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# The Effect of Temperature and Aprotinin During Cardiopulmonary Bypass on Three Different Methods of Activated Clotting Time Measurement

David Machin, BSc (Hons), ACP;* Philip Devine, MSc†

**Perfusion Department, Theatres, and †Medical Statistician, Glenfield Hospital, Leicester, United Kingdom*

**Abstract:** The activated clotting time (ACT) is used frequently for monitoring blood anticoagulant response with heparin before, during, and after cardiopulmonary bypass (CPB). Many cardiac procedures involving CPB require reduction of the patient's blood temperature and use of the serine protease inhibitor, aprotinin. Three different methods of ACT measurement were compared to show the effects of different CPB temperatures and the presence of aprotinin. A total of 42 patients were included in the study: 14 received CPB at 28°C, 14 received CPB at 32°C, and 14 normothermic (37°C) CPB. Within each temperature group, seven received aprotinin. The ACT in each group of patients was measured by a celite activator (C-ACT), a kaolin activator (K-ACT), and a celite, kaolin and glass activator (MAX-ACT). All three methods of ACT measurement showed significant increases ($p < .05$) in clotting times at hypothermic CPB compared with normothermic groups. During heparinization the C-ACT was significantly increased ($p < .05$) in the presence of aprotinin. Comparability between the 3 ACT measurement methods showed a very high correlation between C-ACT and K-ACT clotting times ($R^2 = .8962$), and slightly lower correlation between MAX-ACT and C-ACT ($R^2 = .7780$), and MAX-ACT and K-ACT ($R^2 = .7827$). All ACT measurements are affected by changes in blood temperature. The C-ACT measurement is prolonged with aprotinin, whereas the MAX-ACT and K-ACT method of measurement in the presence of aprotinin are not significantly altered. It appears that the MAX-ACT produces lower values and may necessitate additional heparin therapy for ACT target values considered safe during CPB. Further study is required from these additional findings. **Keywords:** temperature, MAX-ACT, aprotinin, cardiopulmonary bypass. *JECT. 2005;37:265–271*

During cardiopulmonary bypass (CPB), the extracorporeal circuit exposes circulating blood to an extensive additional surface area that is devoid of endothelium. The natural anticoagulant properties of the vascular endothelium are therefore not sufficient to prevent the hemostatic balance from being upset, with the potential for massive stimulation of coagulation activation and clot formation as blood flows over the nonbiological surface. Unfractionated heparin is given intravenously before CPB in a sufficient dose of typically 300–400 IU/kg to protect the extracorporeal circuit from extensive clot formation (1).

Aprotinin, a naturally occurring serine protease inhibitor derived from bovine lung, often is used during CPB because of its anti-inflammatory roles and proven efficacy in reducing perioperative bleeding and blood product transfusions in cardiac patients (2–4). The mechanisms for improving hemostasis are not fully understood but include inhibition of the serine proteases trypsin, plasmin, and kallikrein and various protective actions on platelet function (5,6). Formation of reversible aprotinin–proteinase complexes at the active serine site of the enzyme attenuates coagulatory contact activation, thrombin generation, and fibrinolysis (7,8). Aprotinin potency is expressed in kallikrein inactivator units (KIU) and is either administered as a high-dose or low-dose regimen, to give average plasma concentrations of 250 KIU and 137 KIU, respectively (calculated for a patient with 6 L of blood volume). High-dose regimen consists of a $2 \times 10^6$ KIU loading dose, $2 \times 10^6$ KIU into the CPB circuit, and $5 \times 10^5$ KIU per hour continuous infusion during cardiac surgery (9). The low-dose regimen contains a $1 \times 10^6$ KIU loading dose, $1 \times 10^6$ KIU in the CPB circuit, and $2.5 \times 10^5$ KIU per hour continuous infusion (10).

Activated clotting time (ACT) tests achieved major recognition in CPB during the late 1970s, where many studies were undertaken to determine ACT values during CPB. It was concluded that an ACT of more than 480 seconds provided a safe level of anticoagulation regardless of individual differences in response (11). The theoretical basis of the ACT test is to saturate the blood sample with a

Address correspondence to: David Machin, BSc (Hons), ACP, Perfusion Department, Theatres, Glenfield Hospital, Groby Road, Leicester LE3 9QP, United Kingdom. E-mail: machin_david36@hotmail.com
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particulate activator to ensure Factor XII is converted to Factor XIIa. Particulate activators are negatively charged particles and are commercially available as celite and kaolin for measuring high heparin levels (11,12) and glass beads, which are more sensitive to low heparin concentrations (13).

