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THE EFFECTS OF CARDIOPULMONARY BYPASS ON HEMOSTASIS

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dysfunction characterized by the prolongation of the bleeding time. The cardiopulmonary bypass-induced defect in platelet function has not been completely characterized. Although decreases in platelet surface GPIb (the von Willebrand factor receptor) and the GPIIb-IIIa complex (the fibrinogen receptor) have been described, recent evidence suggests that the "platelet function defect" of cardiopulmonary bypass is the result of a lack of factors extrinsic to the platelet. Hypothermia is an important factor in the genesis of the platelet function defect and accounts, in part, for the reversibility of the prolonged bleeding time. The release of large platelets into the circulation also accounts, in part, for the reversal of the prolonged bleeding time. The pathophysiological significance of the cardiopulmonary bypass-induced increase in platelet membrane microparticles remains to be determined. Previously reported evidence of selective platelet  $\alpha$ -granule release from circulating platelets during cardiopulmonary bypass has not been supported by recent studies in whole blood. Hyperfibrinolysis following the administration of heparin and the institution of cardiopulmonary bypass also contributes to the hemostatic defect observed during and following cardiac surgery. There is a direct relationship between postoperative bleeding time, temperature, and postoperative blood loss in patients undergoing cardiopulmonary bypass. The duration of cardiopulmonary bypass is the main determinant of the postoperative bleeding time and blood loss. Adequate rewarming and expeditious surgery should reduce the hemostatic abnormality and the postoperative blood loss in cardiac surgery. Of the pharmacologic interventions, aprotinin appears to be the most promising in reducing post-cardiopulmonary bypass blood loss, but the mechanism of this salutary effect remains to be elucidated.

12/01/92 THE EFFECTS OF CARDIOPULMONARY BYPASS  
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## I. INTRODUCTION

The vast majority of cardiac surgical operations are performed with cardiopulmonary bypass. Blood contact with the extracorporeal circuit that is used during cardiopulmonary bypass elicits a wide spectrum of pathophysiological changes that affect a variety of organ systems. In a broad sense, the hematologic changes brought about by cardiopulmonary bypass are probably the most important of these pathophysiologic states because they result in the most pronounced clinical abnormality, increased postoperative bleeding, and because they impact on several of the abnormal clinical manifestations of other organ systems (e.g. increased capillary permeability leading to respiratory abnormalities). It has long been recognized that cardiopulmonary bypass results in abnormal hemostasis that sometimes leads to excessive postoperative bleeding. The exact nature of this hemostatic abnormality remains the subject of intense investigation, although platelet function abnormalities, hyperfibrinolysis, and other hematologic abnormalities have been identified as possible culprits. The purpose of this chapter is to provide a description of our current knowledge of most of the hematologic changes observed in the course of cardiopulmonary bypass, to review our understanding of the nature of the hemostatic defect elicited by contact with the extracorporeal circuit, and to address clinical issues related to postoperative blood loss, the most important clinical outcome of these pathophysiologic states.

## II. THE EFFECTS OF CARDIOPULMONARY BYPASS ON BLOOD ELEMENTS

### A. Platelets and the Blood Vessel Wall

#### 1. *Normal Platelet Physiology (Figure 1)*

Platelets are essential for normal hemostasis. The main functions of platelets are adhesion to damaged blood vessel walls, aggregation to form a platelet plug, and promotion of fibrin clot formation. The mechanisms of platelet activation are reviewed in detail in other chapters; the following serves as a brief summary to provide background for the clinical abnormalities of cardiopulmonary bypass.

Platelet adhesion is mediated primarily by the adhesive molecule von Willebrand factor (vWF) which binds both to a specific receptor on the platelet surface glycoprotein (GP) Ib-IX complex and to exposed subendothelial components.<sup>1,2</sup> Platelet-to-platelet aggregation is primarily mediated by fibrinogen binding to its receptor on the platelet surface GPIIb-IIIa complex.<sup>3</sup> Normal circulating platelets are in a resting state and bind neither plasma vWF nor plasma fibrinogen. *In vitro*, the cationic antibiotic ristocetin induces binding of vWF to its receptor on GPIb,<sup>1</sup> but the *in vivo* analogue of ristocetin remains uncertain. Shear stress and/or fibrin (monomer) interacting with vWF may serve as the physiologic stimulus to initiate GPIb-vWF interaction. Thrombin and other physiological platelet agonists (e.g. adenosine diphosphate [ADP], epinephrine) induce exposure of the fibrinogen receptor on

the platelet surface GPIIb-IIIa complex.<sup>3</sup> These agonists also stimulate platelets to change shape, secrete the contents of their granules (e.g.  $\beta$ -thromboglobulin [BTG], platelet factor 4, thrombospondin), and to aggregate. Secreted thrombospondin binds to a receptor on the platelet surface membrane, as well as to fibrinogen, thereby stabilizing platelet-to-platelet aggregates.<sup>4</sup> P-selectin<sup>5</sup>, also known as GMP-140<sup>6</sup> and PADGEM protein<sup>7</sup>, is a component of the  $\alpha$ -granule membrane of resting platelets that is only expressed on the platelet plasma membrane after platelet activation and secretion.<sup>8</sup> Platelet surface expression of P-selectin is therefore a very precise marker of platelet secretion. Although its physiologic role remains speculative, P-selectin is known to mediate *in vitro* adhesion of activated platelets to monocytes and neutrophils.<sup>9,10</sup> In contrast to its effect on P-selectin and the fibrinogen receptor on the GPIIb-IIIa complex, thrombin downregulates the platelet surface expression of the vWF receptor on the GPIb-IX complex.<sup>11,12,13,14</sup>

## 2. *Time-course of Changes in Platelet Parameters During and Following Cardiopulmonary Bypass*

The Bleeding Time: The bleeding time is markedly prolonged during cardiopulmonary bypass.<sup>15,16</sup> Previously published data indicate that it normalizes within 24 hours postoperatively.<sup>15</sup> The operator-dependence of the measurement of the bleeding time and its recently demonstrated temperature-dependence<sup>17</sup> have contributed to a lack of consistency in the data reported by various investigators. As

evidenced from uniform measurements made in 87 patients undergoing uncomplicated myocardial revascularization procedures, systemic anticoagulation with 3 mg/Kg of heparin prior to the institution of cardiopulmonary bypass elicits a modest but significant rise in the bleeding time (Figure 2). The bleeding time is markedly prolonged during cardiopulmonary bypass and remains so for 2 hours after bypass. Although it reverses mostly between 2 and 24 hours after cardiopulmonary bypass, it may not normalize until 72 hours postoperatively (Figure 2). The marked prolongation of the bleeding time in the course of cardiopulmonary bypass and its subsequent reversal suggest that a primary culprit in the hemostatic defect induced by cardiopulmonary bypass is platelet dysfunction.

The Platelet Count: Thrombocytopenia is consistently observed during and following cardiopulmonary bypass<sup>15,16,18,19,20,21</sup> (Figure 3). It occurs as early as 5 minutes following the institution of cardiopulmonary bypass<sup>21</sup> and reaches its nadir by 25 minutes.<sup>16</sup> The platelet count remains depressed throughout the postoperative period (Figure 3) and may continue to be depressed for several days after the bypass procedure.<sup>15,22</sup>

Hemodilution and platelet adhesion to synthetic surfaces are the two primary contributors to the thrombocytopenia observed during cardiopulmonary bypass. During extracorporeal circulation for cardiac surgery, dilution of blood occurs as a result of priming the extracorporeal perfusion system with either crystalloid (5% dextrose in saline, Ringer's lactate) or colloid (albumin, dextran, hetastarch solutions, plasma). This hemodilution is generally

considered to be the major cause of thrombocytopenia during cardiopulmonary bypass.<sup>18,19</sup>

However, the degree of thrombocytopenia observed during and following cardiopulmonary bypass is more severe than that which might be expected from hemodilution alone.<sup>16</sup> A loss of platelets during cardiopulmonary bypass occurs which is proportional to the flow rate and the surface area of the extracorporeal circuit.<sup>23</sup> Platelets have been shown by scanning electron microscopy to adhere to extracorporeal synthetic surfaces.<sup>24</sup> Fibrinogen appears to be the most important cofactor in platelet adhesion to synthetic surfaces<sup>25</sup>, as it is for platelet-to-platelet aggregation.<sup>3</sup> Plasma fibrinogen is preferentially adsorbed onto synthetic surfaces<sup>26,27</sup> and platelet reactivity with these surfaces has been reported to be directly proportional to the adsorbed fibrinogen concentration<sup>28</sup>, although this supposition is disputable.<sup>29</sup> The mechanism(s) by which platelets are initially activated within extracorporeal perfusion systems is not completely clear, but possible causes include: direct surface contact, thrombin, and ADP. Thrombin, which is generated in small amounts despite the presence of heparin during cardiopulmonary bypass surgery, is adsorbed onto synthetic surfaces<sup>30</sup> and likely binds to the adsorbed fibrinogen on the extracorporeal surface. ADP is stored in platelet-dense granules and is released both by platelet lysis and platelet activation. Hemolysis of red cells also releases ADP.

Platelet activation results in exposure of fibrinogen receptors on the GPIIb-IIIa complex<sup>31</sup> and permits binding to

fibrinogen molecules previously adsorbed onto the surface.<sup>32</sup> Data of Gluszko et al.<sup>29</sup> show that exposure of fibrinogen receptors associated with the GPIIb-IIIa complex contributes to platelet consumption during cardiopulmonary bypass. These investigators<sup>29</sup> demonstrated that patients with Glanzmann's thrombasthenia (an inherited deficiency of the GPIIb-IIIa complex) had reduced cardiopulmonary bypass-induced thrombocytopenia, whereas patients with Bernard-Soulier syndrome (an inherited deficiency of GPIb, GPIIX, and GPV) did not.

