

Stability Studies of Twenty-Four Analytes in Human Plasma and Serum

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Background: The stability and stoichiometric changes of analytes in plasma and serum after prolonged contact with blood cells in uncentrifuged Vacutainer® tubes were studied.

Methods: We simultaneously investigated the stability of 24 analytes (*a*) after prolonged contact of plasma and serum with blood cells and (*b*) after immediate separation of plasma and serum (centrifuged twice at 2000g for 5 min). We verified biochemical mechanisms of observed analyte change by concomitant measurement of pH, P_{CO_2} , and P_{O_2} . Hemolysis was qualitatively and semiquantitatively assessed. All specimens were maintained at room temperature (25 °C) and analyzed in duplicate 0.5, 4, 8, 16, 24, 32, 40, 48, and 56 h after collection. Statistically significant changes from the 0.5 h mean were determined using repeated-measures ANOVA. The significant change limit was applied to determine clinically significant changes in measured analytes.

Results: Fifteen of 24 analytes in plasma and serum maintained in contact with cells showed clinically relevant changes, with the degree of change more pronounced in most plasma specimens. All analytes in plasma and serum immediately separated from cells after collection were stable.

Conclusion: Storage of uncentrifuged specimens beyond 24 h caused significant changes in most analytes investigated because of (*a*) glucose depletion and Na^+, K^+ -ATPase pump failure; (*b*) the movement of water into cells, causing hemoconcentration; and (*c*) leakage of intracellular constituents and metabolites. Immediate separation of plasma or serum from cells provides optimal analyte stability at room temperature. When prolonged contact of plasma or serum with cells is

unavoidable, use of serum is recommended because of the higher instability of plasma analytes.

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A general problem facing clinical laboratories is the integrity of uncentrifuged specimens for chemical analyses. Because prolonged contact of plasma or serum with cells is a common cause of spurious test results, plasma and serum should ideally be separated from cells as quickly as possible to prevent ongoing metabolism of cellular constituents as well as active and passive movement of analytes between the plasma or serum and cellular compartments. In the past, issues regarding serum analyte stability were a major concern because serum was the specimen preferred by most laboratories. However, some laboratories are switching to plasma because serum specimens have several inherent problems: (*a*) an increase in turnaround time because of the time necessary for clot formation, especially in patients receiving anticoagulant therapy; and (*b*) the risk of fibrin clot interference on automated analyzers, especially those with a common sample probe and no clot detection ability. However, although the changes that occur with analytes of uncentrifuged serum specimens are reasonably well investigated, no such data exist for uncentrifuged plasma specimens. Moreover, the changes in plasma analyte concentrations with and without prolonged contact with cells have yet to be simultaneously compared with those seen with serum analytes under identical conditions.

Published literature pertaining to chemical analyte stability has addressed many issues related to serum specimens but largely neglected plasma. The stability of 72 analytes after prolonged contact of serum with cells has been described (1–7). The effects of prolonged storage on the stability of 31 analytes in plasma and serum separated from cells by a gel barrier are also known (6, 8–10). Lastly, the stability of 30 analytes in serum immediately separated from cells has been described (11–15), but no similar studies are available on plasma.

Our study addressed (*a*) the simultaneous measurement and determination of stability for 24 analytes after prolonged contact of plasma and serum with cells; (*b*) the

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simultaneous measurement and determination of stability for 24 analytes after immediate separation of plasma and serum from cells with subsequent delays before analysis; (c) verification of certain biochemical processes within uncentrifuged specimens by measurement of pH, P_{CO_2} , and P_{O_2} ; (d) qualitative and semiquantitative measurement of free hemoglobin to indirectly assess erythrocyte membrane integrity and rule out hemolytic interference with our testing methodologies; and (e) determination of clinically relevant changes using the significant change limit (SCL)¹ approach.