During this study, anticoagulation in heparinized CPB patients was monitored by ACT values obtained from the automated ACT technique using patient blood samples contained in celite activator ACT test tubes (C-ACT; International Technidyne, Edison, NJ), which are routinely used in our institute. ACT values also were measured by two other types of particulate activator ACT test tubes; a kaolin activator (K-ACT; International Technidyne), and a tube containing three activators: kaolin, celite, and glass beads (MAX-ACT; Array Medical, Kent, United Kingdom). Many procedures during CPB involve a reduction in the patient's core temperature and the administration of aprotinin. During this study, the C-ACT, K-ACT, and MAX-ACT were performed simultaneously on the same patients before, during, and after CPB. The effect of temperature and the presence or absence of low-dose aprotinin on the three different methods of ACT measurement were compared. The purpose of this study was to determine the most applicable ACT test tube activator for use in CPB.

## MATERIALS AND METHODS

### Study Design

After obtaining institutional review board approval, 42 patients undergoing CPB for elective cardiac surgery were chosen for participation in this study. Patients included were those receiving coronary artery bypass grafts with or without concomitant heart valve replacement and a presurgical hematocrit greater than 24%. Patients with known coagulopathies, renal disease, thrombocytopenia, metabolic disorders, and any medication known to effect the clotting cascade (for example, warfarin and heparin), were excluded from the investigation. From the 42 patients investigated, 14 received hypothermic CPB at 28°C (nasopharyngeal temperature), 14 received mild hypothermic CPB at 32°C, and the remainder had normothermic CPB. Within each specific CPB temperature group, seven patients received aprotinin that was administered as a low dose regimen. Thus, a total of six patient groups were involved in the study.

All patients were anesthetized following the standard regime of fentanyl, etomidate, and pancuronium, with supplemental inhalation of isoflurane as necessary. CPB was performed using an adult CPB circuit constructed for each patient, consisting of a roller pump, hollow fiber membrane oxygenator, arterial filter, closed venous reservoir, cardiotomy reservoir, and polyvinyl chloride tubing (Lifestream International, West Sussex, United Kingdom). The CPB circuit was primed with 1400 mL of priming solution consisting of 700 mL of plasmalyte-A solution, 500 mL of gelofusine, 200 mL of 10% mannitol, 40 mL of 8.4% sodium bicarbonate, and 5000 IU of porcine intestinal heparin. The patient's blood was anticoagulated initially with 300 IU/kg of porcine intestinal heparin given intravenously and additional quantities were administered if required, to maintain a C-ACT greater than 480 seconds using a Hemochron 401 ACT machine (International Technidyne). After termination of CPB, heparinization was reversed by protamine sulfate and verified by an ACT comparable to pre-heparinization.

### Sampling and Measurement

ACT measurements on each patient were taken using celite activator ACT tubes (C-ACT); kaolin activator ACT tubes (K-ACT); and ACT tubes containing three activators: celite, kaolin, and glass (MAX-ACT). All ACT tubes were pre-warmed to 37°C before ACT measurement. Three Hemochron 401 ACT machines were used for the three different ACT tubes to enable simultaneous ACT measurements on each patient. Quality controls (QCs) were performed daily on the Hemochron ACT machines using Electronic System Verification (ESV) and control plasma QCs on each batch of ACT tubes.

ACTs of patients were measured from the radial artery catheter after anesthetic induction (Pre-Heparin); 2 minutes after aprotinin administration (aprotinin groups only); 2 minutes after heparin administration (Post-Heparin); 2 minutes after initiation of CPB (On-CPB); at specific CPB temperature (At Temp); initiation of rewarming in 28°C and 32°C groups (Rewarm); at the end of CPB (End-CPB); and 2 minutes after protamine administration (Post-Protamine).

During each ACT measurement the patient's nasopharyngeal and venous temperatures were recorded, time taken to cool, maintain at required temperature, rewarm, and perform CPB. In addition, the patient's demographic data, platelet count, fibrinogen level, hematocrit, urine output, and heparin, blood product and protamine usage were recorded.