Activated platelets, in addition to adhering to the synthetic surfaces of the cardiopulmonary bypass tubing, are also more likely to adhere to injured endothelial surfaces, to deposit in the heart after cardioplegic arrest<sup>33</sup>, and to be removed by the reticulo-endothelial system. The concept that platelet activation, adherence, and/or clearance is important in the etiology of thrombocytopenia during cardiopulmonary bypass is supported by findings that infusion during cardiopulmonary bypass of Prostaglandin (PG)E<sub>1</sub>, PGI<sub>2</sub>, Iloprost, or dipyridamole, drugs which inhibit platelet activation, can result in a marked reduction in platelet adherence to the synthetic surfaces, maintenance of platelet counts at near-normal levels, and reduction in postoperative blood loss.<sup>29,34,35,36,37,38,39,40,41</sup> An adsorbed protein layer that reduces the affinity of synthetic surfaces for platelets may eventually form.<sup>42,43</sup> Although the exact physiological basis for this process, termed *passivation*<sup>18</sup>, remains unclear, support for the concept derives from studies with PGI<sub>2</sub>.<sup>34,35</sup> When PGI<sub>2</sub> was used to

inhibit platelets during 2 hours of recirculation in a membrane oxygenator system, platelet activation was inhibited only during the first hour.<sup>34,35</sup> PGI<sub>2</sub> is extremely unstable in plasma, and after 1 hour the recirculated platelets regain their ability to aggregate in the presence of ADP and epinephrine, yet do not react with the synthetic surface.<sup>34,35</sup>

Oxygenators, and to a lesser extent filters, contain the largest surface areas in contact with blood and therefore are the most prominent sites of platelet deposition.<sup>18</sup> The degree of thrombocytopenia observed during cardiopulmonary bypass is related more to the turbulence, flow rate, and amount of suction in the circuit<sup>23,44,45</sup> than to the type of oxygenator employed.<sup>16,46</sup>

Three less common causes of thrombocytopenia during cardiopulmonary bypass are disseminated intravascular coagulation, heparin-induced thrombocytopenia, and cyanotic congenital heart disease. Although probably uncommon, as distinct from primary fibrinolysis<sup>47</sup>, disseminated intravascular coagulation is a cause of thrombocytopenia and possibly of increased bleeding following cardiopulmonary bypass.<sup>48,49</sup> Disseminated intravascular coagulation is rarely encountered during cardiopulmonary bypass but is more likely to occur later, in association with septicemia or low cardiac output.<sup>18</sup> Heparin-induced thrombocytopenia is discussed later in this chapter and in Chapter G3. The mechanism of the association between cyanotic congenital heart disease and thrombocytopenia<sup>50,51</sup> is unclear.

Mean Platelet Volume and Platelet Mass: The mean platelet volume (MPV) decreases significantly after the institution of cardiopulmonary bypass and reaches its nadir approximately two hours after discontinuation of bypass (Figure 3). There is a progressive and significant increase in MPV between 2 and 72 hours postoperatively, accompanied by a significant rise in platelet mass<sup>16</sup>, suggesting that larger platelets are selectively removed during the extracorporeal circulation.<sup>16,52</sup> This is an important finding in light of the fact that platelet size has been shown to relate directly to platelet function.<sup>53</sup> An increase in the mean platelet volume between 2 and 72 hours postoperatively, accompanied by a relatively stable platelet count, suggests that a postoperative release of large platelets into the peripheral circulation is responsible in part for the improvement in the bleeding time seen during this period.

Alpha Granule Release: When platelets adhere to synthetic surfaces, they are activated and degranulated, and the contents of their alpha granules are released into the circulation. Levels of plasma BTG and platelet factor 4, both of which are contained within alpha granules, are markedly increased during and immediately following cardiopulmonary bypass.<sup>15,54,55,56,57,58</sup> Levels return to normal within the 24 hour postoperative period<sup>15,16</sup> (Figure 4). These changes in the plasma levels of platelet-specific proteins reflect an initial irreversible activation and lysis of a relatively small number of platelets which are removed from circulation within the first 24 hours following cardiopulmonary bypass; these changes

are not indicative of continued platelet activation occurring during cardiopulmonary bypass.<sup>59</sup>

Thromboxane B<sub>2</sub> and 6-keto PGF<sub>1α</sub>: Studies conducted in the baboon and in man in which thromboxane B<sub>2</sub> (the stable metabolite of thromboxane A<sub>2</sub>) and 6-keto PGF<sub>1α</sub> (the stable metabolite of prostacyclin) were measured in blood shed from the skin at the site of the bleeding time have yielded valuable information on platelet function.<sup>60,61,62</sup> In patients undergoing cardiopulmonary bypass, a marked reduction in the level of thromboxane B<sub>2</sub> in the shed blood has been observed soon after the institution of cardiopulmonary bypass and is indicative of a platelet dysfunction<sup>16,17</sup>(Figure 5). Within 2 to 24 hours after the discontinuation of cardiopulmonary bypass, the level of thromboxane B<sub>2</sub> in the shed blood is significantly increased while the systemic plasma level of thromboxane B<sub>2</sub> is decreased.<sup>16</sup> The increase in the shed blood thromboxane B<sub>2</sub> postoperatively is not a reflection of changes in the plasma concentration of this metabolite, but rather is a reflection of the progressive improvement in platelet function postoperatively which is paralleled by an improvement in the bleeding time and an increase in the mean platelet volume. Changes in the shed blood 6-keto PGF<sub>1α</sub>, both during and after cardiopulmonary bypass, are generally opposite those observed in the shed blood thromboxane B<sub>2</sub>, and are probably reflective of the systemic changes in plasma 6-keto PGF<sub>1α</sub>.<sup>16</sup>

Platelet Aggregation: Blood samples obtained from patients during cardiopulmonary bypass have shown markedly reduced platelet

aggregation in response to *in vitro* stimulation with various agonists.<sup>19,46,63,64,65,66,67</sup> However, the *in vitro* nature of this test, wide variability in the reported responses to the various agonists, and the wide variability in the data reported by various investigators, underscore the unreliability of this measurement in the quantification of the degree of platelet dysfunction elicited by cardiopulmonary bypass.

### 3. *The Role of Platelet in the Hemostatic Defect Induced by Cardiopulmonary Bypass*

It is clear that a defect in platelet function characterized by a prolongation of the bleeding time is present in cardiopulmonary bypass surgery. That the prolongation of the bleeding time reflects a platelet function defect is established by the normal<sup>68</sup>, or increased<sup>69,70</sup> plasma levels of vWF and by increasing divergence during bypass of the normal relationship between platelet count and bleeding time.<sup>15</sup> The degree of thrombocytopenia alone is usually not severe enough to produce a bleeding diathesis.

What Causes the Platelet Function Defect? Although the cause(s) of the platelet function defect during cardiopulmonary bypass is not entirely clear, possible causes are listed in Table 1, and many are discussed elsewhere in this section. The theory that a circulating platelet inhibitor is present is questionable because of the finding that plasma from patients undergoing cardiopulmonary bypass does not inhibit the function of normal platelets.<sup>18</sup> Although circulating fibrin(ogen) degradation products can interfere with

platelet function<sup>71</sup>, and these are present in the majority of patients undergoing cardiopulmonary bypass surgery<sup>47</sup>, a correlation between the two has not been substantiated.<sup>72,73</sup> A correlation has been observed between high concentrations of denatured plasma proteins and reduced platelet function<sup>74</sup> and during cardiopulmonary bypass, particularly in bubble oxygenator perfusion systems<sup>75,76</sup>, plasma proteins are denatured. Decreases in platelet aggregation in response to ADP, epinephrine and collagen, as well as abnormalities in platelet release, have been observed pre-operatively in patients with cyanotic congenital heart disease.<sup>77,78</sup>

Why has it been Difficult to Characterize the Defect in Platelet Function? The precise nature of the defect(s) in platelet function during and after cardiopulmonary bypass remains obscure. The variability in the reported defects in platelet function during and after bypass result in part from differences in equipment and techniques (Table 1). However, methodological problems may also be involved. During the process of separating platelets from whole blood for functional assays, the platelets are susceptible to membrane alterations<sup>79</sup> and to *ex vivo* activation. The popular use of plasma assays of the secretion products of platelet  $\alpha$ -granules (platelet factor 4 and BTG) to study the platelet defect in cardiopulmonary bypass is questionable because a 1% increase in platelet secretion may cause as much as a 30-fold increase in the plasma level of platelet factor 4.<sup>80</sup> Moreover, plasma assays of platelet factor 4 and BTG indicate not only the number of circulating activated platelets but also the number of lysed

platelets and non-circulating activated platelets that adhere to synthetic surfaces or vessel walls.

Do Platelet Microparticles and/or Platelets with Partial  $\alpha$ -Granule Release Circulate? Whole blood assays have been developed that do not involve any separation or manipulation of platelets.<sup>11,14,81</sup> George et al.<sup>11</sup> used <sup>125</sup>I-labeled monoclonal antibodies to directly measure platelet surface glycoproteins in immediately fixed whole blood samples and were able to define two prototypic types of acquired abnormalities of platelet surface glycoproteins. Patients with adult respiratory distress syndrome were shown to have an increased concentration of P-selectin and thrombospondin on the surface of their platelets, demonstrating *in vivo* platelet secretion, but no increase in platelet microparticles (see Chapter C4) in their plasma. In contrast, following cardiac surgery with cardiopulmonary bypass, patients exhibited changes consistent with membrane fragmentation without secretion: a decreased platelet surface concentration of GPIb and GPIIb, no increase in platelet surface P-selectin or thrombospondin, and an increased plasma concentration of platelet membrane microparticles.<sup>11</sup> The production of platelet microparticles was assumed to be the result of turbulence and shear stress.<sup>11</sup> Harker et al.<sup>15</sup>, using an electron microscopic analysis of fixed, separated platelets and plasma assays of platelet factor 4 and BTG, reported evidence of platelet  $\alpha$ -granule secretion during cardiopulmonary bypass. However, a recent study by Abrams et al.<sup>82</sup> supported the findings of George et al.<sup>11</sup> Utilizing a whole blood flow cytometric assay<sup>81</sup>,

these authors<sup>82</sup> also found that cardiopulmonary bypass results in platelet fragmentation with the production of microparticles, but not platelet secretion as determined by a P-selectin-specific monoclonal antibody. Dechavanne et al.<sup>83</sup>, also in agreement with George et al.<sup>11</sup>, demonstrated a reduction in the binding of a monoclonal antibody directed against the platelet GPIIb-IIIa complex, with no evidence of degranulation, as evidenced by electron microscopy, and lack of binding of monoclonal antibodies directed against an  $\alpha$ -granule membrane glycoprotein and thrombospondin.