Materials and Methods

INSTRUMENTATION

Alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALK), aspartate aminotransferase (AST), direct bilirubin, total bilirubin, calcium, total carbon dioxide (tCO_2), chloride, total cholesterol, creatinine, creatine kinase (CK), γ -glutamyltransferase (GGT), glucose, lactate, lactate dehydrogenase (LD), Mg^{2+} , P_i , K^+ , Na^+ , total protein, triglycerides, uric acid, and urea were measured with the Dade Dimension RxL (Dade International Inc.). We used the Gem Premier 2.2 Blood Gas Analyzer (Mallinckrodt Sensor Systems, Inc.) to measure pH, P_{O_2} , and P_{CO_2} . Free hemoglobin was assessed by the Hitachi 747 (Boehringer Mannheim Corp.).

SPECIMEN COLLECTION AND HANDLING

The investigational proposal was approved by the Institutional Review Board. All volunteers were informed of the rationale for the study, and consent was obtained. Venous blood was collected from each of 10, nonfasting volunteers after antecubital venipuncture into 15 sterile (unopened) 5-mL Vacutainer[®] Serum Separator Tubes with clot activator (SST; Becton Dickinson) and 15 sterile (unopened) 4.5-mL Vacutainer Plasma Separator Tubes containing 15–20 IU/mL lithium heparin (PST; Becton Dickinson); samples were collected sequentially, and tubes were filled completely. Blood was collected from a different volunteer each day over 10 contiguous days. Ambulatory volunteers were seated in an upright posture ~1 min before venipuncture and throughout specimen collection. The tubes were randomized to negate the effect of draw order with the exception that all SSTs were collected before the PSTs to prevent anticoagulant contamination (16). The tourniquet used during collection was removed after 1 min to prevent hemoconcentration and the production of metabolic byproducts (17). Total collection time per volunteer was 10–15 min.

Cell-free plasma and serum were obtained by allowing

seven PSTs and seven SSTs from each volunteer to stand for 0.5 h at room temperature (25 °C), after which tubes were centrifuged (2000g for 5 min). The plasma and serum from six centrifuged PSTs and six centrifuged SSTs from each volunteer were pooled into separate 15-mL sterile red-top no-additive Vacutainer tubes, recapped, and centrifuged. From each 15-mL red-top tube, the double-spun plasma (DSP) and serum (DSS) were subsequently aliquoted into nine respective 1.5-mL samples cups, capped, and wrapped with Parafilm (American National Can). One double-spun aliquot of plasma and serum from each volunteer was immediately analyzed on the Dimension RxL for the above-mentioned analytes to serve as the double-spun, 0.5 h baseline value. The remaining eight double-spun aliquots of plasma and serum from each volunteer were stored upright at room temperature (25 °C) in the dark, where they remained unopened until analysis. The plasma and serum of the remaining centrifuged PST and SST from each volunteer were immediately analyzed on the Gem Premier for pH, P_{CO_2} , and P_{O_2} , then on the Dimension RxL for use as the 0.5 h baseline value for each analyte with prolonged contact with cells.

Plasma and serum specimens in prolonged contact with cells were obtained by allowing the remaining eight uncentrifuged PSTs and eight uncentrifuged SSTs from each volunteer to sit at room temperature (25 °C) in the dark, where they remained unopened until analysis. Of the remaining uncentrifuged specimens and double-spun aliquots, one of each was analyzed at 4, 8, 16, 24, 32, 40, 48, and 56 h. At each designated time an uncentrifuged PST and SST was centrifuged, and the plasma and serum were immediately analyzed on the Gem Premier, then on the Dade Dimension RxL. DSP and DSS aliquots were analyzed only on the Dimension RxL. Specimens analyzed on the Gem Premier were run singly, whereas all specimens analyzed on the Dade Dimension RxL were run in duplicate (back to back). Two levels of commercial reference sera were run in conjunction to monitor instrument variation.

Qualitative assessment of free hemoglobin was graded by visual inspection of each sample at all time intervals. Semiquantitative measurement of free hemoglobin was performed on the Hitachi 747 in a preliminary study, where 2 of the 10 volunteers in the current investigation were randomly selected to undergo the same specimen collection and handling procedures as explained previously. Their samples for all time intervals were analyzed in duplicate on the Hitachi 747.