### Statistics

All numerical data was expressed as mean ± standard deviation of the mean. The ACT values measured in the different CPB temperature groups were compared using the one-way analysis of variance and Duncan's post-hoc multiple range test. Comparison of ACT between the various sample time intervals in each CPB temperature group was performed using the paired Student *t* test. The ACT values measured in patients receiving aprotinin and those not receiving aprotinin were analyzed using the independent *t* test. The relationship of ACT between the three

different methods of ACT measurement were compared using correlation analysis. All statistical analysis was performed using the SPSS 8.0 software (SPSS Inc., Chicago, IL), where a $p$ value of less than .05 was considered significant.

## RESULTS

The age (43–79 years) and weight (53–108 kg) of patients were comparable in each study group; however, the number of male ($n$ = 31) to female ($n$ = 11) participants was considerably greater. Total heparin, protamine administration and urine output also were similar. With reference to the CPB timed events in each group, the period of cooling and rewarming were similar, with CPB times between 67 ± 16 minutes (normothermic CPB non-aprotinin group) and 109 ± 39 minutes (28°C CPB aprotinin group). The hematocrit, platelet counts, and fibrinogen levels were comparable between the patient groups and dropped consistently as would be expected.

### The Effect of Temperature

The baseline (pre-heparin) ACT with the three different methods were similar in the patient groups, except for the MAX-ACT non-aprotinin (Figure 3B) temperature group ($p < .05$ between 28°C and 37°C groups). Upon patient heparinization, there was some insignificant variation in ACT among the six groups of patients (Figures 1–3).

On initiation of CPB an overall increase in ACT with the three different ACT tubes was observed in the hypothermic CPB temperature groups. A significant difference was observed ($p < .05$) in the non-aprotinin groups between 28°C and 37°C with all ACT tubes (Figures 1B, 2B, and 3B), and 32°C and 37°C using K-ACT and MAX-ACT (Figures 2B and 3B). Comparison of the 'On-CPB' ACT with the 'Post-Heparin' ACT showed significant increases in ACT ($p < .05$) with all ACT methods in the aprotinin and non-aprotinin 28°C temperature groups (Figures 1–3), and the K-ACT and MAX-ACT 32°C non-aprotinin groups (Figures 2B and 3B).

When reaching the required CPB temperature, the ACT was increased during hypothermia with the three different ACT tubes ($p < .05$ between 28°C and 37°C CPB aprotinin groups using all ACT tubes, and the 32°C and 37°C non-aprotinin group with K-ACT). Generally, the lower the CPB temperature, the higher the ACT reading (Figures 1–3), except for the MAX-ACT without aprotinin (Figure 3B) and the K-ACT aprotinin group (Figure 2A). However, in these two groups the increased ACT readings at 32°C compared to 28°C were insignificant in comparison to the opposite and more profound effect seen in other patient groups.