#### What is the Mechanism of the Platelet Aggregation Defect?

Circulating platelets have been reported to be less responsive *ex vivo* to agonists such as ADP, collagen, and epinephrine, during cardiopulmonary bypass.<sup>15,19,46,84</sup> A recent report of platelet hyper-reactivity during cardiopulmonary bypass<sup>85</sup> suggests that some of these defects may result from platelet refractoriness to aggregation due to partial activation of the platelets *in vitro* during separation. Another mechanism may be loss of platelet membrane fibrinogen<sup>32,86</sup> and epinephrine<sup>84</sup> receptors, possibly as a result of platelet microparticle formation<sup>11</sup> or of detachment of adherent platelets, with some fibrinogen receptors remaining on still-adherent fragments of platelet membrane.<sup>86</sup>

A decrease in ADP-induced platelet aggregation has been observed following protamine sulfate administration.<sup>19,87</sup> Mammen et al.<sup>19</sup> reported that ristocetin-induced platelet agglutination remains relatively unchanged during cardiopulmonary bypass, but decreases after administration of protamine sulfate and remains abnormal for

24 hours postoperatively. Although the effects of protamine on platelets are well-documented<sup>88,89</sup>, the mechanisms by which these effects are induced are not clear. *In vitro* experiments have suggested that the effects may be mediated by a protamine-heparin complex rather than by protamine alone.<sup>88</sup>

What is the Role of Plasmin-induced Cleavage of Platelet Surface GPIb? As discussed in detail in section II.B.2. of this chapter, the majority of patients undergoing cardiopulmonary bypass surgery have a primary fibrino(geno)lytic state, resulting in the generation of plasmin.<sup>47</sup> Plasmin cleaves platelet surface GPIb *in vitro*.<sup>90,91</sup> This could be important in the hemostatic defect of cardiopulmonary bypass, because GPIb is essential for normal platelet adhesion (via its vWF receptor<sup>1,2</sup>) and activation (possibly via its thrombin receptor<sup>92,93,94</sup>). Mohr et al.<sup>68</sup> reported impaired platelet aggregation to ADP, collagen, and ristocetin after cardiopulmonary bypass surgery in all 20 patients in their study, and found the only aggregation response that correlated with clinical bleeding was the response to ristocetin. Assays for vWF were normal, strongly suggesting that the defect in ristocetin-induced platelet agglutination was due to an abnormality in platelet surface GPIb.<sup>68</sup> Redistribution of intraplatelet stores of GPIb to the platelet surface<sup>95,96</sup> could account for the rapid decrease in the bleeding time that occurs at the end of bypass. It has been demonstrated that aprotinin *in vitro* inhibits the plasmin-mediated cleavage of platelet surface GPIb.<sup>90</sup> Furthermore, it has been suggested that the *in vivo* effectiveness of aprotinin in decreasing

bleeding during and after cardiopulmonary bypass surgery<sup>97,98</sup> (CPB) may relate to inhibition of plasmin-induced cleavage of platelet surface GPIb.<sup>99,100,101</sup>

Is the Platelet Function Defect Intrinsic or Extrinsic?     a

a recent study<sup>85</sup>, it was found that cardiopulmonary bypass surgery does not result in circulating activated platelets, loss of platelet surface GPIb or the GPIIb-IIIa complex, or loss of *in vitro* platelet reactivity. The finding that during cardiopulmonary bypass platelets were not reactive *in vivo* but were reactive *in vitro* suggests that the "platelet function defect" of cardiopulmonary bypass is an extrinsic defect. The extrinsic defect appears to be related, in part, to hypothermia and an *in vivo* lack of availability of platelet agonists including thromboxane A<sub>2</sub> and thrombin (neutralized by heparin). The role of hypothermia in eliciting a reversible platelet dysfunction is discussed in Section III below.

B. The Fluid Phase: Coagulation and Fibrinolysis

1. *Time Course of Changes in Plasma Proteins During and Following Cardiopulmonary Bypass*

Most of the plasma proteins are adsorbed to the cardiopulmonary bypass circuit in small and inconsequential amounts. Fibrinogen, on the other hand, is preferentially adsorbed to synthetic surfaces and is the dominant protein on these surfaces.<sup>26,27</sup> The institution of cardiopulmonary bypass elicits nearly a 50% decrease in the

concentration of plasma proteins, mainly as a result of hemodilution (Table 2). Because of the complexity of cardiac surgery and its demand for frequent administration of various types of fluids and blood component transfusions, the exact contribution of hemodilution to the observed concentration of the various plasma proteins, during and following cardiopulmonary bypass, is difficult to ascertain. As reflected by the changes in hematocrit (Figure 6), hemodilution is most pronounced within minutes after the institution of cardiopulmonary bypass; it is sustained, to a lesser degree, for several days postoperatively. Protein concentrations, which fall during cardiopulmonary bypass and remain low throughout the initial postoperative days, may be reflective of hemodilution and not of sustained intraoperative consumption.

Although plasma protein changes during and following cardiopulmonary bypass should be interpreted in light of the diluted state, it is not appropriate to correct for hemodilution by simply relating the plasma protein concentration to the corresponding hematocrit because the proportion of plasma to whole blood is different from that of the red cells to whole blood. Correcting for hemodilution during and following cardiopulmonary bypass without due consideration of this fact is another source of difficulty in the interpretation of published plasma protein concentration data. The following is a more appropriate formula for use in the calculation of the concentration of a plasma protein independent of the dilution effect elicited by the institution of cardiopulmonary bypass:

$$DCP = BP [(BL \text{ Hct} / BP \text{ Hct} - BL \text{ Hct}) / (1 - BL \text{ Hct})]$$

Where DCP = dilution-corrected protein concentration during cardiopulmonary bypass, BP = actual protein concentration during cardiopulmonary bypass, BL = baseline (prebypass) value, and Hct = hematocrit.

This formula was used to correct for hemodilution in 49 patients undergoing cardiopulmonary bypass (Table 2). The pump prime was a crystalloid solution, free of colloids. At 25 minutes after the institution of cardiopulmonary bypass, the hemodilution-corrected concentrations of total proteins, albumin, plasminogen, fibronectin, and anti-thrombin III were significantly increased compared to baseline. The concentration of the complement C<sub>3</sub>, which is only present in the intravascular space, and the concentration of IgM, which has a large molecular weight, were both decreased compared to baseline (Table 2). The concentrations of IgG and vWF did not change significantly after correcting for hemodilution. Fibrinogen could not be accurately measured because of the presence of heparin in the samples. These observations suggest that, during the initial period of cardiopulmonary bypass, there is a net influx of interstitial proteins into the intravascular space, probably as a consequence of a complement-induced alteration in capillary permeability and changes in colloid osmotic gradients. Extracorporeal circulation has been shown to cause alterations in vascular integrity which may result in a diffuse perivascular leak in the perioperative period.<sup>102</sup> Although a net "plasma-refill" following the institution of cardiopulmonary bypass has not been previously described, a net increase in intravascular albumin concentration

following hemorrhage and crystalloid hemodilution has been reported by several investigators<sup>103,104,105,106,107</sup>, and several theories and mathematical models have been proposed to explain it.<sup>108,109,110,111,112</sup>

The Oncotic and Opsonic Proteins: Total protein concentrations reflect the diluted state observed during and following cardiopulmonary bypass.<sup>16</sup> Albumin is minimally adsorbed to the extracorporeal circuit. During the period between 2 and 72 hours postoperatively, albumin remains depressed, paralleling the hematocrit (Figure 6). The opsonic proteins (IgG, IgM, C<sub>3</sub> and fibronectin) also decrease significantly during and following cardiopulmonary bypass; they remain depressed for at least three days postoperatively (Figures 7A & 7B). C<sub>3</sub> increases significantly during the 24-72-hour post-CPB period but it does not return to its baseline value (Figure 7A). The decrease in the opsonic proteins during and following cardiopulmonary bypass is dilutional but has also been attributed to generalized opsonic consumption during prolonged cardiopulmonary bypass<sup>113,114,115</sup>, fibronectin-mediated removal of macrocellular aggregates by the reticuloendothelial system<sup>116</sup>, protein degradation by proteolytic enzymes<sup>117</sup>, and cold-induced precipitation of fibronectin with fibrinogen.<sup>118</sup>

The Clotting and Fibrinolytic Proteins: Plasma fibrinogen levels are elevated above normal in patients with heart disease.<sup>119,120,121</sup> Likewise, they are elevated preoperatively in patients undergoing cardiac surgery.<sup>16,54</sup> Two hours following cardiopulmonary bypass, fibrinogen levels are decreased, but between 2 and 72 hours postoperatively there is a progressive increase in

plasma fibrinogen, resulting in levels which are significantly higher than baseline (Figure 8). Likewise, there is a progressive increase in the concentrations of Factor VIII and vWF between 2 and 72 hours following CPB, resulting in levels which are significantly higher than baseline (Figure 8). Thus, unlike the opsonic proteins which remain depressed for few days postoperatively, the clotting proteins increase well above baseline during this period.<sup>16,122,123,124,125</sup>

Fibrinolytic activity increases significantly during and following cardiopulmonary bypass<sup>72,73,87,126,127,128,129,130,131,132,133,134,135</sup> and contributes to increased postoperative blood loss.<sup>47,87,128,131,136</sup> Fibrinolytic activity is actually observed shortly after systemic heparinization *before* the institution of cardiopulmonary bypass. Heparin induces a significant rise in plasma plasmin activity,<sup>20,133</sup> which is sustained (at a lower level probably as a reflection of hemodilution) throughout the duration of cardiopulmonary bypass. Plasmin activity returns to normal at the completion of cardiopulmonary bypass. Alpha-2 antiplasmin, a specific inhibitor of plasmin, is also affected by systemic heparinization prior to institution of cardiopulmonary bypass. Both systemic heparinization and the institution of cardiopulmonary bypass effect a progressive decrease in the antiplasmin activity which is sustained throughout the first 24 hours postoperatively. The antiplasmin activity during bypass is significantly lower than that expected on the basis of hemodilution alone.<sup>133</sup> While plasmin activity returns to normal immediately after discontinuation of cardiopulmonary bypass,

antiplasmin levels do not return to normal before 48-72 hours postoperatively.

