

Original Article

Does Hyperfibrinolytic Activity Occur During Cardiopulmonary Bypass From Blood Exposed to Pleural Surfaces?

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ABSTRACT

During cardiac surgery with cardiopulmonary bypass (CPB), fibrinolytic activity may be stimulated when blood exposed to pleural surfaces is suctioned into the extracorporeal circuit (ECC). The purpose of this study was to determine the effect of reinfused blood exposed to pleural surfaces on systemic fibrinolytic activity. Following Institutional Animal Care Utilization Committee approval, 120 ml of blood was drawn from the femoral artery of 4 pigs and placed in both pleural cavities, where it remained for 120 min during CPB. After this time, the exposed blood was suctioned back into the ECC. Blood samples were drawn at the following times: 40 min prior to median sternotomy, 30 and 90 min during CPB, and 30 min post-suction. Tests performed on the samples included thromboelastography (TEG), D-dimer (DD), fibrin degradation products (FDP), fibrinogen concentration, activated clotting time (ACT), hematocrit, and platelet count. TEG index decreased significantly in the circuit following suction (5.28 ± 0.45 vs. 0.98 ± 1.86 , $p < 0.0007$), while fibrinolytic activity increased ($6.25 \pm 1.50\%$) in the ECC when compared to pleural blood ($2.17 \pm 1.04\%$, $p < 0.01$). The DD and FDP were both elevated in the systemic circulation following suction of the pleural blood, although statistical significance was not achieved. The ACT was significantly elevated in the pleural fluid during CPB (707 ± 213) compared with the ECC (378 ± 32 , $p < .003$), which may indicate an accelerated consumption of coagulation factors. In conclusion, blood exposed to pleural surfaces may have increased fibrinolytic activity, but systemic hyperfibrinolysis was not seen.

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INTRODUCTION

Postoperative hemorrhage following cardiac surgery with cardiopulmonary bypass (CPB) continues to be one of the most important contributors to patient morbidity and mortality despite increased efforts to reduce hemostatic alterations and blood loss. It is well documented that CPB impairs hemostatic mechanisms (1) and stimulates the fibrinolytic system (2), leading to inadequate clot formation and uncontrollable bleeding requiring homologous blood transfusion (3). Current methods used by clinicians to reduce postoperative bleeding and blood transfusion include heparin-coated circuitry (4), anti-fibrinolytic pharmacological agents (5), autologous plasmapheresis (6,7), and autotransfusion with or without cell-saving techniques (8).

Intraoperative whole blood autotransfusion is commonly used in cardiac surgery, as blood collected in the thoracic cavity is suctioned into the extracorporeal circuit (ECC) in an effort to reduce blood loss during the procedure. The pericardial and pleural surfaces contain fibrinolytic activators that induce fibrinolysis in blood that is collected in these cavities (9-12). The introduction of these activators and by-products of fibrinolysis into the systemic circulation may have an effect on systemic hyperfibrinolytic activity during CPB (13-17), although controversy exists as to the degree of stimulation and its clinical importance (8,18,19).

The purpose of this study was to determine the contribution of blood exposed to pleural surfaces to hyperfibrinolytic activity in cardiac surgery. This study will provide a foundation on which clinicians can better evaluate the usage of intraoperative whole blood autotransfusion as a safe and beneficial means of blood conservation during cardiac surgery.

MATERIALS AND METHODS

ANIMAL PROTOCOL

This research protocol was approved by the Institutional Animal Care Utilization Committee at the University of Nebraska Medical Center. All animals used in this study received humane care in compliance with the Guide for the Care and Use of Laboratory Animals as published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985). A total of 4 porcine models (40-50 kg) were used as experimental subjects in this study.

All procedures were conducted as acute studies with the animal anesthetized with ketamine (20 mg/kg) and xylazine (2 mg/kg) given intramuscularly. Once adequate anesthesia was obtained, the animal was intubated with a 6.5 F endotracheal tube, and ventilated with a tidal volume of 20-30 ml/kg body weight at a rate of 15-20 breaths per minute. Electrocardiogram leads were placed and the heart rate continuously monitored. The femoral artery and vein were cannulated with 14 gauge needles for hemodynamic monitoring, medication infusion, and blood sampling. Once these access lines had been established, the ani-

mal was given intermittent doses of pentothal to maintain anesthesia. A median sternotomy was performed and the great vessels dissected free in preparation for cannulation. Prior to cannulation, the animal received a bolus dose of 300 IU/kg of bovine lung heparin and adequate anticoagulation was assured by maintaining an activated clotting time (ACT) of greater than 480 sec. One purse string suture was placed in the aorta for arterial cannulation, and a second was placed in the right atrial appendage to prepare for venous return.