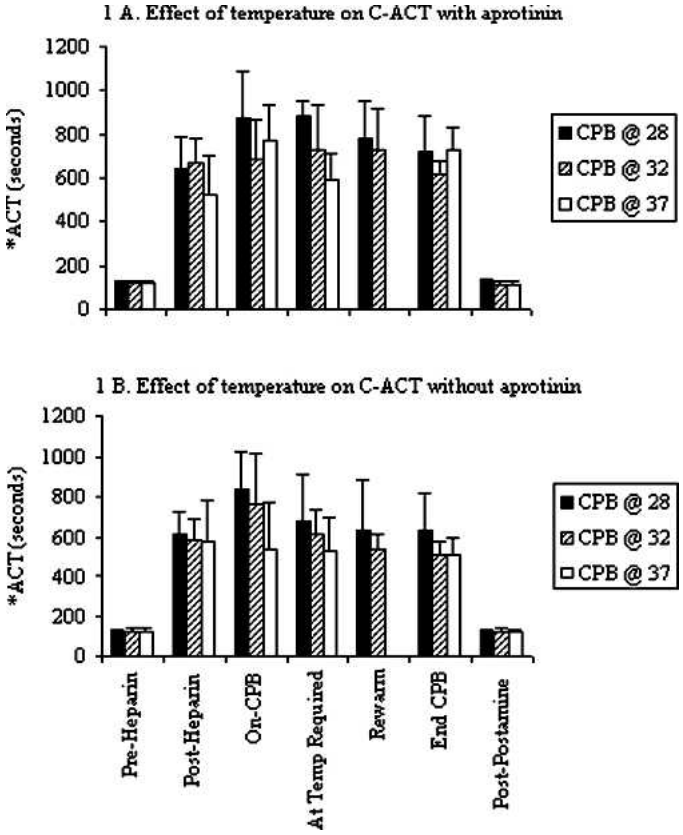


**Figure 1.** The effect on the ACT of different temperatures during CPB using the celite activator (C-ACT) with and without aprotinin administration. *Values shown as mean with error bars representing standard deviation from the mean.

Comparison of the 'On-CPB' ACT with that measured 'At Temp Required' in the same patient groups (Figures 1–3), presented a decrease in many groups ($p < .05$ shown by all types of ACT tubes in 28°C non-aprotinin groups, and normothermic aprotinin group by the C-ACT). Overall, ACT's measured by the various ACT tubes showed an increase in 28°C temperature groups in comparison to 32°C groups.

Hypothermic CPB groups re-warmed to 37°C generally showed higher ACT readings with all ACT tube methods in comparison to normothermic groups (Figures 1–3); suggesting hypothermia still has an effect on the ACT particulate activators, regardless of the patient being re-warmed to 37°C. In addition, CPB at 28°C produced higher ACT values compared to those at 32°C. However, measurement by the C-ACT (Figure 1A) showed an ACT increase in normothermic aprotinin groups.

After heparin reversal with protamine, the ACT values using all ACT tubes were similar between the various temperature groups, except for the C-ACT aprotinin group (Figure 1A) where a significant increase in ACT of patients who underwent CPB at 28°C compared to normothermic CPB group ($p < .05$) was seen.

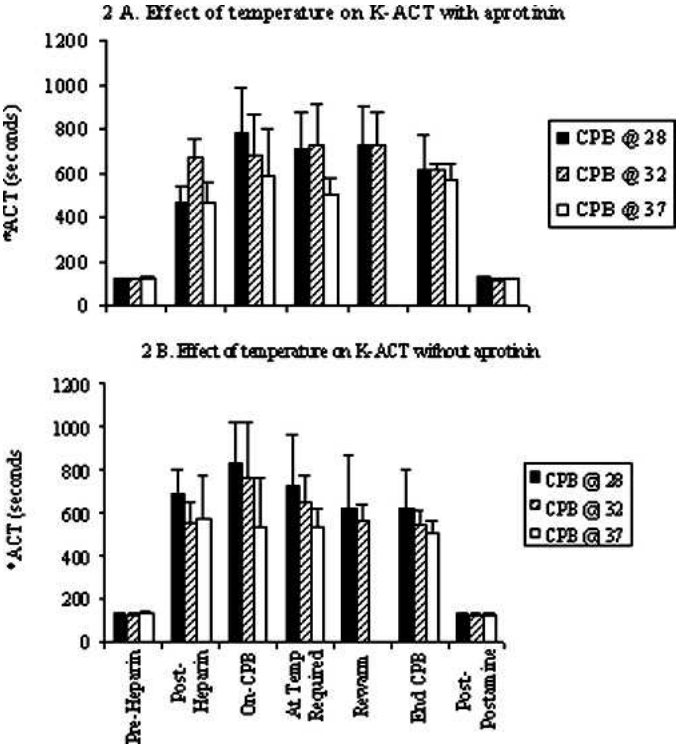


**Figure 2.** The effect on the ACT of different temperatures during CPB using the kaolin activator (K-ACT) with and without aprotinin administration. *Values shown as mean with error bars representing standard deviation from the mean.