The concentration of tissue plasminogen activator (t-PA) increases during cardiopulmonary bypass and returns to normal rapidly thereafter<sup>126,129,131</sup>; it is not affected by systemic heparinization. This suggests that cardiopulmonary bypass is a major stimulus for the release of t-PA from the vascular endothelium, in addition to the other known stimuli such as exercise, hypotensive shock, pharmacologic agents, and Protein C.<sup>137</sup> Plasma plasminogen and Antithrombin III decrease significantly during cardiopulmonary bypass and remain depressed from their baseline levels throughout the first 72 hours postoperatively (Figure 9). They parallel the changes in hematocrit and are probably reflective of the dilutional state.<sup>134</sup>

Fibrinogen/fibrin degradation products and D-Dimer increase during and following cardiopulmonary bypass.<sup>127,128,132,135</sup> At the completion of cardiopulmonary bypass, the FDP level is markedly reduced and no FDPs are detected beyond two hours following cardiopulmonary bypass. D-dimer, a product of the degradation of cross-linked fibrin, increases after the administration of protamine and reaches its peak two hours following the discontinuation of cardiopulmonary bypass (Figure 10).

## **2. *The Role of Coagulation and Fibrinolytic Factors in the Hemostatic Defect Induced by Cardiopulmonary Bypass***

There are a number of pathways that could lead to increased fibrinolysis during cardiopulmonary bypass surgery.<sup>138,139</sup> The contact of blood with a large artificial surface leads to activation of the contact phase of coagulation and kallikrein generation (see Chapter B2). Kallikrein directly, and indirectly via bradykinin, stimulates release of tissue plasminogen activator from endothelial cells.<sup>138,139</sup> Kallikrein can also convert the inactive zymogen pro-urokinase into urokinase. Release of tissue plasminogen activator from endothelial cells during CPB may also be stimulated by the elevated levels of thrombin, epinephrine, angiotensin II, leukotrienes, and hypoxia.<sup>138</sup> Heparin can also induce a significant rise in plasma plasmin activity and fibrin(ogen) degradation products before the institution of CPB.<sup>133</sup> In addition to the fibrinolytic state *per se*, circulating fibrin(ogen) degradation products can interfere with thrombin activity, fibrin polymerization, and platelet function.<sup>47</sup> The precise role of fibrinolysis in the genesis of the hemostatic defect induced by CPB remains to be determined. However, it is of great interest that the blood loss during and after CPB surgery can be reduced by the administration of aprotinin, an inhibitor of plasmin, kallikrein, and urokinase.<sup>97,98</sup> Administration of aprotinin (see Chapter L3) during CPB surgery prevents the formation of fibrin(ogen) degradation products<sup>128,140</sup> and the reduction in  $\alpha_2$  antiplasmin activity.<sup>128</sup> (The possible effect of plasmin on platelet surface glycoproteins during CPB surgery, and the reversal of this effect by aprotinin is discussed in section II. A. 3. above.) The clinical effectiveness of aprotinin in decreasing blood loss

suggests that fibrinolysis is important in the hemorrhagic diathesis associated with CPB surgery.

The reductions in plasma levels of clotting factors during CPB surgery are primarily due to hemodilution<sup>15,141</sup> (see section II. B. 1. above). Only factor V levels decrease to a level lower than that predicted by dilution alone.<sup>15,19</sup> For all coagulation factors, including factor V, levels observed during CPB surgery rarely fall into a range in which hemostasis would be compromised.<sup>16,19,47,48,134</sup>

Investigators have reported conflicting results regarding the concentration of vWF during and following cardiopulmonary bypass.<sup>16,68,69,70,142</sup> As shown in Figure 8, vWF decreases during cardiopulmonary bypass but not to levels below those considered adequate for hemostasis.<sup>16,142</sup> Pre-operatively, high-molecular-weight multimers of vWF may be selectively deficient in patients with valvular heart disease and noncyanotic congenital heart disease.<sup>142,143</sup>

### C. Leukocyte and Complement Activation

In the course of cardiac surgery, complement is activated via both the classical and the alternate pathways. Blood contact with the artificial surfaces initiates complement activation through the alternate pathway. The administration of protamine to reverse the heparin effect after the discontinuation of cardiopulmonary bypass results in the formation of immune complexes which trigger complement activation through the classical pathway. Complement activation results in complex adverse sequelae which include the

activation and aggregation of granulocytes. A detailed description of the effect of artificial surfaces, including cardiopulmonary bypass, on complement activation and leukocyte function is provided in Chapter XX.

### III. HYPOTHERMIA: ITS ROLE IN HEMOSTASIS AND BLOOD CELL ACTIVATION DURING AND FOLLOWING CARDIOPULMONARY BYPASS

#### A. Hypothermia and Hemostasis

Over the years, surgeons have intuitively recognized that hypothermia tended to increase the bleeding in the surgical patient and that rewarming the patient improved hemostasis. Until recently, however, patient studies which elucidated the role of hypothermia in hemostasis have been scarce. Canine studies have demonstrated that hypothermia at a temperature of 20 °C causes thrombocytopenia, sequestration of platelets in the hepatic sinusoids, and a marked decrease in collagen-induced platelet aggregability.<sup>144,145</sup> In addition, hypothermia in the dog causes a marked activation of the plasma fibrinolytic system.<sup>145</sup> In the baboon, local hypothermia causes a significant increase in the bleeding time and a decrease in the concentration of thromboxane B<sub>2</sub> in the blood shed from the site of the measurement of the bleeding time.<sup>60</sup> These changes are completely reversed with rewarming.<sup>60</sup> Rewarming beyond 37°C does not elicit any further changes in bleeding time and the shed blood thromboxane B<sub>2</sub>.

These observations in the hypothermic baboon have been confirmed in a clinical study of 25 patients undergoing cardiopulmonary bypass with systemic hypothermia (25°C).<sup>17</sup> In these patients, one arm was kept warm with a water jacket throughout the intra and postoperative periods while the temperature in the other arm reflected the systemic changes. As demonstrated in Figure 11, the bleeding time was significantly prolonged in the cold arm compared to the warm arm; likewise, the concentration of shed blood (from the site of the measurement of the bleeding time) thromboxane B<sub>2</sub> was significantly lower in the cold arm than in the warm arm. These reversible changes in platelet function provided, for the first time in humans, a definitive demonstration of the effect of hypothermia on hemostatic parameters in the course of cardiopulmonary bypass. These data, along with recent data demonstrating relationships between temperature and postoperative blood loss (see below), underscore the importance of adequate rewarming following cardiopulmonary bypass to prevent platelet dysfunction and to reduce blood loss following cardiopulmonary bypass.

The effect of moderate and profound hypothermia on the hemostatic mechanism in man cannot be safely investigated without the institution of cardiopulmonary bypass. Hence it is difficult, in clinical studies, to differentiate between the effects of hypothermia and the effects of cardiopulmonary bypass per se on the hemostatic mechanism.

It has been recently reported that hypothermia reversibly inhibits human platelet activation in normal volunteers *in vitro* and

*in vivo*.<sup>146</sup> These results suggest that rewarming a hypothermic bleeding patient can reduce the need for the less safe alternative of transfusion of platelets and other blood components.

#### B. Hypothermia and Complement Activation

Systemic hypothermia, hemodilution, and the administration of heparin during cardiopulmonary bypass have been shown to protect the patient, in part, from the adverse effects of complement activation by reducing both the generation of C<sub>3</sub>a/C<sub>5</sub>a and the subsequent cellular response of neutrophil activation.<sup>147</sup>

### IV. CLINICAL CONSIDERATIONS

#### A. Anticoagulation During Cardiopulmonary Bypass

##### 1. Systemic Anticoagulation with Heparin

Anticoagulation with heparin is central to the conduct of cardiopulmonary bypass. As described in Chapter M2, heparin elicits its anticoagulant effect by catalyzing the action of antithrombin III with a resultant inhibition of thrombin formation. Heparin has also been shown to induce a platelet defect.<sup>148,149</sup> The contribution of heparin to the platelet defect during cardiopulmonary bypass is yet to be elucidated.

