

Quantitative Evaluation of Hypothermia, Hyperthermia, and Hemodilution on Coagulation

Scott A. Kmiecik, MPS; Jun-Li Liu, MPS, MD, PhD; Tal S. Vaadia, BS; Jeffery D. Nichols, MPS; Ryan J. Kohtz, MPS; Nancy J. Mills, MPS; Craig M. Petterson, MPS; Alfred H. Stammers, MSA, CCP

Division of Clinical Perfusion Education, School of Allied Health Professions, University of Nebraska Medical Center, Omaha, Nebraska

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Abstract: The purpose of this study was to investigate the effects of temperature change on the coagulation time of blood at two different hematocrit levels by using various coagulation-monitoring devices. The devices used in this study were the Bayer Rapid Point Coag Analyzers, Hemochron Jr. Signature, Hemochron Response, Medtronic ACT II, and Haemoscope Thrombelastograph. One unit of human bank blood was used in this study. The hematocrit level was adjusted to 40% and 20%. A control bath and experimental bath were set up. Control blood was maintained at 37°C and tested every 45 ± 15 min throughout the experimental period of 6 h to demonstrate the stability of the model. The experimental blood was tested at temperature points of 37, 32, 27, 32, 37, 42, and 37°C. Activated clotting time (ACT) tended to increase when the temperature was initially decreased from 37 to 27°C, which reached a statistically significant level

when measured by the Hemochron Response at both the 20% (147 ± 10.7 to 159.3 ± 11.0, $p < .0332$) and 40% hematocrit level (130 ± 14.9 to 152.1 ± 19.7, $p < .0148$). ACT was decreased significantly ($p < .05$) when the temperature was increased to 42°C as measured by all machines except the Hemochron Jr. Signature at the 20% hematocrit level. ACT was significantly higher ($p < .05$) at a 20% hematocrit level as compared to that at a 40% hematocrit level on all devices for the majority of temperature points. These data suggested that hypothermia only increased ACT when measured by a macrosample device requiring a milliliter sample (Hemochron Response). However, hemodilution induced anticoagulatory effects and hyperthermia caused an acceleration in coagulation by all devices utilized in this study. **Keywords:** coagulation, CPB, heparin, hemodilution, hypothermia, hyperthermia. *JECT. 2001;33:100-105*

Exposure of blood to artificial surfaces during cardiopulmonary bypass (CPB) causes activation of blood, platelets and the coagulation system resulting in excessive thrombin generation and thrombus formation (1). However, the large doses of heparin required to prevent clot formation during CPB may induce such serious side effects as platelet dysfunction and bleeding (1, 2).

In addition to the use of anticoagulants, coagulation is affected by numerous variables that include both physical and biological processes. It is well documented that patients undergoing hypothermic CPB demonstrate a tendency toward increased intraoperative and postoperative blood loss, as well as an increased rate of reoperation for bleeding (3-5). Mild hypothermia of only 0.5°C, a reduction to 36.5°C core temperature, has been shown to slow blood clotting significantly (6, 7). Watts et al. reported that 34°C was the critical point at which coagulation enzyme activity slowed significantly and at which significant alter-

ation in platelet activity was seen (8). Alternatively, unintentional hyperthermia can occur during the rewarming phase of CPB. Pivalizza et al. demonstrated an accelerated fibrin formation when whole-body hyperthermia was implemented (9). In addition, hemodilution induces marked nonheparin-induced anticoagulation attributable to the dilution of clotting factors and platelets (10).

Accurate and precise monitoring of coagulation with changes in temperature and hematocrit level could provide clinicians with valuable information concerning hemostatic potential during CPB. However, little has been documented on the variation of coagulation with the physical alterations occurring during CPB. Therefore, the purpose of the current study was to investigate the effects of hypothermia, hyperthermia, and hemodilution on coagulation by using modern point-of-care coagulation monitors.