STATISTICAL ANALYSIS

To determine time-dependent changes of analytes in plasma and serum with and without prolonged contact with cells, the mean from all 10 volunteers for each respective analyte was obtained for each time interval. Statistically significant changes were determined for each analyte by repeated-measures ANOVA. Clinically signifi-

¹ Nonstandard abbreviations: SCL, significant change limit; ALT, alanine aminotransferase; ALK, alkaline phosphatase; AST, aspartate aminotransferase; tCO_2 , total CO_2 ; CK, creatine kinase; GGT, γ -glutamyltransferase; LD, lactate dehydrogenase; SST, serum separator tube; PST, plasma separator tube; USD, usual SD; DSP, double-spun plasma; and DSS, double-spun serum.

icant changes were determined by the SCL (18) approach, defined as:

$$\text{SCL} = \text{Initial value} \pm 2.8 \text{ USD}$$

and is based on the assumption that the usual SD (USD) is representative of the inherent day-to-day variability of the method. In our study, the calculated mean for each analyte at 0.5 h represented the initial value. The USD was obtained by averaging the SD of the quality-control data for the last 7 months for each respective analyte (18). The quality-control reference serum whose target mean most closely matched the 0.5 h mean for each analyte was used to determine the USD. For simplicity, the SCL was computed for each analyte by establishing the range (± 3.0 USD) from the volunteer mean at 0.5 h. Statistical analyses were performed by Quattro Pro, Ver. 9.0 (© WordPerfect Office 2000; Corel Corp.); Microsoft Excel, Ver. 9.0 (© Microsoft Office Professional; Microsoft Corp.); and Analyze-It, Ver. 1.63 for Microsoft Excel (© Analyze-It Software, Ltd.).

Results

The statistics for plasma and serum analytes that exceeded the SCL because of prolonged contact with cells are shown in Table 1. The other analytes in plasma and serum that were exposed to prolonged contact with cells remained stable over the 56-h period; their 0.5-h mean and greatest change were as follows: ALK (U/L), plasma (81 ± 3), serum (85 ± 3); ALT (U/L), serum (47 ± 1); AST (U/L), plasma and serum (21 ± 2); direct bilirubin (mg/L), plasma and serum (1.0 ± 0); total bilirubin (mg/L), plasma and serum (6.0 ± 1); calcium (mg/L), serum (92 ± 2); tCO₂ (mmol/L), serum ($26.3 - 2.1$); chloride (mmol/L), plasma ($103 - 3$), serum ($103 - 2$); cholesterol (mg/L), serum ($1950 + 40$); CK (U/L), plasma ($111 + 8$), serum (115 ± 3); GGT (U/L), serum ($41 + 1$); Mg²⁺ (mmol/L), serum ($0.74 + 0.08$); Na⁺ (mmol/L), plasma ($139 + 3$), serum ($140 + 2$); total protein (g/L), serum ($75 + 1$); triglycerides (mg/L), plasma ($1940 + 70$), serum ($1990 + 30$); urea (mg/L), plasma and serum ($150 + 10$).

All 24 analytes in DSP and DSS were stable (by SCL) over the 56-h period; their 0.5-h mean and greatest change were as follows: ALT (U/L), DSP ($47 - 2$), DSS ($47 - 1$); albumin (g/L), DSP ($41 + 2$), DSS ($42 + 2$); ALK (U/L), DSP and DSS (85 ± 2); AST (U/L), DSP ($21 + 1$), DSS ($22 - 1$); direct bilirubin (mg/L), DSP and DSS (1.0 ± 0); total bilirubin (mg/L), DSP and DSS (6.0 ± 0); calcium (mg/L), DSP ($90 + 2$), DSS ($91 + 3$); tCO₂ (mmol/L), DSP ($25.1 - 1.2$), DSS ($26.7 - 1.7$); chloride (mmol/L), DSP and DSS (103 ± 0); total cholesterol (mg/L), DSP ($1870 + 20$), DSS ($1940 - 20$); creatinine (mg/L), DSP and DSS (10 ± 0); CK (U/L), DSP ($112 + 3$), DSS ($115 - 3$); GGT (U/L), DSP ($31 + 5$), DSS (40 ± 0); glucose (mmol/L), DSP (6.7 ± 0), DSS (6.4 ± 0); lactate (mmol/L), DSP and DSS (1.9 ± 0); LD (U/L), DSP (137 ± 1), DSS ($143 - 2$); Mg²⁺ (mmol/L), DSP and DSS (0.74 ± 0); P_i (mmol/L), DSP and DSS (1.1 ± 0); K⁺ (mmol/L), DSP (4.0 ± 0), DSS (4.1 ± 0); Na⁺ (mmol/L),

