Lactate Dehydrogenase-Immunoglobulin G Complex in the Serum of the Postburn Patient, Zejun Liu,* Changsheng He, Xingyu Huang, and Mingjing Wei (Department of Laboratory Medicine, Southwestern Hospital, Chongqing, 400038 People’s Republic of China; * author for correspondence: fax 8623-68754405, e-mail zjliuzj@public.cta.cn)

Lactate dehydrogenase (LDH)-immunoglobulin (Ig) complex has been reported in various diseases (1). Here we describe circulating LDH-IgG complex in a postburn patient; the complex appeared to be related to the progression of burn in this case.

A 13-year-old Chinese male was admitted to our hospital with burns over 20% of his body. Some of the relevant laboratory test results and reference intervals (in parentheses) for serum analysis were as follows: alanine aminotransferase, 44 U/L (4 – 50 U/L); aspartate aminotransferase, 62 U/L (5– 45 U/L); γ-glutamyltransferase, 17 U/L (4–50 U/L); and alkaline phosphatase, 110 U/L (32–140 U/L). LDH analysis showed high LDH activity (407 U/L; reference interval, 110–240 U/L), and LDH isoenzyme analysis showed abnormal values for the five isoenzymes (LDH-1, 11.8%; LDH-2, 15.8%; LDH-3, 17.6%; LDH-4, 42.5%; and LDH-5, 5.4%) and an extra band, LDH-6 (6.8%; Fig. 1A).

LDH activity was measured by a spectrophotometric assay with a CX-7 automatic biochemistry analyzer (Beckman). The assay conditions were largely based on the method described by Wroblewski and LaDue (2). LDH isoenzymes in serum were electrophoretically fractionated with the Paragon electrophoretic system. Bands containing activity were made visible by use of d,l-lactate and NAD+ as the substrate, phenazine methosulfate as the intermediate, and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide as the final hydrogen acceptor. Isoenzyme bands were scanned with the Appraise densitometer system (Beckman).

A broad band in the LDH-4 isoenzyme region characterized the patient’s LDH isoenzyme pattern, with the apparent appearance of an extra band, LDH-6 (Fig. 1). The patient’s erythrocyte LDH isoenzyme patterns were normal; therefore, it was not a genetic mutation that caused this anomaly, because a genetic mutation changes erythrocyte and serum LDH isoenzyme patterns (3). The presence of LDH-IgG complex in the patient’s serum was demonstrated by counter immunoelectrophoresis (Fig. 1B) (4).

With his symptoms improved, the patient’s serum LDH activity and isoenzymes gradually returned to reference values. One month after admission, his serum LDH concentration was normalized to 165 U/L, and his LDH isoenzymes were almost within health-related values (LDH-1, 20.8%; LDH-2, 28%; LDH-3, 27%; LDH-4, 19.1%; and LDH-5, 4.2%). Other laboratory test data were as follows: alanine aminotransferase, 26 U/L; aspartate aminotransferase, 34 U/L; γ-glutamyltransferase, 18 U/L; and alkaline phosphatase, 83 U/L. The LDH-Ig complex might be associated with the pathophysiology of this case.

There have been many reports on abnormal isoenzyme patterns of plasma LDH in various diseases (5–7). However, in China there are few reports on LDH-Ig complexes. To our knowledge, ours is the first case of LDH-Ig complex in the serum of postburn patients. The exact mechanism of the formation of LDH-Ig complex under disease conditions must be elucidated.
Automated Enzymatic Assay for the Determination of Sucrose in Serum and Urine and Its Use as a Marker of Gastric Damage, Bernard Vinet,1* Benoit Panzini,2 Mathieu Boucher,1 and Julie Massicotte1 (Departments of 1 Biochemistry and 2 Gastroenterology, Centre hospitalier de l’Université de Montréal, Montreal, Quebec, Canada H2L 4M1; * address correspondence to this author at: Département de biochimie, Centre hospitalier de l’Université de Montréal (CHUM), Campus Notre-Dame, 1560, rue Sherbrooke est, Montreal, Quebec, Canada H2L 4M1; fax (514) 869-4651)

Sugars have been used as probes for intestinal damage because healthy gastrointestinal tissue, unlike damaged mucosa, is almost totally impermeable to disaccharides (1, 2). Sutherland et al. (3) proposed the use of sucrose, which is degraded in the small intestine by a specific disaccharidase, for the detection of mild upper gastrointestinal damages. Therefore, even with intestinal disorders, the presence of sucrose in urine or blood after oral ingestion (100 g) is indicative of gastric permeability. At a cutoff of 180 mg sucrose/5 h urine, the test has a sensitivity of 84% for the detection of gastric ulcers and a specificity of 96% for normal endoscopy (3). The presence of Helicobacter pylori does not significantly affect the permeability to sucrose (4). In children, the specificity and sensitivity for gastric damage were 90% and 83%, respectively (5). This assay was also applied to patients suffering from malaria (6) and used in various animal studies (7, 8).