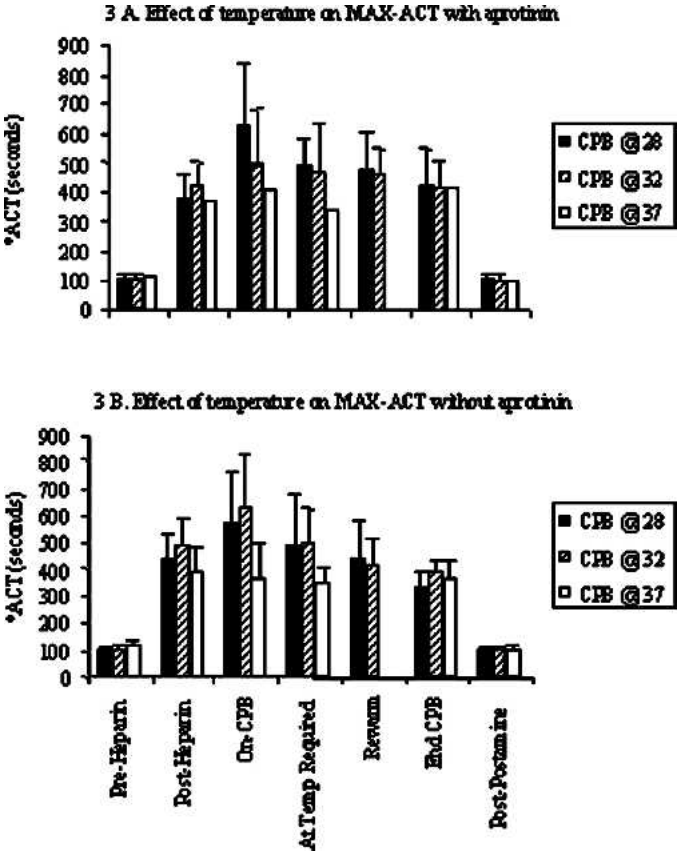


**Figure 3.** The effect on the ACT of different temperatures during CPB using the celite, kaolin, and glass activators (MAX-ACT) with and without aprotinin administration. *Values shown as mean with error bars representing standard deviation from the mean.

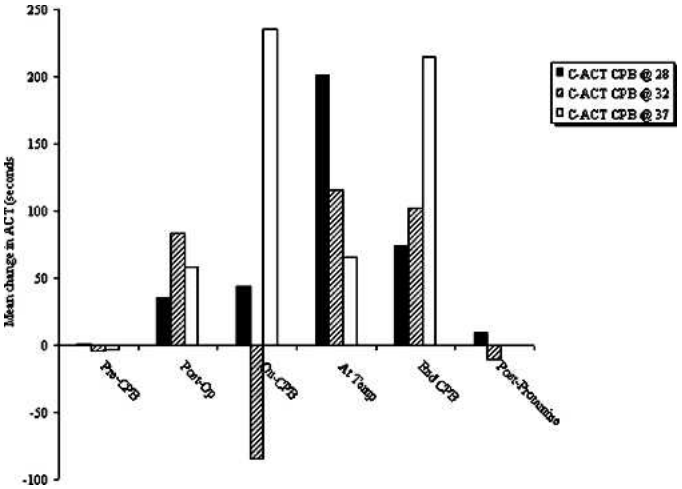


**Figure 4.** Change in ACT using the celite activator (C-ACT) with aprotinin administration in the different CPB temperature groups.

### The Effect of Aprotinin

Aprotinin administration before heparinization did not significantly affect the various ACT methods (Figures 4–6). After heparinization and during CPB, C-ACT readings generally were elevated in the presence of aprotinin (Figure 4). C-ACT was significantly elevated ($p < .05$) during the 'On-CPB' sampling period in normothermic groups (773 ± 161 s) compared with the same temperature non-aprotinin groups (538 ± 234 s). Aprotinin significantly increased the C-ACT by 202 seconds ($p < .05$) in the 28°C CPB group 'At Temp Required' sampling interval, and the 'End- CPB' interval in the 32°C (increase of 102 s) and normothermic groups (increased by 214 s).

K-ACT and MAX-ACT values were not prolonged in the presence of aprotinin after the administration of heparin (Figures 5 and 6). However, there was a significant drop ($p < .05$) in ACT between the K-ACT non-aprotinin (684 ± 105 s) and aprotinin 28°C temperature groups (471 ± 72 s). During CPB and after heparin reversal, aprotinin showed no significant effect in either the K-ACT or MAX-ACT measurements (Figures 5 and 6).