DSP ($140 - 1$), DSS ($141 - 1$); total protein (g/L), DSP (76 ± 0), DSS ($75 - 1$); triglycerides (mg/L), DSP (1940 ± 10), DSS (1990 ± 20); uric acid (mg/L), DSP ($61 - 1$), DSS ($60 + 1$); urea (mg/L), DSP and DSS (150 ± 0).

Qualitative assessment of free hemoglobin was negative for all specimens. Semiquantitative measurement of free hemoglobin in the double-spun specimens for plasma and sera at 0.5 h was 40 mg/L with variation of ± 15 mg/L, and ± 10 mg/L for plasma and serum, respectively. Semiquantitative analysis of specimens with prolonged contact with cells yielded free hemoglobin values at 0.5 h of 80 mg/L for plasma and 60 mg/L for serum with variations from 50 to 110 mg/L and from 40 to 90 mg/L, respectively, over the 56-h study.

All commercial reference materials for the 24 analytes and blood gas values were within ± 2 SD of their respective target means during the entire investigation.

Discussion

FREE HEMOGLOBIN

According to Dade International package inserts, free hemoglobin concentrations of 500 and 2500 mg/L would be required to interfere with the direct bilirubin and CK assays, respectively, with the remaining 22 analytes subject to interference if free hemoglobin concentrations were ≥ 5000 mg/L. Because the highest quantitative measurement of free hemoglobin in our study was 110 mg/L, we conclude that free hemoglobin was not at sufficient concentrations to interfere with any methodology for the 24 analytes included in our study.

DSP AND DSS WITH PROLONGED INCUBATION AT ROOM TEMPERATURE

All 24 analytes in plasma and sera immediately separated from cells were stable over the 56-h period. Our results for serum analytes were consistent with those obtained by previous authors (11–15) who investigated these serum analytes under similar conditions, with a few notable exceptions: Felding et al. (14) and Berg et al. (15) noted instability of total bilirubin, with the latter stating photodegradation as a likely etiology; Baer and Krause (11) reported calcium instability but cited possible bacterial contamination as an etiology. Our observations of plasma analyte stability are new and demonstrate that plasma is equal to serum when stored under similar conditions over 56 h.

PROLONGED CONTACT OF PLASMA AND SERA WITH CELLS AT ROOM TEMPERATURE

Variation of metabolically active analytes. Glucose concentrations decreased rapidly to 24 h, then decreased more slowly to 56 h. Although the decrease was more pronounced in plasma, a stoichiometric yield of lactate was observed in both plasma and serum via glycolysis (see Table 1). The net loss of glucose was 5.6 mmol/L for plasma and 3.6 mmol/L for serum, and the net gain of lactate was 12.6 mmol/L for plasma and 8.9 mmol/L for

Table 1. Statistical analysis of selected analytes at room temperature.