At the termination of each experiment, the animal was euthanized by the concurrent administration of barbiturate and potassium chloride directly into the aortic root.

CARDIOPULMONARY BYPASS

The CPB circuit consisted of a hollow fiber membrane oxygenator^a, a cardiotomy/venous reservoir^b, an arterial line filter^c, polyvinyl chloride tubing, and a twin head positive displacement roller pump. The circuit was primed with 1200 ml of a balanced electrolyte solution, 50 ml of 8.4% sodium bicarbonate (50 mEq), and 3000 IU of bovine lung heparin.

Initial heparinization was accomplished with 300 IU/kg of bovine lung heparin and supplemented as needed to maintain an ACT of 480 sec or greater. Moderate hypothermic CPB was conducted with the perfusate being allowed to drift downward and then maintained at 32°C. Alpha stat blood gas management was employed throughout the CPB procedure, and lab values were maintained within normal physiologic ranges of pH (7.35-7.45), pCO₂ (35-45 mmHg), pO₂ (100-200 mmHg during CPB), and base excess (0 ± 2). Venous saturations were maintained at a value greater than 75%. The mean arterial blood pressure was maintained between 50 and 80 mmHg with the use of either phenylephrine or isoflurane as necessary. After 120 min of CPB, the subjects were actively warmed to a core temperature of 37°C.

EXPERIMENTAL PROTOCOL

1. Pre-median sternotomy: A 15 ml sample of blood was drawn into a syringe from the femoral artery catheter and injected into the appropriate tubes for laboratory evaluation of baseline systemic fibrinolytic activity (description of tests to follow).

2. Post-median sternotomy: Two milliliters of fluid was drawn from the pericardial space and mixed with 6 ml of blood from the femoral artery, and evaluated for baseline pericardial fibrinolytic activity. As a control, 2 ml of normal saline was mixed with 6 ml of blood from the femoral artery and evaluated for fibrinolytic activity as well.

3. Pre-cardiopulmonary bypass: 120 ml of blood was drawn from the femoral artery into a heparin-coated syringe, and placed in the pleural cavities (60 ml each). The blood was allowed to pool in these cavities for 120 min.

a Univox Gold, Bentley Laboratories, Baxter Healthcare, Irvine, CA
 b HSVR-CR, Bentley Laboratories, Baxter Healthcare, Irvine, CA
 c ALF-40, Bentley Laboratories, Baxter Healthcare, Irvine, CA

4. Cardiopulmonary bypass procedure: Two samples of 15 ml each were drawn from the pleural cavities during the CPB procedure (30 and 90 min), to determine fibrinolytic activity. Two samples of 15 ml each were also drawn from the ECC at these times and evaluated for systemic fibrinolytic activity. After 120 min of CPB, the blood from the pleural cavities was returned to the ECC via cardiotomy suction. After 150 min of CPB (30 min post infusion of pleural blood into the CPB circuit), one 15 ml sample of blood was drawn from the circuit to determine the extent of systemic fibrinolytic activity.

LABORATORY EVALUATION

A panel of 4 tests was used to determine fibrinolytic activity: fibrinogen concentration (FIB), fibrin degradation product concentration (FDP), D-dimer concentration (DD), and thromboelastography (TEG). Platelet count (PLT) was also tested with the fibrinolytic indicators. Hematocrit (HCT) and ACT were monitored throughout this experiment to ensure adequate anticoagulation and oxygen carrying capacity (Figure 1).

STATISTICAL ANALYSIS

Parametric data was analyzed using one-way analysis of variance. Fisher's least significant difference was used when significant ratios were reached. Nonparametric data were analyzed using the chi square test. Statistical significance was accepted at the $p < 0.05$ level. All data is presented as mean \pm standard deviation.

RESULTS

There was a significant reduction in FIB observed across all sample points when com-

pared to baseline ($p < 0.0001$), and FIB was significantly higher in the pleural cavity at 30 min as compared with the circuit at 30 min ($p < 0.02$), and following suction of the pleural blood ($p < 0.02$) (Figure 2).