Heparin is administered prior to the institution of cardiopulmonary bypass in an initial intravenous dose of 3 mg/Kg. The

activated clotting time (ACT) is the most widely used measure of anticoagulation with heparin during extracorporeal circulation. Its routine use in gauging the doses of heparin required in the course of cardiopulmonary bypass offers distinct advantages over the unmonitored protocol-directed administration of heparin.<sup>150</sup> Baseline ACT levels, to which post-heparin-reversal levels are compared, must be established *after* the induction of anesthesia and the opening of the chest since anesthesia and surgery have been shown to reduce the ACT.<sup>151</sup> Although a number of alternatives to the use of the ACT have been advocated (e.g. thromboelastography and Sonoclot analysis<sup>152</sup> and *in-vivo* heparin protamine titration<sup>137</sup>), the clinical utility of this test has been amply demonstrated. ACT levels correlate well ( $R = 0.886$ ) with actual heparin levels<sup>153</sup> and provide adequate management during long term anticoagulation for extracorporeal respiratory assistance.<sup>154,155</sup> However, the clinician should realize that the ACT is prolonged by hypothermia<sup>156</sup> and by hemodilution.

The optimal level at which the ACT should be kept during cardiopulmonary bypass has been debated. In most centers, after the initial heparin dose, the ACT is maintained above 480 seconds by the periodic administration of 1 mg/Kg of heparin. Because compensated subclinical plasma coagulation activity<sup>157</sup> and gross fibrinous material within the pump oxygenator have been observed in patients with ACT levels around 400 seconds, some centers now maintain ACT levels during cardiopulmonary bypass around 600 seconds in an attempt to prevent subclinical consumptive coagulopathy. Others

have found it safe to maintain the ACT below 400 seconds<sup>158</sup>, and even between 250 and 300 seconds.<sup>159</sup>

The anticoagulant response to heparin is influenced by platelets, fibrin, vascular surfaces, and plasma proteins.<sup>160</sup> It is also influenced by hypothermia and hemodilution. The response is variable among patients and is disproportionately dependent on the dose and the duration of the treatment.<sup>160</sup> Patients receiving intravenous infusions of heparin prior to cardiac surgery require larger than usual doses of heparin to achieve adequate ACT levels during cardiopulmonary bypass. The plasma biologic half-life of an intravenous injection of heparin is not uniform but dose dependent<sup>161,162</sup>; for a dose of 1 mg/Kg it is 56 minutes, while for a dose of 4 mg/Kg it is 152 minutes.<sup>161</sup> Intravenous nitroglycerine has been considered to induce heparin resistance<sup>163,164</sup> but this is still uncertain.

The anticoagulant effect of heparin can be reversed at the termination of cardiopulmonary bypass by protamine sulfate in incremental doses until the ACT is brought back to its pre-heparinization level. Protamine has to be administered slowly because of its frequent hemodynamic effects, including hypotension.<sup>165</sup> Contrary to previous beliefs, the rapid administration of protamine into the aorta has not been shown to be safer than the administration into the central venous system and does not prevent or reduce adverse hemodynamic effects.<sup>166,167</sup>

Although not common, the administration of protamine may elicit a severe hemodynamic derangement, characterized by marked

pulmonary vasoconstriction, acute pulmonary hypertension and peripheral vascular collapse, which can be effectively treated with intravenous administration of prostaglandin E<sub>1</sub>.<sup>168</sup> Protamine sulfate has been shown to elicit complement activation by the classic pathway, and a correlation exists between the severity of complement activation and the subsequent hemodynamic derangements.<sup>169,170,171,172</sup> High levels of anti-protamine immunoglobulin E antibody have been identified in the serum of a sensitized patient with protamine-induced fatal anaphylaxis and in the serum of diabetic patients who had received insulin-containing protamine, indicating that the routine administration of protamine to susceptible individuals is inadvisable.<sup>173</sup>

An increase in the bleeding following cardiopulmonary bypass may occur secondary to "heparin rebound" which is thought to be due to 1) an increase in circulating levels of heparin, 2) increased amounts of antithrombin III, or 3) heparin-protamine complexes formed as a result of excess protamine.<sup>174</sup> Probably the most frequent cause of heparin rebound is the transfer of cold heparin-containing extracellular fluid from the periphery into the central circulation, which occurs as a result of rewarming and vasodilation in the postoperative period. Inadequate systemic rewarming prior to termination of cardiopulmonary bypass predisposes the patient to this phenomenon.

The transfusion of fresh frozen plasma may produce an increase in antithrombin III levels. Heparin may remain complexed in the presence of excess protamine, but is subsequently liberated as the protamine is metabolized, resulting in increased antithrombin III

activity. Increased protamine by itself does not have an anticoagulant effect. Although, when protamine is complexed with heparin, heparin rebound with increased postoperative bleeding may occur.<sup>174</sup> When chemical rather than biologic measurements were made of post-cardiopulmonary bypass heparin levels in samples from 27 patients undergoing routine coronary revascularization, there was no evidence of persistent heparin in 99.6% of the samples, raising doubts as to the actual presence of heparin rebound.<sup>175</sup>

## *2. Alternatives to Systemic Anticoagulation with Heparin*

*In vitro* and animal studies have clearly documented the biocompatibility of oxygenators and tubings coated with end-point attached heparin.<sup>176,177,178,179,180,181,182,183</sup> These experimental studies have demonstrated that, compared to non-coated circuits, heparin-coated surfaces (either in the absence or in the presence of low-level systemic heparinization) result in reduced complement and platelet activation, increased hematocrit and platelet count, decreased plasma hemoglobin level, improved intraoperative hemodynamics, and reduced postoperative blood loss and blood requirements. Early clinical experience with heparin-coated cardiopulmonary bypass circuits has been encouraging.<sup>181,182,184</sup> Although total cardiopulmonary bypass with a heparin-coated circuit and without systemic heparinization has not yet been achieved, the use of heparin-coated membrane oxygenators and tubings has reduced the need for heparin, has improved postoperative hematocrit, and has

decreased postoperative bleeding.<sup>181</sup> Partial (left heart) bypass with heparin-coated equipment without systemic heparinization has been successfully used in patients undergoing resection of thoracoabdominal aneurysms.<sup>182</sup> Heparin-coating is a promising technology; its clinical application should expand rapidly in the near future, reducing the need for full systemic heparinization.

Experimental studies also have demonstrated the feasibility of interposing in the bypass circuit an immobilized heparinase reactor filter<sup>185</sup> and an immobilized protamine bio-reactor filter<sup>186,187</sup>, which would eliminate heparin from the blood returned to the patient and reduce both heparin anticoagulant activity and the need for protamine (with its potential adverse reactions). This technology has not yet been used clinically.

Decreasing the average molecular weight of heparin decreases its antithrombin activity while retaining its anti-Xa activity<sup>188,189</sup>(see Chapter M2). Hence, a variety of low-molecular-weight heparins, heparin fractions and heparinoids are currently being explored as alternatives to unfractionated heparin for anticoagulation during cardiopulmonary bypass. The ability of these compounds to anticoagulate blood is measured by their anti-Xa activity in units/kg body weight. Experimental studies in animals, which included dogs placed on cardiopulmonary bypass, have demonstrated that these compounds are associated with a lower incidence of hemorrhage than standard heparin at equivalent anti-Xa activity.<sup>190,191,192</sup> However, low molecular weight heparins may produce complications when emergency neutralization procedures are

required.<sup>193</sup> When used as anticoagulants for cardiopulmonary bypass, severe postoperative hemorrhage has been encountered.<sup>194,195</sup> The use of fractionated low molecular weight heparins and heparinoids for anticoagulation during cardiopulmonary bypass should be restricted to situations in which a standard heparin regimen cannot be used, such as in severe heparin-induced thrombocytopenia and thrombosis.<sup>189,194,195,196,197</sup>

Hirudin and ancrod are two other pharmacologic agents that may potentially replace heparin during cardiopulmonary bypass. Hirudin and hirudin fragments are antithrombin III-independent thrombin inhibitors which, unlike heparin, can inactivate thrombin bound to fibrin.<sup>198,199,200</sup> Recombinant hirudin has been used effectively as an anticoagulant during cardiopulmonary bypass in experimental animals.<sup>201</sup> Clinical cardiopulmonary bypass studies using hirudin and its analogs in humans are currently in progress. The specificity of these compounds for thrombin and their ability to penetrate formed clots make them very promising for use as heparin substitutes.

Initial studies with ancrod, a defibrinogenating enzyme, in animals and in man have demonstrated it to be safe and effective as a substitute to heparin during cardiopulmonary bypass.<sup>202</sup> Confirmatory studies are necessary to determine the safety and efficacy of ancrod (arvin).

### ***3. Heparin-Induced Thrombocytopenia***

Heparin-induced thrombocytopenia is of two types.<sup>203</sup> One type is a transient thrombocytopenia of immediate onset and mild degree and accompanies heparin therapy in approximately 5% of patients.<sup>203</sup> The mechanism is probably non-immune and related to a direct proaggregatory effect of heparin.<sup>204</sup> The other type occurs much less frequently in patients receiving heparin and is a delayed, severe and probably immune-mediated thrombocytopenia that may occur in association with platelet activation, aggregation, and, on occasion, massive arterial thrombosis.<sup>203</sup> Heparin-induced thrombocytopenia is discussed in detail elsewhere in Chapter G3. Patients known to be predisposed to heparin-induced thrombocytopenia and thrombosis present a major challenge if they need to be placed on cardiopulmonary bypass. Management of these patients has included pretreatment with aspirin and dipyridamole<sup>205</sup>, pretreatment with iloprost<sup>37</sup>, and anticoagulation with low-molecular-weight heparins and heparinoids.<sup>185,188,195,196,197</sup> Of particular promise in the treatment of this difficult patient group is the use of ancrod<sup>206</sup> or hirudin<sup>201</sup> instead of heparin for anticoagulation during cardiopulmonary bypass.