### Additional Findings

The blood clotting times measured by the MAX-ACT were consistently lower than those obtained by the K-ACT and C-ACT. In the absence of aprotinin, the C-ACT and K-ACT clotting times were very highly correlated ($R^2 = .8962$). Whereas a slightly weaker correlation was shown between the MAX-ACT and K-ACT values ($R^2 = .7827$), and the MAX-ACT and C-ACT values ($R^2 =$

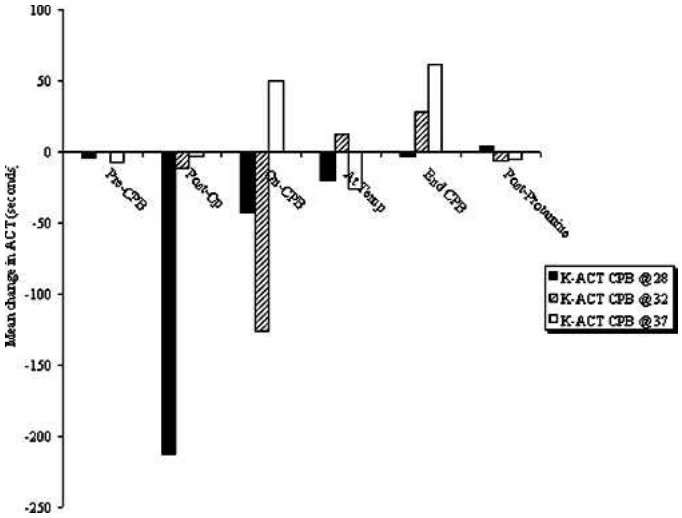


**Figure 5.** Change in ACT using the kaolin activator (K-ACT) with aprotinin administration in the different CPB temperature groups.

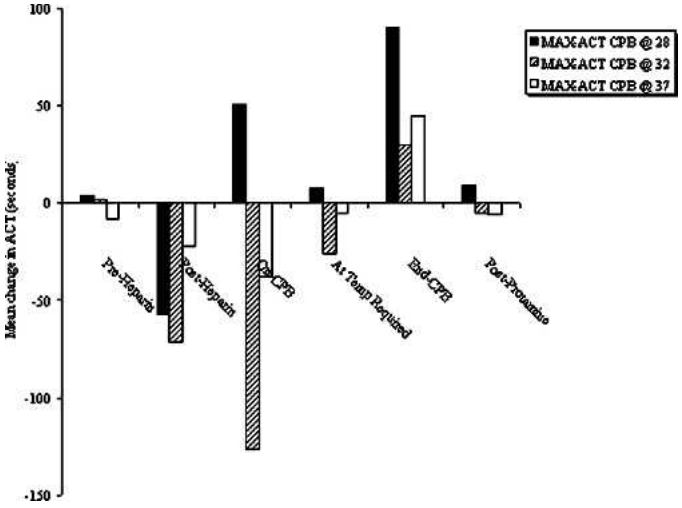


**Figure 6.** Change in ACT using the celite, kaolin, and glass activator (MAX-ACT) with aprotinin administration in the different CPB temperature groups.

.7780). Thus, the C-ACT and K-ACT clotting times in the absence of aprotinin yielded a strong relationship and seemed highly comparable.

## DISCUSSION

Hypothermic CPB has an effect on prolonging the ACT when using the C-ACT, K-ACT, or MAX-ACT with or without the use of aprotinin. It can be seen that the more profound the hypothermia, the greater the ACT measurement using all types of ACT tubes. These findings seem to agree with those by Tian and co-workers (14), who made a comparison between the same patient group during hypothermic CPB and when rewarmed.

The increase in ACT after the commencement of CPB is most probably the result of the hemodilution effect from the CPB circuit prime. This phenomenon was demonstrated by the concurrent drop in measured hematocrit, platelet, and fibrinogen levels, which also are influenced by the extent of activation and deposition of blood products within the CPB circuit (15,16). The decrease in ACT with CPB progression in the same patient groups is presumably attributed to the continual degradation of heparin and fluid loss from the vascular circulation. Thus, concentrating clotting factors and increasing blood clotting capability as demonstrated by the gradual increase in hematocrit, platelet count and fibrinogen levels throughout CPB.

Theoretically, the hypothermic effect on ACT could be attributed to a number of factors. Hypothermia induces reversible platelet membrane dysfunction, partially inhibits platelet aggregation, and reduces platelet count and function rate (17–19). Disordered fibrinolytic cascade activity has been observed in patients undergoing hypothermic CPB, thus reducing the blood clotting process (19). Decreasing blood temperature most probably reduces the activity rate of coagulant enzymic systems (20). Anticoagulation substances also may be released during hypothermia, which specifically inhibit factor Xa (21). It also seems reasonable to accept that hemodilution contributes to prolonged ACTs. This effect was demonstrated by the addition of fibrinogen into blood samples diluted with 75% saline, which did not reduce the ACT significantly (22).