MATERIALS AND METHODS

Experimental Set-up

One unit of human bank blood anticoagulated with CPDA-1 was drawn and delivered from Interstate Blood

Address correspondence to: Scott A. Kmiecik, MPS, Division of Clinical Perfusion Education, University of Nebraska Medical Center, 985155 Nebraska Medical Center, Omaha, NE 68198-5155. E-mail: skmiecik@unmc.edu

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Bank, Inc. (Memphis, TN), the day before the experiment. Blood was filtered through a 110-micron gross filter utilizing a blood collection reservoir [Cobe Brat® Collection Reservoir (Ref:007401), Arvada, CO]. Twenty milliequivalents of sodium bicarbonate were added to the blood to adjust the pH to 7.4 ± 0.05 . Hematocrit (HCT) levels were adjusted to 40% and 20% $\pm 2\%$ by diluting with a balanced electrolyte solution. Blood was stored in 50-mL polypropylene test tubes for the duration of the experiment.

Two Bayer Rapidpoint™ Coag Analyzers (Chorion Diagnostics, Raleigh, NC) (BRP), two Haemoscope 5000 Thromboelastographs (Haemoscope Corp., Skokie, IL) (TEG), two Hemochron® Jr. Signatures (International Technidyne Corp., Edison, NJ) (SIG), two Hemochron® Responses (Medtronic Inc., Minneapolis, MN) (RES), and one Medtronic ACT II (Medtronic Inc., Minneapolis, MN) (ACT II) were used to monitor blood coagulation in this study. The TEG samples were measured by celite activation and required 340 microliters of whole blood. The BRP and SIG devices utilized low range celite activation as well, but required only a drop of whole blood per sample. Celite activated as well, the ACT II and RES required 200 microliters and two milliliters respectively. All devices were battery charged at least 16 hours before initiation of the experiment and were operated in accordance with manufacturer instructions for use. Normal and abnormal liquid quality controls were performed and found to be within range on all devices 1 h before initiation of the experiment.

Control Groups

To monitor the stability of the preparations, four test tubes (two at 40% HCT, two at 20% HCT) of blood were used as control groups. The control blood was continuously agitated and maintained at a temperature of $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ in a water bath. Control samples were taken every 45 min (± 15 min) beginning just before initiation of the experiment until termination to demonstrate stability of the model. To ensure mixture homogeneity test tubes were inverted 10 times immediately before the samples were drawn. Samples were recalcified with a 0.2 M calcium chloride solution by adding 20 microliters of calcium chloride to 340 microliters of blood, and inverted 10 times before performing each test. Heparin was not used in any portion of the experiment to eliminate variables other than those being manipulated. The activated clotting times (ACTs) of both the 40% and 20% HCT levels were measured on the ACT II at every time interval. In addition, samples of 20% hematocrit control blood were taken at the onset, midway point, and conclusion of experimental testing. Samples were taken at the same time intervals and sent to a separate laboratory (Department of Pathology and Microbiology Laboratory) and assayed for fibrinogen

count, platelet count, partial thromboplastin time (PTT), and prothrombin time (PT).

Experimental Groups

Eight test tubes (four at 40% HCT and four at 20% HCT) of blood were placed in the experimental bath. The temperature of the water bath was manipulated in 5°C increments with an initial baseline of 37°C and proceeding as follows: 37°C - 32°C - 27°C - 32°C - 37°C - 42°C - 37°C . The experimental period was approximately 6 h in duration. A temperature probe was placed in one of the test tubes in the experimental bath, and no samples were drawn from this tube. Once the desired temperature was reached in the monitoring port, all experimental blood was allowed to equilibrate for 3 min. ACTs were measured in eight replications per device at both the 40% and 20% HCT levels at every temperature point. Devices utilized to measure ACTs at these intervals included the BRP, SIG, RES, and the ACT II. In addition, four replications of each the 40% and 20% HCT levels were taken from the experimental bath and run on the TEG at the following temperature points: 37°C - 27°C - 37°C - 42°C - 37°C . TEG variables included the reaction time (R), kinetic time (K), angle (α), and maximum amplitude (MA), which were measured by 1% celite activation. A coagulation index (CI) was calculated according to the manufacturers discriminant analysis ($\text{CI} = -7.792 - .326 \times \text{R} - .189 \times \text{K} + .122 \times \text{MA} + .076 \times \alpha$). The CI demonstrates the over-all coagulation status of the blood. The blood was calcified and drawn in the same manner as in the control groups. All samples were randomized with respect to the well used to run the samples.