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Analyte ^a	Units	Specimen type ^b	Time of analysis, h										USD value	SCL range ^c
			0.5	4	8	16	24	32	40	48	56			
K ⁺	mmol/L	Plasma	3.9	3.8 ^d	3.9	4.0	4.0	4.4 ^{d,e}	5.1 ^{d,e}	5.9 ^{d,e}	6.8 ^{d,e}	0.08	3.7–4.1	
		Serum	4.0	4.1	4.2 ^d	4.0	4.2 ^d	4.5 ^{d,e}	4.9 ^{d,e}	5.6 ^{d,e}	6.2 ^{d,e}	0.08	3.8–4.2	
CO ₂	mmol/L	Plasma	26.0	25.4	26.1	25.7	24.7 ^d	23.9 ^d	23.7 ^d	23.0 ^d	21.9 ^{d,e}	1.23	22.3–29.7	
Glucose	mmol/L	Plasma	6.5	5.7 ^{d,e}	4.7 ^{d,e}	3.1 ^{d,e}	2.0 ^{d,e}	1.5 ^{d,e}	1.1 ^{d,e}	0.9 ^{d,e}	0.9 ^{d,e}	0.10	6.2–6.8	
		Serum	6.5	5.8 ^{d,e}	5.4 ^{d,e}	4.6 ^{d,e}	4.0 ^{d,e}	3.4 ^{d,e}	3.0 ^{d,e}	3.0 ^{d,e}	2.9 ^{d,e}	0.10	6.2–6.8	
Creatinine	mg/L	Plasma	10	9 ^d	9 ^d	9 ^d	11 ^d	15 ^{d,e}	19 ^{d,e}	21 ^{d,e}	21 ^{d,e}	0.4	9–11	
		Serum	10	10	10	9 ^d	10	12 ^{d,e}	14 ^{d,e}	15 ^{d,e}	16 ^{d,e}	0.4	9–11	
TP	g/L	Plasma	76	76	77	77	78 ^d	79 ^{d,e}	79 ^{d,e}	79 ^{d,e}	80 ^{d,e}	0.75	74–78	
Albumin	g/L	Plasma	41	41	41	42 ^d	43 ^d	43 ^d	43 ^d	44 ^{d,e}	44 ^{d,e}	0.73	39–43	
		Serum	42	42	43 ^d	43 ^d	43 ^d	44 ^d	44 ^d	45 ^{d,e}	45 ^{d,e}	0.73	40–44	
Calcium	mg/L	Plasma	90	90	91 ^d	92 ^d	93 ^d	94 ^d	96 ^{d,e}	96 ^{d,e}	95 ^{d,e}	1.3	86–94	
		Plasma	1.0	1.0	0.9 ^d	0.8 ^{d,e}	1.1	1.7 ^{d,e}	2.6 ^{d,e}	3.3 ^{d,e}	3.9 ^{d,e}	0.03	0.9–1.1	
P _i	mmol/L	Serum	1.1	1.1	1.1	1.0 ^d	1.2	1.6 ^{d,e}	2.2 ^{d,e}	2.8 ^{d,e}	3.3 ^{d,e}	0.03	1.0–1.2	
Urate	mg/L	Plasma	61	61	60 ^d	60 ^d	60 ^d	59 ^d	57 ^{d,e}	56 ^{d,e}	56 ^{d,e}	0.8	59–63	
		Serum	60	60	60	59 ^d	59 ^d	57 ^{d,e}	57 ^{d,e}	56 ^{d,e}	55 ^{d,e}	0.8	58–62	
LD	U/L	Plasma	144	147	152	153 ^{d,e}	161 ^{d,e}	153 ^{d,e}	156 ^{d,e}	170 ^{d,e}	186 ^{d,e}	2.87	136–152	
		Serum	142	144	150 ^d	155 ^{d,e}	160 ^{d,e}	166 ^{d,e}	164 ^{d,e}	168 ^{d,e}	171 ^{d,e}	2.87	134–150	
ALT	U/L	Plasma	46	46	46	46	46	47	45	38 ^{d,e}	37 ^{d,e}	1.11	43–49	
TC	mg/L	Plasma	1880	1870	1900 ^d	1920 ^d	1950 ^d	1990 ^{d,e}	1990 ^{d,e}	2000 ^{d,e}	2040 ^{d,e}	33.9	1778–1982	
Mg ²⁺	mmol/L	Plasma	0.74	0.74	0.78 ^d	0.78 ^d	0.78 ^d	0.82 ^d	0.82 ^d	0.86 ^{d,e}	0.86 ^{d,e}	0.03	0.64–0.84	
		Plasma	30	30	31 ^d	32 ^d	34 ^d	35 ^d	36 ^d	37 ^{d,e}	38 ^{d,e}	2.12	24–36	
GGT	U/L	Plasma	1.8	3.6 ^{d,e}	5.4 ^{d,e}	8.6 ^{d,e}	11.1 ^{d,e}	12.6 ^{d,e}	13.2 ^{d,e}	14.3 ^{d,e}	14.4 ^{d,e}	0.076	1.6–2.0	
		Serum	1.8	3.2 ^{d,e}	4.4 ^{d,e}	6.1 ^{d,e}	7.5 ^{d,e}	8.9 ^{d,e}	10.0 ^{d,e}	10.2 ^{d,e}	10.7 ^{d,e}	0.076	1.6–2.0	
Lactate	mmol/L	Plasma	7.43	7.37 ^{d,e}	7.32 ^{d,e}	7.24 ^{d,e}	7.18 ^{d,e}	7.13 ^{d,e}	7.10 ^{d,e}	7.07 ^{d,e}	7.05 ^{d,e}	0.013	7.39–7.47	
		Serum	7.42	7.39 ^d	7.34 ^{d,e}	7.30 ^{d,e}	7.28 ^{d,e}	7.24 ^{d,e}	7.20 ^{d,e}	7.20 ^{d,e}	7.17 ^{d,e}	0.013	7.38–7.46	
pH	mmHg	Plasma	44	50 ^d	53 ^{d,e}	63 ^{d,e}	69 ^{d,e}	73 ^{d,e}	80 ^{d,e}	82 ^{d,e}	82 ^{d,e}	1.93	38–50	
		Serum	45	47 ^d	52 ^{d,e}	55 ^{d,e}	58 ^{d,e}	62 ^{d,e}	65 ^{d,e}	64 ^{d,e}	68 ^{d,e}	1.93	39–51	
Pco ₂	mmHg	Plasma	85	65 ^{d,e}	74 ^{d,e}	68 ^{d,e}	74 ^{d,e}	74 ^{d,e}	62 ^{d,e}	66 ^{d,e}	65 ^{d,e}	2.33	78–92	
		Serum	76	65 ^{d,e}	72	68 ^{d,e}	72	66 ^{d,e}	62 ^{d,e}	64 ^{d,e}	64 ^{d,e}	2.33	69–83	

