of isomorphic erythrocytes was significantly higher in the menstrual phase than in the other 2 phases, and almost all dysmorphic erythrocytes were observed in the proliferative and secretory phases (Fig. 1A). Leukocyte size was significantly larger in the menstrual phase than in the other 2 phases (Fig. 1B). The number of small, round epithelial cells gradually increased after men-

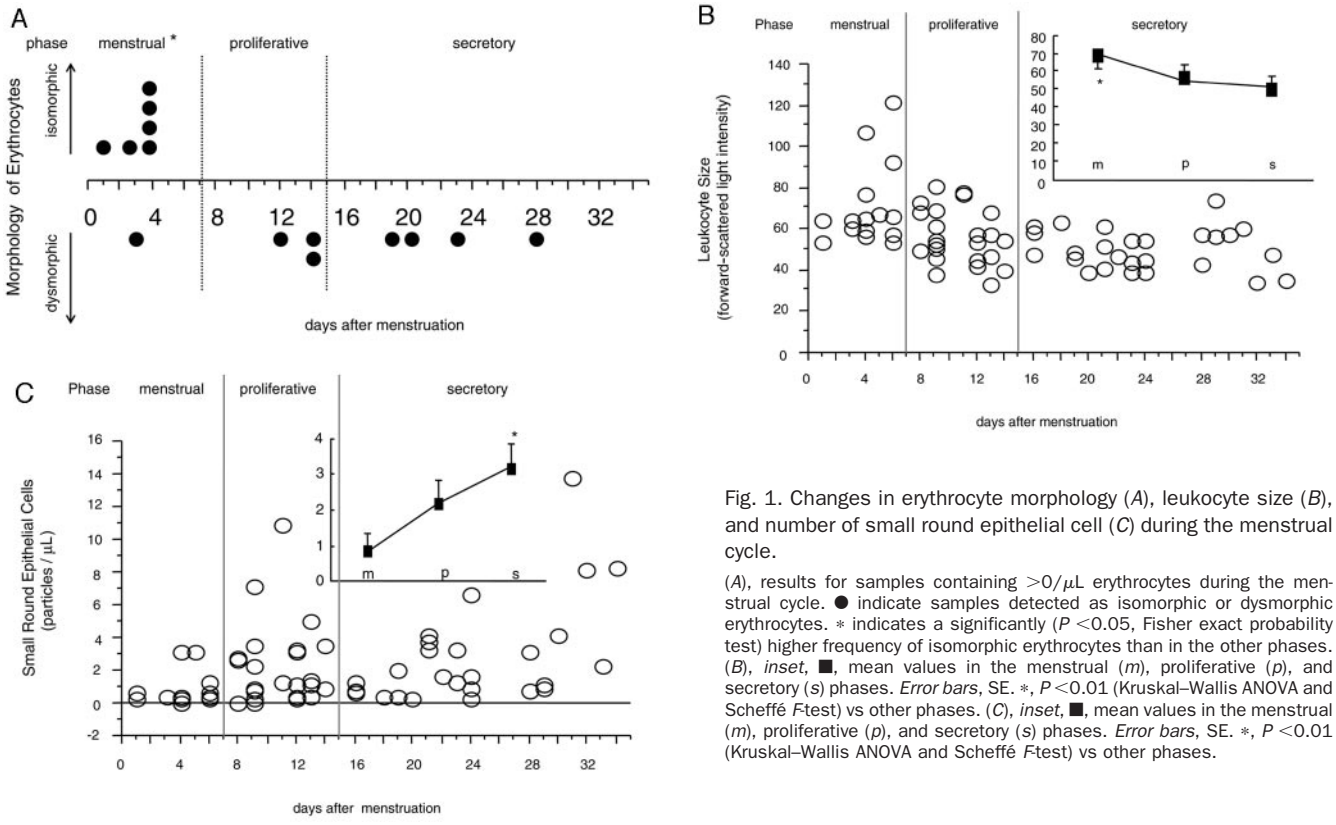

Fig. 1. Changes in erythrocyte morphology (*A*), leukocyte size (*B*), and number of small round epithelial cell (*C*) during the menstrual cycle.

(*A*), results for samples containing >0/μL erythrocytes during the menstrual cycle. ● indicate samples detected as isomorphic or dysmorphic erythrocytes. * indicates a significantly ($P < 0.05$, Fisher exact probability test) higher frequency of isomorphic erythrocytes than in the other phases. (*B*), *inset*, ■, mean values in the menstrual (*m*), proliferative (*p*), and secretory (*s*) phases. *Error bars*, SE. *, $P < 0.01$ (Kruskal–Wallis ANOVA and Scheffé *F*-test) vs other phases. (*C*), *inset*, ■, mean values in the menstrual (*m*), proliferative (*p*), and secretory (*s*) phases. *Error bars*, SE. *, $P < 0.01$ (Kruskal–Wallis ANOVA and Scheffé *F*-test) vs other phases.