To maintain a consistent hyperthermia period of all blood samples, upon reaching the second of three 37°C temperature sample points, only enough volume of blood for one complete trial of samples at 42°C was left in the experimental bath, with the remaining blood transferred to the control bath. This transferred blood remained in separate tubes from the control blood. The temperature of the experimental bath was then increased from 37°C to 42°C and equilibrated for 3 min. ACT, R, K, α , and MA were measured. The previously cooled blood was then transferred back from control bath to experimental bath for another complete trial. This step was repeated until all tests at 42°C were completed.

For the final 37°C samples, enough previously cooled blood for one complete trial was transferred from control bath to experimental bath and allowed to equilibrate at 42°C . Aliquots were then transferred back to the control bath and allowed to equilibrate at 37°C . Once the temperature remained at 37°C for the predetermined 3-min stabilization period, samples were completed. This step was repeated until all tests for the final 37°C were completed.

Statistical Analysis

The differences among groups were tested by one-way analysis of variance (ANOVA). When significant differences were found, Fisher's protected least significant post-hoc analysis was completed. Statistical significance was accepted at a $p \leq .05$. All data are expressed as mean \pm standard deviation (SD).

RESULTS

Experimental results of the effects of hemodilution on ACT are shown in Table 1, which compares 40% HCT to 20% HCT at every temperature point for all devices. At least two of the four devices tested showed significantly ($p < .05$) elevated ACTS at 20% HCT compared to 40% HCT level at every temperature point.

ACTS increased significantly ($p = .0165$) during cooling from 37°C to 32°C in the RES at the 40% HCT level (Figure 1). The coldest tested temperature (27°C) resulted in significantly increased ACTS in the RES at both the 40% HCT ($p = .0148$) and 20% HCT ($p = .0332$) levels (Figure 1). Contrary to hypothermia, hyperthermia attenuated ACTS to a significant level ($p < .05$) in all devices at both HCT levels, with the exception of the SIG at 20% HCT (Figures 2-4). Significantly reduced ACT values from baseline were also seen after the temperature was decreased from 42°C to 37°C at the 40% HCT level in both the SIG ($p = .0001$) and the RES ($p = .002$), and at the 20% HCT level in the BRP ($p = .0003$).

The CI, R, K, α , and MA values from the TEG are listed in Table 2. Hypothermia of 27°C induced a significant reduction in the CI at 20% HCT; whereas, hyperthermia of 42°C caused a significant increase in the CI at 40% HCT (Figure 5). The stability of the model was well maintained through the experiment, which is shown in Figure 6 and Table 3.

DISCUSSION

Hemodilution and hypothermia have both been shown to independently alter values disrupting the correlation of anticoagulation and heparinization (10-12). Cohen et al.