#### B. The Clinical Utility of the Measurement of the Bleeding Time

The bleeding time at two hours post-cardiopulmonary bypass correlates with the preoperative bleeding time, the skin temperature, the duration of cardiopulmonary bypass, and the initial postoperative blood loss.<sup>16</sup> (Figure 11) Thus, the bleeding time

measurement is useful in the diagnosis and management of excessive non-surgical post-cardiopulmonary bypass blood loss. Although the preoperative bleeding time correlates with the postoperative bleeding time, it does not predict the amount of postoperative blood loss under normal circumstances.<sup>16,47,69,207,208,209,210,211,212,213,214</sup> Marked extensions in the preoperative bleeding time, however have tended to result in increased postoperative blood loss.<sup>207</sup> It has not yet been established whether or not prolonged preoperative bleeding times in patients receiving preoperative aspirin are predictive of excessive bleeding post-cardiopulmonary bypass. There are data to suggest that preoperative aspirin ingestion prolongs the preoperative bleeding time but does not influence post-cardiopulmonary bypass bleeding time.<sup>215</sup> Conversely, a blinded randomized, placebo-controlled Veterans Administration Cooperative Study has demonstrated a significant increase in post-cardiopulmonary bypass blood loss in patients receiving preoperative aspirin.<sup>216</sup> Unfortunately, no bleeding time measurements were obtained in this study. The validity of published observations related to this issue is limited by a) the recent demonstration of the temperature-dependence of the bleeding time measurement,<sup>17,146,217</sup> and b) the failure in most of the published studies to differentiate and quantify properly and prospectively non-surgical blood loss (see Section IV.C. below). Properly conducted prospective studies are needed to demonstrate the relationship between an excessively prolonged preoperative bleeding time and the postoperative blood loss. It is imperative, however, that the clinical measurement and

interpretation of the bleeding time take into account the correlation between the bleeding time and skin temperature.<sup>217</sup>

### C. Determinants and Treatment of Blood Loss Following Cardiopulmonary Bypass

Increased blood loss following the institution of cardiopulmonary bypass may be either "surgical" or "nonsurgical" in nature. Blood loss from a specific anatomic site as a result of the surgical procedure itself is referred to as "surgical", while diffuse blood loss which is not associated with a specific anatomic site and which reflects a generalized bleeding diathesis is referred to as "non-surgical". Most of the reported clinical studies which have attempted to quantify postoperative blood loss have been deficient in that they have either failed to differentiate between these two types of blood loss or have assumed that post-cardiopulmonary bypass blood loss can be accurately measured only by measuring postoperative chest drainage in the intensive care unit.

#### 1. *Quantification and Determinants of Post-Cardiopulmonary Bypass Blood Loss*

The hemostatic defect elicited by cardiopulmonary bypass may lead to excessive "non-surgical" postoperative blood loss which may be difficult to control. Quantifying this type of blood loss should include accurate measurements that are made intraoperatively, while the chest is still open, after complete neutralization of heparin as evidenced by the ACT. Quantification should include measurement of the volume of blood aspirated into the wall suction and/or the Cell

Saver during this period as well as the volume of blood collected into mediastinal and chest tubes if present. In addition, all sponges and laparotomy pads used during this period should be weighed and their blood contents recorded and added to the total blood loss.

Any discussion of the treatment of post-CPB blood loss must stress the importance of the prevention of excessive post-CPB blood loss. Preventive measures include 1) performing an adequate preoperative workup of the cardiac surgical patient, 2) paying meticulous attention to proper intraoperative surgical techniques, and 3) understanding the determinants of postoperative blood loss following cardiopulmonary bypass. The preoperative workup should include an assessment of the patient's bleeding tendency and related family history.<sup>218</sup> Preoperative ingestion of aspirin increases post-cardiopulmonary bypass bleeding<sup>215</sup> but need not deter the cardiac operation. Preoperatively it is reasonable to obtain a partial thromboplastin time, prothrombin time, platelet count and a template bleeding time.<sup>219</sup>

Probably the single most important factor in the prevention of excessive post-cardiopulmonary bypass blood loss is the meticulous attention to surgical technique intraoperatively. An adequate preoperative workup coupled with good intraoperative surgical technique have enabled the performance of routine cardiac surgery without the need for administering blood products in up to 50% of the patients.

An understanding of the determinants of post-cardiopulmonary bypass blood loss<sup>16,17</sup> is important for both the prevention and the treatment of excessive postoperative blood loss. The most important determinant of post-cardiopulmonary bypass blood loss is the duration of the period of cardiopulmonary bypass, underscoring the importance of expeditious surgery in the prevention of postoperative blood loss.<sup>16</sup> As shown in Figure 12, the postoperative blood loss during the initial four hours following cardiopulmonary bypass is related directly to the postoperative bleeding time and inversely related to the postoperative hematocrit; the intra- and postoperative esophageal and wound temperatures, platelet count and platelet mass, and the postoperative level of plasma C<sub>3</sub>.

## 2. *Treatment of "Non-surgical" Postoperative Blood Loss*

a. Observation of good surgical technique should reduce to a minimum any "surgical" bleeding, thus making it possible to ascertain that the observed generalized coagulopathy is indeed "non-surgical" in nature.

b. Adequate rewarming and maintenance of normothermia should be the first measure in the prevention and treatment of excessive postoperative blood loss. The rationale for this was elucidated in Section III above and is based on the observed relationships between temperature and post-cardiopulmonary bypass platelet function and blood loss.

c. Transfusion of miscellaneous blood products should be administered following significant blood loss. Autologous blood, collected over a period of 3-4 weeks preoperatively, is the optimal replacement although it may not be logistically feasible in a number of patients.<sup>220,221,222</sup> Phlebotomy of whole blood immediately prior to the institution of cardiopulmonary bypass and its subsequent postoperative reinfusion has not been consistently beneficial.<sup>223,224,225,226,227</sup> In 1986, a consensus conference sponsored by the National Institutes of Health judged that the routine use of platelets in cardiac surgery is unnecessary.<sup>228</sup> Newer methods and techniques have made possible the sequestration of platelet rich plasma (with retransfusion of the red blood cells) immediately prior to institution of cardiopulmonary bypass and reinfusion of this plasma postoperatively after discontinuation of bypass. This technique spares the platelets, in part, from being affected by the extracorporeal circuit. Preservation of platelet number, a decrease in the postoperative blood loss, and a decrease in the requirement for homologous blood transfusions have been reported with this technique.<sup>229,230,231,232</sup> Cell savers are routinely used in the course of open heart surgery, and washed red cells are autotransfused after completion of cardiopulmonary bypass. Blood shed from the pleura and mediastinum through the chest tubes during the first 24 hours is either washed or not before reinfusion in an effort to reduce the need for postoperative homologous blood transfusion.<sup>233,234,235,236</sup> Transfusion of up to a liter of unwashed shed mediastinal blood is safe and except for its possible effect on

the hematocrit does not impact on any of the hematologic or related parameters of the recipient patient.<sup>237</sup> The safety of larger transfusions of unwashed, shed mediastinal blood has yet to be determined. In the clinical setting, it is almost impossible to specifically identify the cause of excessive postoperative "non-surgical" bleeding. Hence the treatment of this condition usually entails the transfusion of platelets (see Chapter L1), cryoprecipitate, and fresh frozen plasma (see Chapter L2). Platelets administered to bleeding patients with thrombocytopenia and thrombocytopathies must be compatible, viable and functional. Actively bleeding patients who lack adequate numbers of functional platelets should receive platelets that have been stored at room temperature for no more than 24 hours.<sup>238</sup>

d. Various pharmacologic agents have been used to reduce bleeding after cardiopulmonary bypass. Prostaglandin E<sub>1</sub> and prostacyclin have been shown to reduce platelet loss in in-vitro simulation of extracorporeal circulation.<sup>34,35,239</sup> However, randomized double-blind controlled trials of prophylactic prostacyclin administration in patients undergoing CPB have not shown clear evidence of benefit.<sup>38,39,64,65,239,240,241,242</sup> Furthermore, prostacyclin and its analogue Iloprost cause severe vasodilation and hypotension.<sup>38,39,64,65,239</sup> Both dog<sup>40</sup> and human<sup>41</sup> studies have also shown that the preoperative administration of dipyridamole preserves the platelet count and reduces blood loss following cardiac surgery. The routine clinical use of these pharmacologic agents, however, has not been established.<sup>65</sup>

Since excessive fibrinolysis is an important component of the hemostatic defect of cardiopulmonary bypass, antifibrinolytic agents (Chapter L3) have been proposed. The use of amino caproic acid (Amicar) in selected patients has proved to be effective in controlling excessive postoperative blood loss following cardiopulmonary bypass.<sup>131,136,243</sup> Its prophylactic use in all patients, however, is not justified.<sup>244</sup> Tranexamic acid (Cyclokapron) is an isomer of epsilon aminocaproic acid, with 7 to 10 times its inhibitory activity.<sup>245</sup> It has been recently shown to be effective in reducing blood loss when administered prophylactically to patients undergoing cardiac surgery.<sup>246</sup> This promising report, however, awaits confirmation.

Intravenous desmopressin acetate (DDAVP) (Chapter L3) has been used to reduce post-cardiopulmonary bypass blood loss because of its potential to raise the plasma levels of the vWF and its multimers. However, inconsistent and apparently contradictory results have been reported with the use of this drug in the postoperative period.<sup>70,247,248,249,250</sup> The original report by Salzman et al.<sup>247</sup> showed DDAVP to be beneficial in increasing the vWF level and in reducing the postoperative blood loss in patients undergoing complex valvular heart surgery. Subsequent studies in patients undergoing routine coronary artery revascularization<sup>70</sup> and in children undergoing cardiac operations<sup>250</sup> showed that the administration of DDAVP did not increase the levels of the vWF over and above the rise ordinarily observed postoperatively in the cardiac patient, nor did it reduce the postoperative blood loss. These latter results, along with the

finding that the level of vWF in a large group of patients undergoing coronary and valvular operations did not correlate with post-cardiopulmonary bypass blood loss<sup>16</sup>, indicate that DDAVP should not be used as a routine adjunct in cardiac surgery.