^a TP, total protein; TC, total cholesterol.

^b Plasma and serum in contact with cells until time of analysis.

^c Mean at 0.5 h ± 3.0 USD.

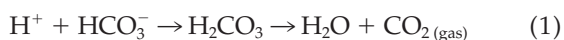
^d Statistically significant difference from 0.5-h concentration (repeated-measures ANOVA, *P* < 0.05).

^e SCLs have been exceeded.

^a TP, total protein; TC, total cholesterol.^b Plasma and serum in contact with cells until time of analysis.^c Mean at 0.5 h ± 3.0 USD.^d Statistically significant difference from 0.5-h concentration (repeated-measures ANOVA, $P < 0.05$).^e SCLs have been exceeded.

serum. The ratio of net glucose loss to net lactate production was 1:2.25 for plasma and 1:2.47 for serum, somewhat higher than the theoretical molar ratio of 1:2. The deviation from theoretical was most likely attributable to the high concentration of initial, steady-state glycolytic intermediates (19) within the erythrocytes at the time of specimen collection, which subsequently underwent glycolysis to yield additional lactate.

Chloride and $t\text{CO}_2$ showed a steady decrease over 56 h, the degree of change being more pronounced for plasma $t\text{CO}_2$ (see Table 1). Our observations were consistent with previous studies of these serum analytes under similar conditions (2, 4–7). The decreases in chloride and $t\text{CO}_2$ were likely attributable to the chloride-bicarbonate shift with subsequent buffering of H^+ ion (from lactic acid) by bicarbonate with production of CO_2 gas (see Eq. 1)



Corroborating our observation was the stoichiometric increase of $P\text{CO}_2$ in plasma and serum over the 56-h period, with the measured net increases in plasma $P\text{CO}_2$ of 38 mmHg and serum $P\text{CO}_2$ of 23 mmHg comparing favorably with the Henderson–Hasselbalch predicted net $P\text{CO}_2$ increases of 35 and 22 mmHg, respectively. Interestingly, at 24 h, plasma pH (7.18) and serum pH (7.28) were roughly equal to the typical, intracellular pH (7.20) of the erythrocyte (19). PO_2 showed a rapid decrease from 0.5 to 4 h, then demonstrated a variable course extending to 56 h.