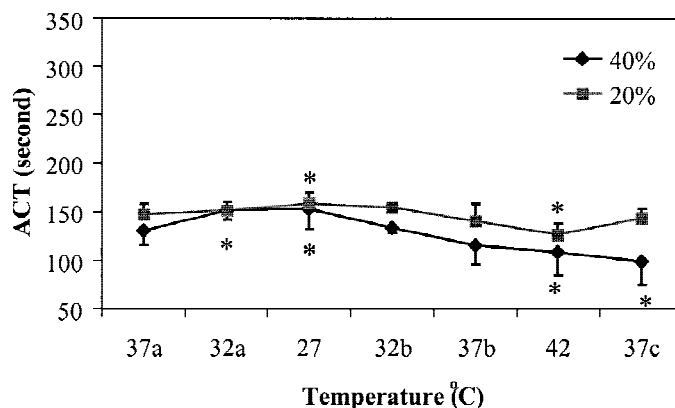


Figure 1. Response: effects of temperature. ACT—Activated clotting time; *—significantly different from the initial 37°C; 37a—initial 37°C; 32a—initial 32°C; 32b—32°C after cooling to 27°C; 37b—37°C after cooling; 37c—37°C after heating; N = 8 at both hematocrit levels for every temperature point.

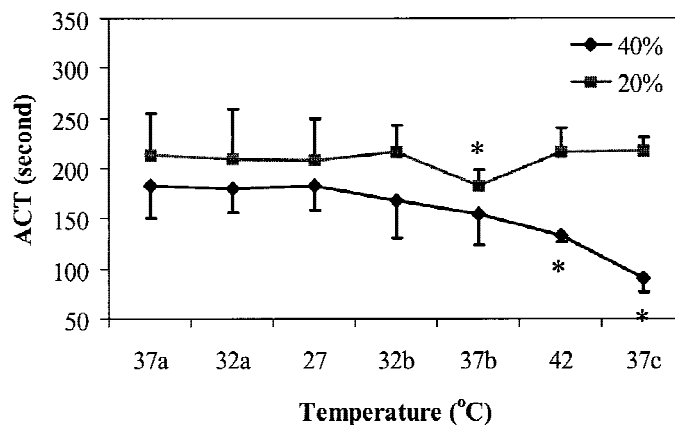


Figure 2. Signature: effects of temperature. ACT—activated clotting time; *—significantly different from the initial 37°C; 37a—initial 37°C; 32a—initial 32°C; 32b—32°C after cooling to 27°C; 37b—37°C after cooling; 37c—37°C after heating; N = 8 at both hematocrit levels for every temperature point.

demonstrated that although heparin concentration decreased an average of 27%, once CPB was initiated, ACTs extended only an average of 8.4% (13). They further demonstrated, with protamine titration to measure heparin concentration, that the ACT was prolonged when heparin

Table 1. Effects of hemodilution on ACT.

Temperature (°C)	Response		ACT II		Signature		Bayer Rapid Point	
	40%	20%	40%	20%	40%	20%	40%	20%
37	130.0 ± 14.9	147.0 ± 10.7*	122.3 ± 18.6	162.1 ± 11.7*	183.0 ± 42.0	212.8 ± 32.2	225.3 ± 10.1	257.3 ± 25.0
32	151.8 ± 9.6	151.9 ± 7.5	135.7 ± 12.7	162.1 ± 11.5*	179.9 ± 49.1	209.9 ± 24.3	211.8 ± 21.6	286.5 ± 32.2*
27	152.0 ± 9.6	159.3 ± 11.0	125.4 ± 19.6	172.0 ± 16.4*	182.1 ± 40.7	208.0 ± 23.0	201.3 ± 27.8	269.0 ± 10.1*
32	133.9 ± 6.0	154.1 ± 4.5*	129.6 ± 8.2	155.3 ± 20.5*	179.4 ± 40.5	205.0 ± 39.1	212.8 ± 23.5	291.8 ± 19.3*
37	115.9 ± 19.9	140.5 ± 18.2*	130.0 ± 15.5	151.6 ± 28.7	154.8 ± 15.6	182.9 ± 30.7*	196.5 ± 14.8	262.8 ± 24.3*
42	108.1 ± 24.1	125.5 ± 11.8*	101.7 ± 12.1	118.8 ± 17.1	134.4 ± 25.2	215.7 ± 5.5*	181.3 ± 36.3	167.0 ± 28.2
37	97.4 ± 23.3	143.3 ± 9.8*	107.3 ± 11.9	121.8 ± 9.9	88.5 ± 13.9	218.0 ± 12.7*		

* = significantly different from 40% ($p < 0.05$).