The practicability of the test is limited by the precision of urine collection (9) and the analytical method used for the detection of sucrose in urine. Sucrose measurement in serum instead of in urine could improve the test and allow a faster, more accurate diagnostic tool; however, its concentration in this biological fluid is low. Sucrose has been measured in biological fluids by HPLC with electrochemical/ampereometric detection (7), gas-liquid chromatography (9), and colorimetric assay (8); these methods, however, are slow and/or insensitive. Two enzymatic assays have been described (9, 10), but they were not used for sucrose in serum. In 1984, Birnberg and Brenner (11) described the measurement of sucrose in plant extracts with the following sequence of reactions:

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\text{Sucrose} \rightarrow \text{Sucrose phosphorylase} \rightarrow \text{Glucose-1-phosphate} + \text{fructose} \rightarrow \text{Glucose-1-phosphate} \rightarrow \text{Glucose-6-phosphate} \rightarrow \text{Glucose-6-dehydrogenase} \rightarrow 6\text{-phosphogluconate} + \text{NADH} + \text{H}^+ \quad \text{(340 nm)}
\]

We describe here a modification of this method and its adaptation for the determination of sucrose in serum and urine.

We obtained sucrose phosphorylase (from Leuconostoc mesenteroides; sucrose:orthophosphate \( \alpha \)-d-glucosyltransferase; EC 2.4.1.7; cat. no. S7760), phosphoglucomutase (from chicken muscle; \( \alpha \)-b-glucose 1,6-phosphomutase; EC 5.4.2.2; cat. no. P6156), glucose-6-phosphate dehydrogenase (from Leuconostoc mesenteroides; \( \alpha \)-b-glucose 6-phosphate:NAD(P) \^ {+} \) 1-oxidoreductase; EC 1.1.1.49; cat. no. G8404), NAD\(^{+}\) (\( \beta \)-nicotinamide adenine dinucleotide from yeast; cat. no. N1511), fructose, maltose, bilirubin, caffeine, and other drugs from Sigma Chemical Co. All other reagents were from Fisher Scientific.

To prepare calibrators at 1400 \( \mu \text{mol/L} \), 20 \( \mu \text{L} \) of a 2.40 g/L sucrose solution in deionized water was added to 1.0 mL of a serum pool made from leftover specimens from fasting subjects or to 1.0 mL of freshly voided urine. Calibrators were prepared at 700, 350, or 175 \( \mu \text{mol/L} \) by serial dilution with the serum pool or urine. The phosphate-magnesium buffer was prepared by dissolving 3.5 g of K\(^{2+}\)HPO\(_4\) and 664 mg of MgSO\(_4\)\( \cdot \)7H\(_2\)O in deionized water, and then bringing the final volume to 1 L with deionized water to obtain final concentrations of 20 mmol/L K\(^{2+}\)HPO\(_4\) and 2.7 mmol/L MgSO\(_4\). The pH was adjusted to 7.0 with phosphoric acid. For the NAD-buffer solution, 66 mg of NAD\(^{+}\) was dissolved in 100 mL of phosphate-magnesium buffer to obtain a 1 mmol/L solution. For the enzyme reagent, 80 \( \mu \text{L} \) of the phosphoglucomutase suspension and 6.5 \( \mu \text{L} \) of the glucose-6-phosphate dehydrogenase suspension were added to 10 mL of the NAD-buffer solution. This reagent was stable for at least 9 weeks at 4 °C. For the sucrose phosphorylase solution, 1 mL of deionized water was added to the lyophilized powder. This solution was diluted 1/10 with the phosphate-magnesium buffer to obtain a final enzyme activity of 10 000 U/L. The enzyme is stable for at least 3 days at 4 °C or can be kept at −20 °C for several months.

We used an automated centrifugal analyzer, the Cobas Fara II\(^{10}\) (Roche Analytical Instruments, Inc.) at 30 °C. The enzyme reagent is used as the main reagent (125 \( \mu \text{L} \) and