The ACT was significantly elevated in the pleural fluid after 30 min of CPB compared with the ECC at the same time ($p < 0.003$), but decreased significantly in the pleural cavities at 90 minutes of CPB ($p < 0.02$), and in the ECC after suctioning the pleural blood ($p < 0.008$) (Figure 3).

Figure 1: Sampling timeline

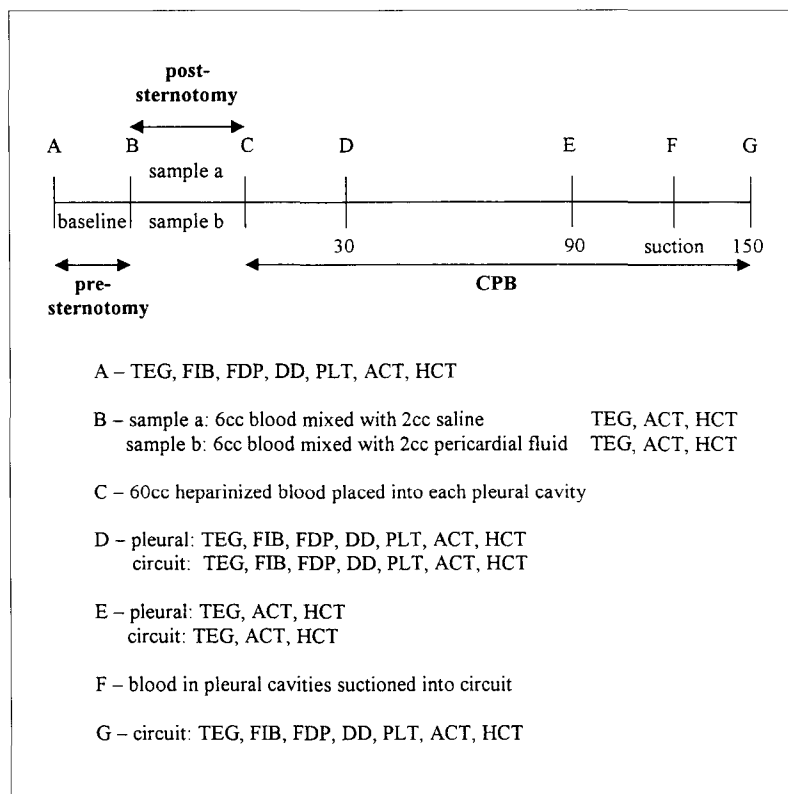


Figure 2: Fibrinogen concentration

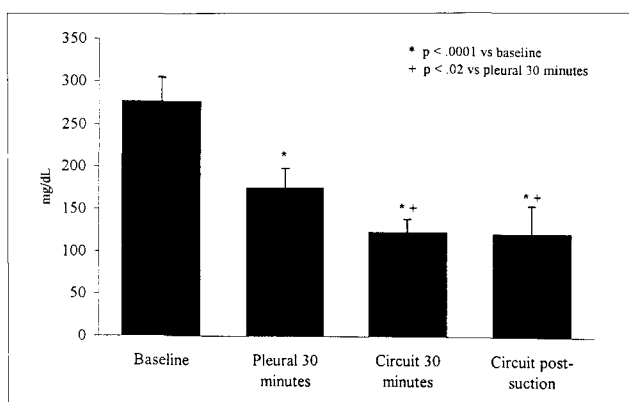
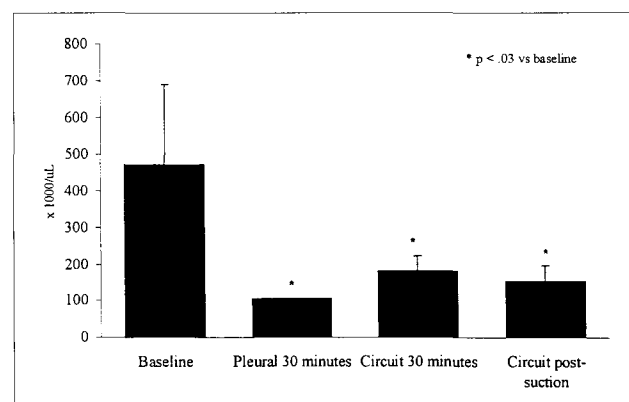


Figure 3: Platelet count



A significant reduction in PLT was observed across all sample points compared with baseline ($p < 0.03$) (Figure 4). However, it is important to note that only one data point was obtained from the pleural cavity at 30 min due to clotting of the specimens.