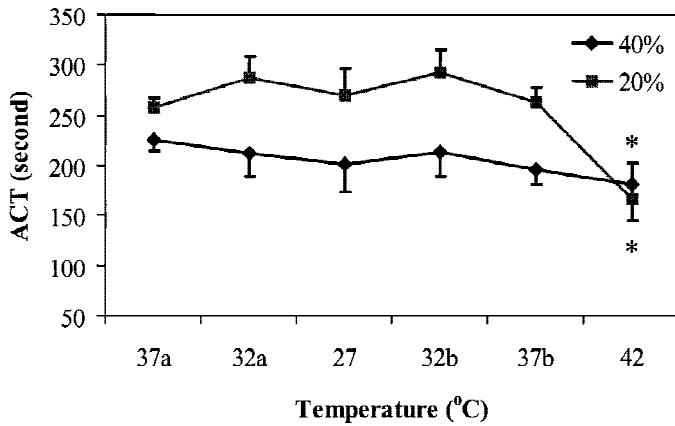


Figure 3. Bayer Rapid Point: effects of temperature. ACT—activated clotting time; *—significantly different from the initial 37°C; 37a—initial 37°C; 32a—initial 32°C; 32b—32°C after cooling to 27°C; 37b—37°C after cooling; 37c—37°C after heating; N = 8 at both hematocrit levels for every temperature point.

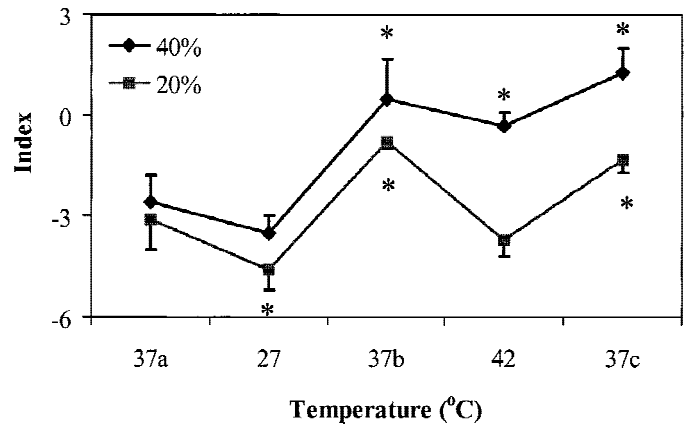


Figure 5. Effects of temperature on TEG index. TEG—thromboelastograph coagulation analyzer; index—over-all coagulation status; *—significantly different from initial 37°C; 37a—initial 37°C; 37b—37°C after cooling; 37c—37°C after heating; N = 4 at both hematocrit levels for every temperature point.

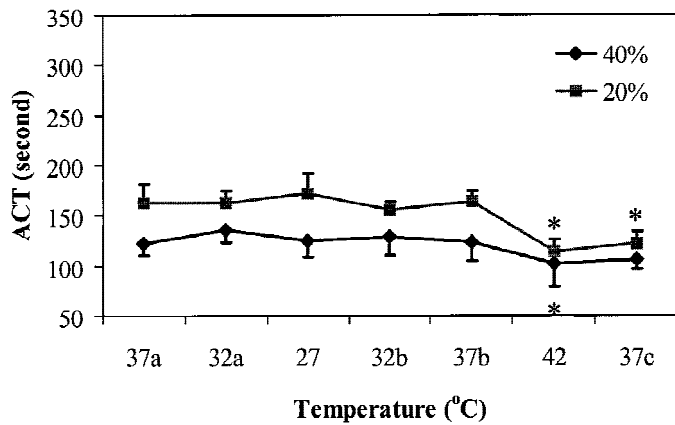


Figure 4. ACT II: effects of temperature. ACT—activated clotting time; *—significantly different from the initial 37°C; 37a—initial 37°C; 32a—initial 32°C; 32b—32°C after cooling to 27°C; 37b—37°C after cooling; 37c—37°C after heating; N = 8 at both hematocrit levels for every temperature point.