Aprotinin, a protease inhibitor, has a more consistent record of significantly reducing postoperative blood loss: reductions of 40 to 50% were observed when this pharmacologic agent was administered in high doses before and throughout the period of cardiopulmonary bypass.<sup>97,98,128,140,251,252</sup> Although the exact mechanism by which aprotinin achieves this impressive salutary effect is not fully understood, there is evidence to suggest that aprotinin inhibits kallikrein, improves platelet function, and reduces fibrinolytic activity.<sup>100,128,140,253,254</sup> The administration of aprotinin in the presence of heparin causes a prolongation of the ACT and the aPTT. This has led some investigators to hypothesize that a synergistic relationship exists between aprotinin and heparin, and to advocate reducing the usual heparin dose in patients receiving aprotinin during cardiopulmonary bypass.<sup>255</sup> In a more recent study, ACT prolongation was found to occur in the presence of aprotinin and heparin when celite-activated tubes were used for the measurement of the ACT but not when a different activating reagent, kaolin, was used.<sup>256</sup> Hence, in the presence of high dose aprotinin during cardiopulmonary bypass, the heparin dose should not be reduced and the ACT should be measured utilizing kaolin-activated tubes. Concern has been expressed about the possibility of aprotinin resulting in increased incidence of post-operative aorto-coronary

graft occlusion. A recent placebo-controlled double blind study conducted in 96 patients showed that early (7-10 day) vein graft patency was not adversely affected by high dose aprotinin.<sup>257</sup> Aprotinin is probably the most promising drug for the prevention or reduction of post-cardiopulmonary bypass blood loss; its routine clinical use in the U.S. currently awaits the approval of the Food and Drug Administration.

## V. SUMMARY

During cardiopulmonary bypass surgery, thrombocytopenia is mainly the result of hemodilution and removal of activated platelets from the circulation. Hemodilution also accounts for the decreases in blood coagulation proteins during cardiopulmonary bypass. Fibrinogen is also adsorbed preferentially to the synthetic surfaces of the bypass circuit. Although the hemostatic defect observed during cardiopulmonary bypass is not fully understood, one known aspect of this defect is a platelet dysfunction characterized by the prolongation of the bleeding time. The cardiopulmonary bypass-induced defect in platelet function has not been completely characterized. Although decreases in platelet surface GPIb (the von Willebrand factor receptor) and the GPIIb-IIIa complex (the fibrinogen receptor) have been described, recent evidence suggests that the "platelet function defect" of cardiopulmonary bypass is the result of a lack of factors extrinsic to the platelet. Hypothermia is an important factor in the genesis of the platelet function defect and accounts, in part, for the reversibility of the prolonged bleeding time. The release of large platelets into the circulation also accounts, in part, for the reversal of the prolonged bleeding time. The pathophysiological significance of the cardiopulmonary bypass-induced increase in platelet membrane microparticles remains to be determined. Previously reported evidence of selective platelet  $\alpha$ -granule release from circulating platelets during cardiopulmonary bypass has not been supported by recent studies in

whole blood. Hyperfibrinolysis following the administration of heparin and the institution of cardiopulmonary bypass also contributes to the hemostatic defect observed during and following cardiac surgery. There is a direct relationship between postoperative bleeding time, temperature, and postoperative blood loss in patients undergoing cardiopulmonary bypass. The duration of cardiopulmonary bypass is the main determinant of the postoperative bleeding time and blood loss. Adequate rewarming and expeditious surgery should reduce the hemostatic abnormality and the postoperative blood loss in cardiac surgery. Of the pharmacologic interventions, aprotinin appears to be the most promising in reducing post-cardiopulmonary bypass blood loss, but the mechanism of this salutary effect remains to be elucidated.

## LEGENDS TO FIGURES

Figure 1. Normal platelet physiology. See text for explanation. Abbreviations:  $\beta$ TG,  $\beta$ -thromboglobulin; P-selectin, granule membrane protein 140; GP, glycoprotein; PF4, platelet factor 4; TSP, thrombospondin; vWF, von Willebrand factor

Figure 2. The means  $\pm$  SEM of the bleeding time (corrected for skin temperature) in 87 patients undergoing isolated coronary artery revascularization at the Brockton/West Roxbury VA Medical Center. Blood samples were obtained in a subgroup of 24 patients. Abbreviations: CBT, corrected bleeding time<sup>217</sup>; CPB, cardiopulmonary bypass; PreCPB, before cardiopulmonary bypass; PreHep, before the administration of heparin; PostHep, 5 minutes after the administration of heparin and before institution of CPB; min, minutes after the institution of CPB; Comp, at completion of CPB; HR POST-CPB, hours after completion of CPB.

Figure 3. The means  $\pm$  SEM of the platelet count (PLT) and the mean platelet volume (MPV) in 77 patients undergoing isolated coronary artery revascularization at the Brockton/West Roxbury VA Medical Center. Abbreviations: Same as in Figure 2.

Figure 4. The means  $\pm$  SEM of the plasma  $\beta$ -thromboglobulin (BTG) and plasma thromboxane B<sub>2</sub> (TXB<sub>2</sub>) in 45 patients undergoing isolated coronary artery revascularization at the Brockton/West Roxbury VA Medical Center. Abbreviations: On CPB, 20 minutes after institution of cardiopulmonary bypass; others same as in Figure 2.

Figure 5. The means  $\pm$  SEM of thromboxane B<sub>2</sub> (TXB<sub>2</sub>) and 6-ketoPGF<sub>1 $\alpha$</sub>  (6-Keto) in the blood shed from the site of the bleeding time determination in 57 patients undergoing isolated coronary artery revascularization at the Brockton/West Roxbury VA Medical Center. Abbreviations: Same as in Figure 2.

Figure 6. The means  $\pm$  SEM of the hematocrit (HCT) in 76 patients and the serum albumin (ALB) in 47 patients undergoing isolated coronary artery revascularization at the Brockton/West Roxbury VA Medical Center. PreCPB, PreHep, PosHep, 5min CPB, and 45min CPB samples were obtained in a subgroup of 24 patients. Abbreviations: Same as in Figure 2.

Figure 7A and 7B. The means  $\pm$  SEM of plasma concentrations of opsonic proteins in 47 patients undergoing isolated coronary artery revascularization at the Brockton/West Roxbury VA Medical Center. Abbreviations: C<sub>3</sub>, complement C<sub>3</sub>; FIB, fibronectin; IgM, immunoglobulin M; IgG, immunoglobulin G; others same as in Figure 2.

Figure 8. The means  $\pm$  SEM of plasma concentrations of coagulation proteins in 61 patients undergoing isolated coronary artery revascularization at the Brockton/West Roxbury VA Medical Center. Samples were obtained in a subgroup of 24 patients at PreHep, PostHep, 5min CPB, and 45min CPB. Abbreviations: FVIII, factor VIII clotting protein; vWF, von Willebrand factor; others same as in Figure 2.

Figure 9. The means  $\pm$  SEM of plasma concentrations of plasminogen and anti-thrombin III (ATIII) in 51 patients undergoing

isolated coronary artery revascularization at the Brockton/West Roxbury VA Medical Center. Abbreviations: Same as in Figure 2.

Figure 10. The means  $\pm$  SEM of serum concentrations of D-Dimer in 22 patients undergoing isolated coronary artery revascularization at the Brockton/West Roxbury VA Medical Center. Abbreviations: Same as in Figure 2.

Figure 11. The shed blood thromboxane B<sub>2</sub> (TxB<sub>2</sub>) level, the skin temperature, and the bleeding time measured simultaneously from the warm and the cold arm during and following hypothermic cardiopulmonary bypass in 37 patients undergoing cardiopulmonary bypass surgery. One arm (WARM ARM) was kept warm with a water-filled blanket set at 40 °C while the other arm (COLD ARM) was allowed to follow the systemic temperature. Hypothermia results in a significant extension of the bleeding time and a significant reduction in the TxB<sub>2</sub> level in the blood shed from the bleeding time site. (*Reproduced with permission from the publishers of the Journal of Thoracic and Cardiovascular Surgery.*)

Figure 12. Preoperative, intraoperative and postoperative variables in tierciles of the blood loss during the initial 4 hours post-cardiopulmonary bypass in 76 patients undergoing valvular and coronary artery surgery. The tiercile levels designated were low: 215-790 ml, n=26; medium: 805-1140 ml, n=26; and high: 1235-2515 ml, n=26. (*Reproduced with permission from the publishers of the Journal of Thoracic and Cardiovascular Surgery.*)

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TABLE 1 Possible Causes Of The Platelet Function Defect During  
Cardiopulmonary Bypass

CAUSE	REFERENCE
Contact with synthetic surfaces	18
Shear force	23
Hypothermia	17
Lack of availability of platelet agonists	85
Plasmin generated by a fibrinolytic state	47
Fibrin(ogen) degradation products	47
Thrombin	30
ADP	14
Oxygenation	47
Denatured plasma proteins	18
Heparin	203
Protamine	88
Aspirin	258
Sodium nitroprusside	259
Cyanotic congenital heart disease	78

TABLE 2 Comparison of Observed and Dilution-Corrected Protein Concentrations During Cardiopulmonary Bypass In 49 Patients<sup>†</sup> Receiving A Crystalloid Pump Prime

<u>PROTEINS</u>	<u>PRE-OP</u>	<u>OBSERVED</u>	<u>ON-BP</u>	<u>CORRECTED</u>
	ALBUMIN (g/dl)	3.4 ± 0.1	1.8 ± 0.1 **	
TP (g/dl)	6.4 ± 0.1	3.3 ± 0.1 **		7.2 ± 0.2 **
vWF (μ/ml)	1.6 ± 0.1	0.8 ± 0.1 **		1.7 ± 0.1 ns
ATIII (%)	103 ± 2	60 ± 3 **		132 ± 7 **
PLMGN (%)	101 ± 3	55 ± 2 **		119 ± 4 **
C <sub>3</sub> (mg/dl)	143 ± 5	61 ± 2 **		134 ± 5 *
IgG (mg/dl)	989 ± 31	453 ± 18 **		997 ± 39 ns
IgM (mg/dl)	143 ± 12	53 ± 4 **		119 ± 10 **
FBN (μg/ml)	396 ± 18	216 ± 13 **		466 ± 21 **

\* p<0.01, \*\* p≤0.0001 Observed/Corrected values compared to Pre-op values

- ns, non-significant

- Pre-Op, prior to anesthesia; ON-BP, during cardiopulmonary bypass

- TP, total protein; vWF, von Willebrand factor antigen; ATIII, antithrombin III; PLMGN, plasminogen; C<sub>3</sub>, complement; IgG, immunoglobulin G; IgM, immunoglobulin M; FBN, fibronectin.