P_i showed a slight decrease from 8 to 16 h with only plasma P_i exceeding the SCL at 16 h; however, this 0.2 mmol/L decrease was not considered clinically significant. After 24 h, plasma and serum P_i concentrations increased continuously, with the degree of change more prominent in plasma (see Table 1). Our observations for serum were consistent with other studies using serum in prolonged contact with cells (3, 4, 6); these studies attributed the increase to hydrolysis of intracellular phosphate esters with subsequent diffusion from the erythrocyte.

K^+ was stable to 24 h, after which a rapid increase was noted; the degree of change was slightly more pronounced in plasma. Our observations for serum were consistent with previous studies using serum in prolonged contact with cells at room temperature (3, 4, 6, 20).

The increase in K^+ after 24 h, the approximate nadir of extracellular glucose, was most likely attributable to Na^+, K^+ -ATPase pump failure, with diffusion of K^+ from the erythrocyte driven by the intracellular to extracellular concentration gradient of 25:1 (19).

Nonmetabolic analytes and hemoconcentration. Analytes demonstrating clinically insignificant increases after 24 h (percentage increase) were total bilirubin (plasma and serum, +17%), Na^+ (plasma and serum, +2%), and urea (plasma and serum, +6%). Analytes demonstrating clinically significant changes after 24 h (percentage increase) were albumin (plasma and serum, +7%), calcium (plas-

ma, +6%), Mg^{2+} (plasma, +16%), and total protein (plasma, +5%). Our observation for serum Na^+ was consistent with similar studies investigating serum with prolonged contact with cells at room temperature (3, 4, 7). Zhang et al. (7) also noted similar observations for albumin, total protein, calcium, Mg^{2+} , and Na^+ with serum specimens. These observations most likely represent hemoconcentration attributable to the movement of water into cells after 24 h. Uric acid showed a decreasing trend over the 56-h period, with the SCL surpassed at 40 h for plasma and 32 h for serum, which may be related to decreased uric acid solubility in the continuously increasingly acidic environment.

Lipids and enzymes. Cholesterol trended upward, with the degree of change more pronounced in plasma than serum. A similar trend was noted previously in serum specimens, and the mechanism proposed was that cholesterol was being continuously removed from blood cells via lecithin: cholesterol acyltransferase (6). Triglycerides were stable over the 56-h period.

LD activity increased continuously over the investigational period, with the SCL surpassed at 16 h and the degree of change more prominent in plasma. Our serum observations were consistent those of authors of similar studies (2–4, 6, 7), who postulated that the increase was attributable to changes in cell membrane integrity. The activities of plasma and serum AST, ALK, and CK were stable over the 56-h period, and our serum observations were consistent with previous studies (2–7).

Variation attributable to methodologic interference. Plasma ALT was stable to 40 h, then lost 20% of activity at 48 and 56 h. This decrease was not observed in serum or the DSP and DSS aliquots and is therefore likely attributable to increased lactate concentrations in plasma specimens after 40 h, which may have interfered with the methodology [ALT-catalyzed conversion of L-alanine + α -ketoglutarate to L-glutamate + pyruvate, with subsequent LD-catalyzed conversion of pyruvate + $\text{NADH}(\text{H}^+)$ to lactate + NAD^+].

The GGT activity in plasma specimens with and without prolonged contact with cells was 27% lower than in serum at 0.5 h; however, GGT activity steadily increased over the 56-h period, with only plasma in prolonged contact with cells exceeding the SCL at 48 h (see Table 1). The discrepancy in plasma and serum activity at 0.5 h was most likely attributable to lithium heparin interference with the methodology (manufacturer's package inserts state interference at or above 280 000 IU/L lithium heparin).

Creatinine increased by 110% in plasma and 60% in serum after 24 h, which was probably attributable to interference of pseudo-creatinines (6) with the kinetic Jaffe reaction. The dramatic increase could not be explained simply by hemoconcentration, because most

other nonmetabolic analytes (i.e., albumin, Na^+ , total protein, and urea) increased by only 2–6%.

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