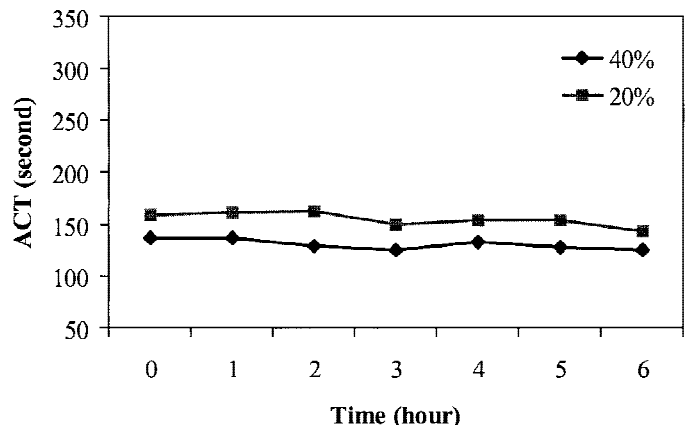


Figure 6. Changes of ACT with time. ACT—activated clotting time.

concentration essentially remained unchanged with cooling (13). This has also been confirmed through the measurement of factor Xa inhibition illustrating that the prolongation in ACT during cooling from 33°C to 28°C was

not attributable to a change in heparin concentration (14). However, Paul and associates reported that profound hypothermia (20°C) for 60 min in dogs could promote a continuous release of a heparin-like factor that reacts as a specific inhibitor of factor Xa (15). Therefore, evaluation of such factors as temperature and hemodilution are of paramount importance for the clinician during CPB.

Table 2. Effects of temperature on TEG variables.

	37	27	37	42	37
R Time (20%)	13.4 ± 1.4	15.3 ± 1.7	10.9 ± 0.3	13.0 ± 2.3	15.8 ± 8.8
R Time (40%)	14.0 ± 0.9	16.5 ± 0.9*	6.8 ± 1.4*	5.9 ± 1.1*	4.7 ± 0.3*
K Time (20%)	4.9 ± 0.5	7.0 ± 0.4*	3.8 ± 0.3*	5.4 ± 0.8	3.5 ± 0.0*
K Time (40%)	4.5 ± 0.8	6.0 ± 0.0*	3.5 ± 0.43	5.3 ± 1.2	3.2 ± 0.3*
Angle (20%)	59.3 ± 4.1	49.6 ± 2.2*	64.0 ± 2.7*	55.5 ± 2.0	62.5 ± 0.0
Angle (40%)	58.6 ± 2.5	52.8 ± 2.6*	65.9 ± 3.7*	65.3 ± 0.8*	66.7 ± 2.5*
MA (20%)	44.6 ± 0.3	46.9 ± 1.4*	52.5 ± 0.6*	38.6 ± 1.5*	48.0 ± 2.1*
MA (40%)	50.3 ± 3.2	55.1 ± 2.1	50.4 ± 2.9	39.0 ± 4.0*	50.2 ± 3.7

*Significantly different from the initial 37°C (p < .05); 37a—initial 37°C; 37b—37°C after cooling; 37c—37°C after heating; N = 4 at both hematocrit levels for every variable at every temperature point.

Table 3. Pathology laboratory control data.