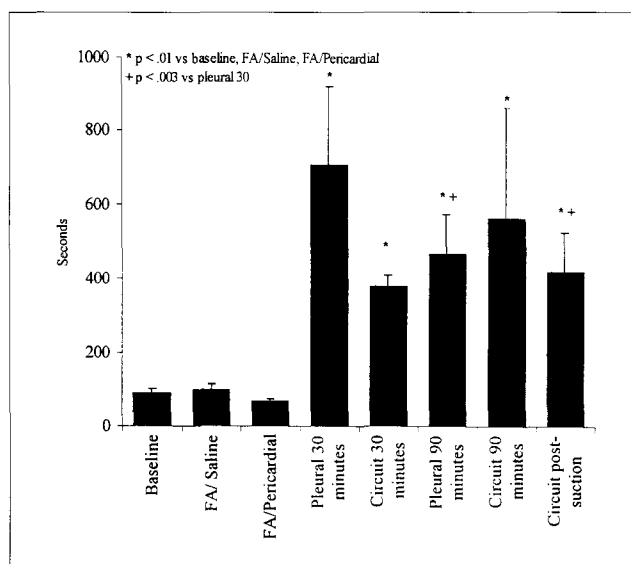
The TEG index is a quantitative summary of all the variables measured by the TEG, with a higher TEG index indicative of increased clotting activity. The TEG index decreased significantly in the ECC from 30 min as compared with the 90 min time point ($p < 0.02$), and in the ECC post-suction ($p < 0.0007$) (Figure 5).

Fibrinolytic activity as measured by TEG was significantly higher in the ECC after 30 min of CPB ($p < .04$) and in the ECC post-suction ($p < .01$) when compared with pleural blood (Figure 6).

The FDP and DD were both elevated in the pleural cavities, although statistical significance was not achieved. Both were also elevated in the systemic circulation following suction of the pleural blood although the difference was not statistically significant (Figures 7 and 8).

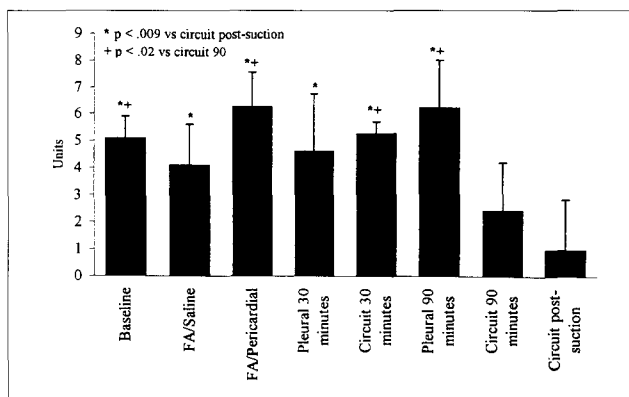
There was a significant decrease ($p < 0.03$) in HCT upon