The observed ACT decrease when patients were rewarmed to 37°C could be attributed to a more efficient activation of coagulation enzymes at 37°C, the hemoconcentration effect as CPB progresses, and/or the elimination of heparin over the course of time. Cold platelets also have demonstrated increased aggregation during warming of the blood (23). Alternatively, hypothermic CPB may reduce the recovery rate of platelet function (18) and could explain increased ACTs in the re-warmed heparinized patients who underwent hypothermic CPB.

The administration of the protease inhibitor drug, aprotinin, prolongs the C-ACT (celite activator) measurement of heparinized blood but does not seem to affect the K-ACT (kaolin activator) or MAX-ACT (glass, celite, and kaolin activator) measurement. This occurs during both hypothermic and normothermic CPB, but is only noticeable in heparinized blood, suggesting that aprotinin does not produce an anticoagulant effect itself. These findings agree with those presented by de Smet et al., who showed an increase in ACT of aprotinin treated patients using the celite activator, and Wang et al., who found no increase in K-ACT and C-ACT in un-heparinized blood containing aprotinin (12,24). Jones and co-workers have postulated that values for kaolin activator tests carried out in-vitro in the presence of high aprotinin concentrations (300 KIU/

mL) are significantly elevated, even in unheparinized aprotinin samples using glass and celite. Interestingly, however, the authors also showed that MAX-ACT is not effected by aprotinin (25). Investigators have suggested that celite ACT values should be maintained more than 750 seconds when aprotinin is used during CPB (26). This is further validated in this study, C-ACT aprotinin groups in the range of 700 s have compared with C-ACT values considered safely anticoagulated in the non-aprotinin groups.

The action of aprotinin on the celite activator resulting in prolongation of the ACT is not fully understood and may be the result of the different structure of kaolin (hydrated aluminium silicate) compared with celite (diatomaceous earth). Both activate factor XII, but kaolin is thought to additionally act on factor XI (27). Thus, the proposed inhibitory effect of aprotinin on factor XII will not affect K-ACT and MAX-ACT values because activation of factor XI can still proceed. It also has been suggested that aprotinin binds to the celite, resulting in prolonged clotting times (28). Conversely, kaolin may bind to aprotinin and so cannot inhibit contact pathway activation (29). As a consequence, the kaolin ACT represents the clinical anticoagulant effect of heparin but not aprotinin. This implies that no prolongation of the celite ACT occurs because it demonstrates the action of aprotinin on factor XII inhibition.

The lower ACT values attained by the MAX-ACT may be the result of the presence of three different activators that could provide optimum factor XII activation. Individuals can differ in their factor XII protein (30), and the use of a single activator may not ensure effective factor XII activation, thereby prolonging ACT. The design of the MAX-ACT tube may allow earlier blood clot detection because a smaller 'gap distance' exists between the magnet and blood clot adhesion post (spindle), enabling the clot to concentrate in a smaller area. If the MAX-ACT values are in agreement with those described by Bull (11), many patients are at levels less than the recommended ACT for CPB. Hence, the patient loading dose will need to be re-evaluated. Lastly, low MAX-ACT readings may reflect increased fibrinolytic and coagulant activity, as indicated by high levels of D-dimers and fibrin during inadequate CPB anticoagulation (31,32).

Whether the C-ACT value in the presence of aprotinin gives an exaggerated prolongation of the ACT, or a true representation of the drug's action on blood coagulation appears to remain controversial. The shorter MAX-ACT values have prompted the need for further study of this method. However, the MAX-ACT may provide a universal method of ACT measurement as a result of the "cocktail: of activators, where its application appears acceptable for high heparin level situations such as CPB with or without aprotinin. Use of the MAX-ACT in situations of low heparin concentrations such as hemodialysis and extracorporeal membrane oxygenation (ie, ECMO) need to be investigated. Overall, factors such as temperature, protease inhibitors, hemodilution, and the type of particulate activator should be considered when interpreting ACT results.

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