Time	PT (sec)	aPTT (sec)	Fibrinogen (mg/dL)	Platelets (X10E3/mcL)
Beginning	9.6	31.2	206	214
Middle	8.4	28.6	204	190
End	8.4	22.1	204	206

In this study, we examined the effects of hypothermia, hyperthermia, and hemodilution on coagulation by using point-of-care coagulation monitors. We found that hypothermia tended to increase ACTS. This increase might be caused by the reduced enzymatic rate for coagulation proteins during hypothermia, which is in agreement with Cohen et al. (13). In addition to enzyme activity, platelet function may be significantly altered during periods of hypothermia as suggested by Watts and colleagues (8). However, the RES was the only device to illustrate significantly increased ACTS under hypothermic conditions, suggesting that this hypocoagulable state induced by hypothermia might be related to sample size. This study suggests that the prewarming phase to 37°C in microsample (≤ 0.2 mL) coagulation devices may negate the hypothermic effects seen in macrosample (2 mL) devices.

In this study, hyperthermia induced decreased ACTs that may be related to the increased rate of coagulation enzymes at hyperthermic conditions. An *in vitro* study by Rohrer and Natale has also demonstrated decreased PT and aPTT in plasma samples at 39°C and 41°C (16). Another explanation is an increase in fibrin formation, which has been suggested by Pivalizza et al. during whole body hyperthermia (9). In contrast, Strother et al. showed that hyperthermic conditions led to a decrease in platelet count, fibrinogen, and plasminogen levels (17). These discrepancies in findings might be attributable to differences in sampling technique, duration of hyperthermia, and differences in the degree of hyperthermia.

The present study illustrated an increase in ACT upon hemodilution from 40% HCT to 20% HCT. This increase is most likely related to the dilution of clotting factors and the cellular elements involved in coagulation. This explanation is consistent with the findings by Rosberg et al. who showed that hematocrit levels, fibrinogen, platelets, factor V, and factor VIII are proportionally reduced upon hemodilution (18).

Interdevice comparison showed a wide range of SD from mean ACT values. Devices requiring the smallest sample volume reported the greatest SD values (SIG-27%, BRP-23%). However, the devices requiring larger sample volumes demonstrated decreased SD values (ACT II-15%, RES-14%). Although the purpose of this study was not to evaluate coagulation devices, differences between the devices illustrate the various mechanisms for clot detection as defined by the manufacturer. The current trend in modern day point-of-care coagulation monitors is

toward an easy-to-use microsample device. Although these devices offer a reduced volume, the clinician must be conscious of the devices' effects on temperature and the variability of these results. The samples ($n = 8$) per device at every temperature point were performed by the same operator throughout the experimental period to limit reproducible variability among the individual devices.

The TEG CI describes the over-all coagulation state of whole blood and is derived from the R (initial fibrin formation), K (clot rate), MA (maximum clot strength), and α (clot strength) of celite-activated whole blood tracings. Normal values for the CI lie between -3.0 and $+3.0$, which is equivalent to three standard deviations from the mean of zero. Positive values outside this range ($CI > +3.0$) indicate that the sample is hypercoagulable, whereas negative values ($CI < -3.0$) indicate hypocoagulability. The CI supports the hemostatic changes upon hemodilution and hypothermia indicated by the ACT devices utilized in our study. Significant CI increases from baseline were reached upon rewarming from 27°C to 37°C. The 40% HCT samples also supported the ACT findings under hyperthermic conditions.

Although the benefits of isolating temperature and hemodilution variables on human whole blood were significant enough to warrant this study, degradation of the blood because of time was of concern. Stability was demonstrated for the duration of the experiment by the modest reductions in fibrinogen and platelets, as well as limited reductions in PT and PTT (Table 3). Relatively constant ACT control values also indicate that the hemostatic changes observed were, in fact, attributable to experimental manipulation.

The following conclusions can be drawn from this study. First, hemodilution produces a hypocoagulable state independent of the device utilized (RES, SIG, BRP, and ACT II). Second, hypothermia increases ACT in the RES, but not in microsample devices (SIG, BRP, and ACT II). Third, hyperthermia decreases ACT independent of the device and HCT level (RES, SIG, BRP, and ACT II).

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