Figure 4: Activated clotting time



FA = femoral artery

Figure 5: Thrombelastograph index



FA = femoral artery

Figure 6: Percent fibrinolysis at 30 min as assessed by thrombelastograph

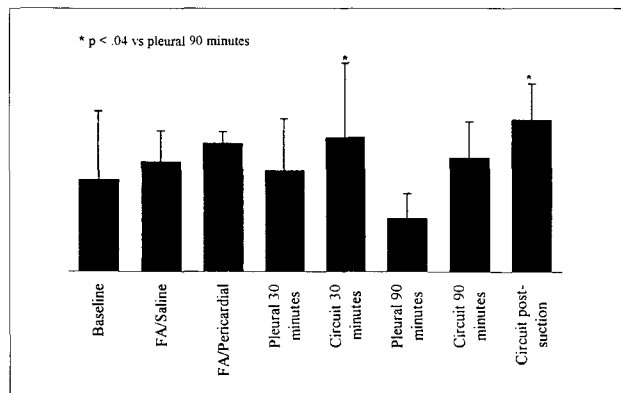


Figure 7: Fibrin degradation product concentration

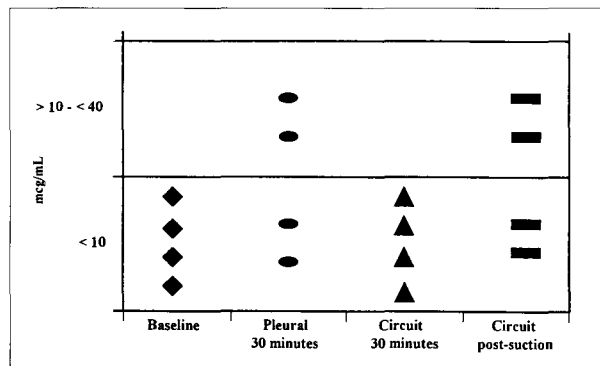
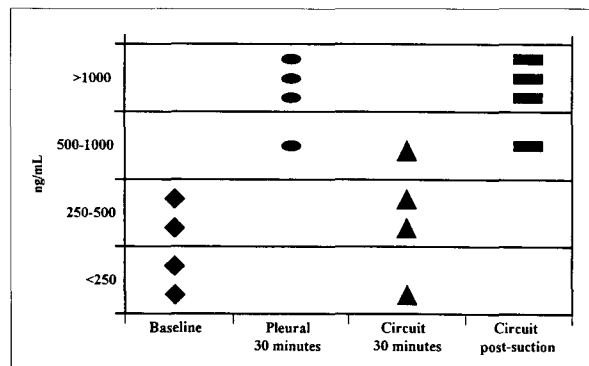


Figure 8: D-dimer concentration



initiation of CPB. The average amount of blood suctioned into the ECC from the pleural cavities was 305 ± 155 ml.

DISCUSSION

Postoperative blood loss is a common complication following cardiac surgery with CPB. The use of CPB involves hemodilution, hypothermia, and activation of the clotting cascade by the ECC. All of these factors induce a host of hemostatic responses that result in an increased potential for bleeding postoperatively. These responses include, but certainly are not limited to: dilutional coagulopathies, qualitative and/or quantitative platelet and coagulation disorders, and excessive fibrinolytic activity. Activation of the clotting system through the intrinsic pathway due to contact activation has been an issue throughout the development of the ECC (4). However, even with improvements in circuit biocompatibility, postoperative blood loss remains a problem (20). Perhaps we need to consider an alternate route of blood activation during CPB, such as excessive fibrinolysis caused by activators other than artificial surfaces.

FIBRINOLYSIS

There are two primary components of the fibrinolytic pathway, plasminogen and plasmin. Plasminogen, a cleaving enzyme, is the circulating precursor of plasmin. Once plasminogen is activated, it is cleaved from a single chained molecule to a two-chained molecule called plasmin, a proteolytic enzyme that binds fibrin, resulting in solubilization of clot (7,21).

As stated previously, plasminogen must first be activated in order to form plasmin and initiate the fibrinolytic pathway. Plasminogen is activated by factor XIIa, prekallikrein, high molecular weight kininogen, exogenous factors, and tissue type plasminogen activator (t-PA) (21). The endothelium produces t-PA, which is the fibrinolytic activator primarily focused upon in this study, as its activity is abundant in the pericardial and pleural mesothelial tissues (9-12). The resultant products of fibrinolysis are called fibrin degradation or split products. There are 5 primary degradation products: fragments X, D, E, Y, and DD. Further utilization of anticoagulation occurs due to the ability of FDP to bind platelet surface membrane receptors, resulting in platelet inhibition (21).

FIBRINOLYTIC NATURE OF PERICARDIUM AND PLEURAL SEROSA

Many studies have been performed to determine the hemostatic nature of mesothelial tissues such as the pericardium and pleural serosa. A number of researchers have identified strong clotting and fibrinolytic systems that are activated by tissue factor and t-PA in human and animal mesothelial tissues (9-13,22). The sample of blood mixed with pericardial fluid in this study did not demonstrate greater clotting activity or fibrinolytic activity as measured by TEG when compared with the control sample mixed with saline. There was also no significant difference ob-

served between these 2 samples in ACT values. However, we did note a trend toward increased clotting and fibrinolytic activity from the baseline femoral artery sample to the pericardial fluid sample.

Studies have shown that tissue factor derived from mesothelial surfaces stimulates thrombin generation more strongly through the extrinsic clotting pathway than does the ECC through the intrinsic pathway (23,24). In our study, FIB was significantly greater in the pleural cavities than in the ECC, although the difference was probably due to hemodilution rather than increased clot formation. The blood in the pleural cavities had been drawn directly from the femoral artery prior to the initiation of CPB, therefore the dilutional factor was not as great in this sample. The ACT values were significantly higher in the pleural cavities at 30 min than in the ECC at this time point. There was no significant difference in TEG index values between the pleural cavities and ECC at the 30 min sample point. However, after 90 min of CPB, the pleural TEG index was significantly greater than in the ECC. Our results indicate that the ECC generates clotting activity more so than the pleural cavity immediately upon the initiation of CPB. However, over time, thrombin generation is stimulated to a greater extent in the pleural cavity than in the ECC.

A number of researchers have demonstrated fulminant fibrinolysis in fluids of the pericardial and pleural cavities due to the activity of t-PA and urokinase (11,13,25). Along with the identification of these activators, high concentrations of FDP and DD were observed in pleural and mediastinal drainage samples, indicating active fibrinolysis occurring within mesothelial spaces (11). Our results also exhibited high concentrations of FDP and DD in the pleural cavities indicating active fibrinolysis. However, there was no statistical significance between the pleural blood and the ECC at the same time period.

REINFUSION OF WHOLE BLOOD COLLECTED FROM MESOTHELIAL SPACES

The effect of retransfusing whole blood collected from the pleural and mediastinal cavities may impair systemic hemostasis. Tabuchi and associates performed a study in which it was hypothesized that local fibrinolytic activation by the pericardial cavity may have consequences for the systemic activation processes during CPB. To test their hypothesis, they interrupted blood suction from the pericardial cavity during CPB in coronary artery bypass operations. The blood in the pericardial cavity exhibited levels of thrombin-antithrombin III complex, t-PA antigen, and FDP that were significantly higher than the systemic blood. When the blood was returned to the systemic circulation an immediate significant increase in these values was observed (13). Similar studies by other researchers have concluded that reinfusion of highly activated suctioned blood exacerbates wound bleeding, and they noted that high concentrations of FDP in this blood may play a role in the observed impaired hemostasis (14,16,17,26-30). In contrast, other studies have shown that an increased level of fibrinolytic activator and FDP in whole

blood reinfusions has no clinically significant effect on the stimulation of systemic fibrinolytic activity. These studies state that autotransfusion of unwashed, filtered blood is a safe and efficacious alternative to homologous blood replacement in these patients (19,31-35). In this study, both FDP and DD increased in the ECC after infusion of the pleural blood, although not significantly. Fibrinolytic activity as measured by TEG increased slightly in the ECC post-infusion of pleural blood, but again, statistical significance was not achieved.

In summary, we did not observe significantly greater clotting or fibrinolytic activity in the pericardial cavity as compared with the systemic circulation prior to CPB. The ECC generated clotting activity more so than the pleural cavity immediately upon initiation of CPB. However, over time, thrombin generation was stimulated to a greater extent in the pleural cavity than in the ECC. High concentrations of FDP and DD were observed in the pleural cavities, although there was no significant difference in the degree of fibrinolytic activation when compared to the ECC prior to infusion of the pleural blood. A trend toward decreased fibrinolytic activity was noted in the pleural blood as well as in the ECC over time.

After suctioning the pleural blood, both FDP and DD concentrations increased in the ECC, although not significantly. The fibrinolytic activity as assessed by TEG did increase in the ECC, but again, statistical significance was not achieved. There was, however, a significant increase in the ECC as compared with the pleural cavity at 90 min. Perhaps the mere presence of fibrinolytic by-products in the pooled blood from the initial fibrinolytic episode seen at 30 min was sufficient to have an additive effect on the systemic fibrinolysis already occurring in the systemic circulation.

Other investigators have felt that impairment of systemic hemostasis only occurs when very large volumes of mediastinal shed blood are reinfused (15,32,34). Perhaps the small volumes suctioned into the ECC in our study were not sufficient to significantly affect the hemostatic mechanisms taking place in the systemic circulation. Another deficiency of this study is that we were unable to follow these subjects through a postoperative course and see if there was in fact a significant clinical effect due to this practice.

In conclusion, this study found that blood exposed to pleural surfaces may have increased fibrinolytic activity, but systemic hyperfibrinolysis was not observed. Clinical studies which have been performed on this topic have resulted in differing conclusions, and therefore more research in this area is indicated.

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