- <sup>†</sup> Undergoing uncomplicated isolated coronary artery bypass graft surgery at the Brockton/West Roxbury VA Medical Center.

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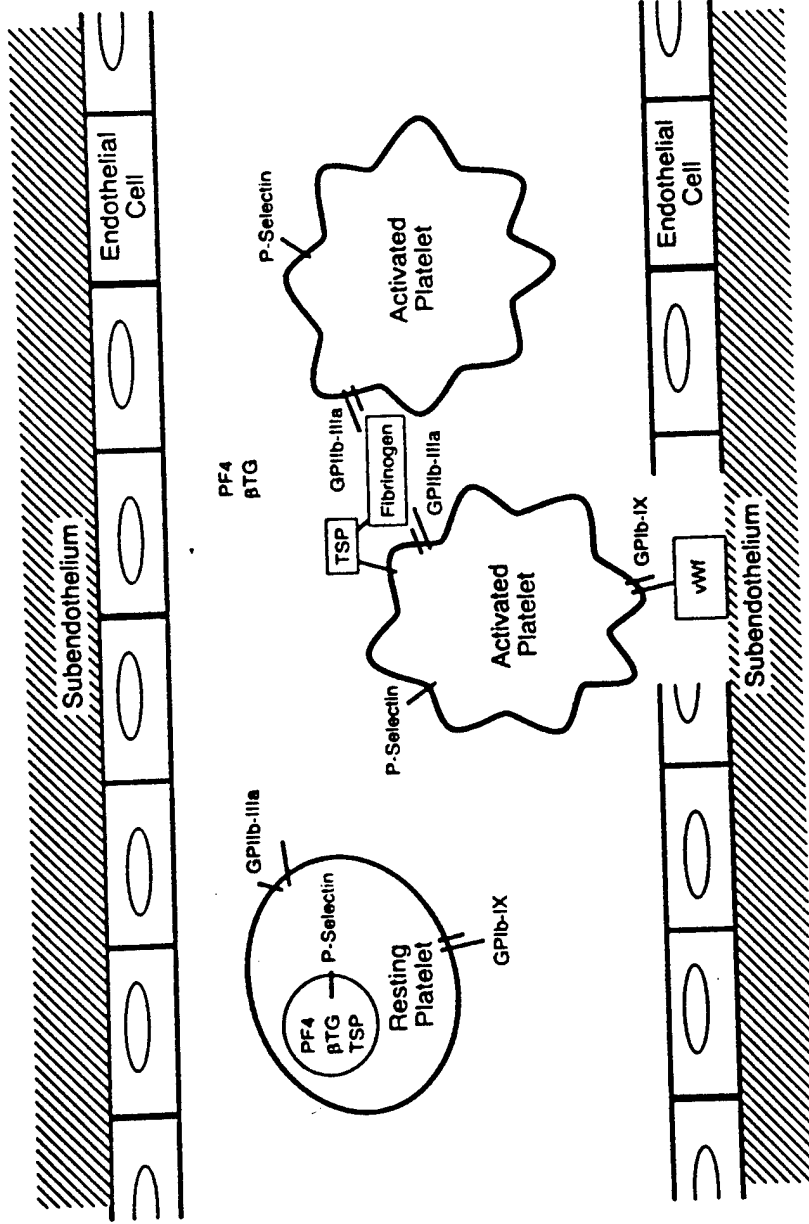
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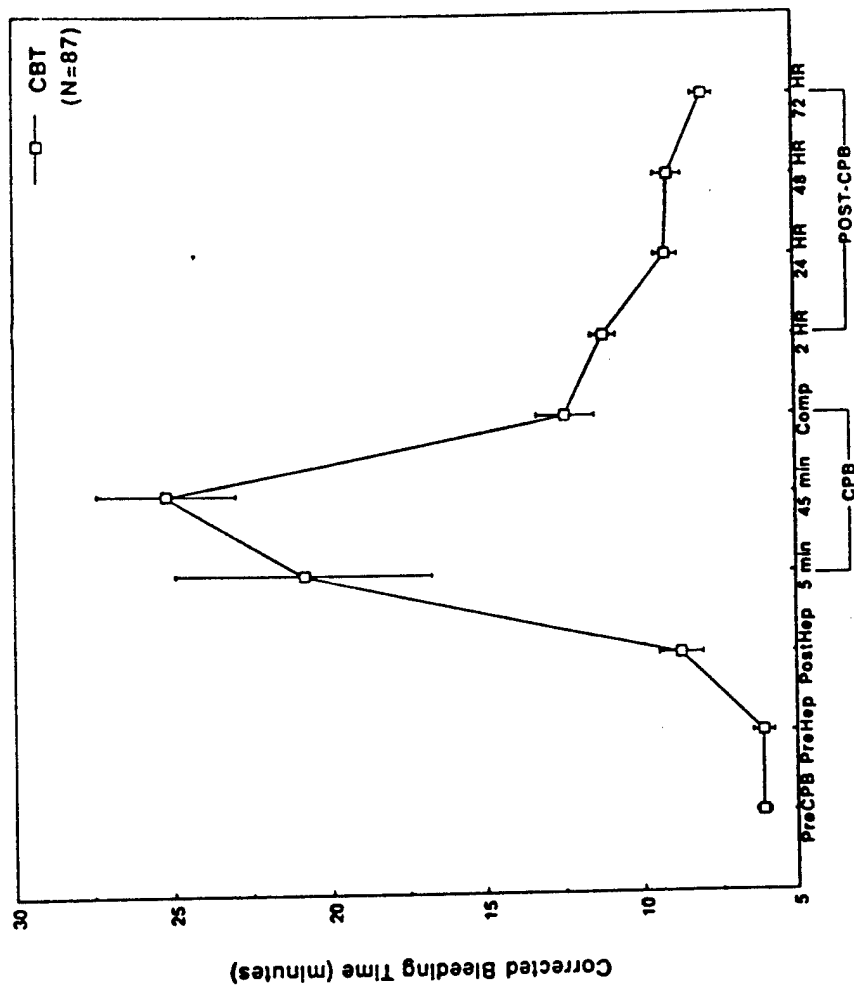
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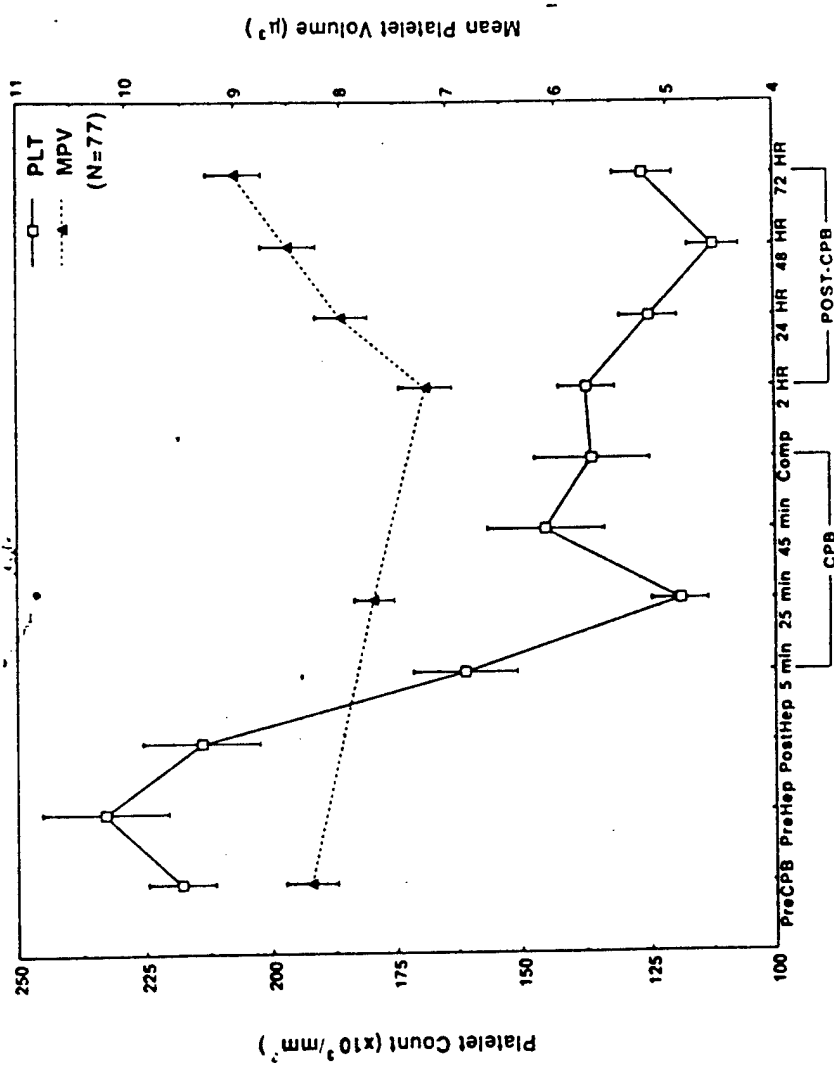
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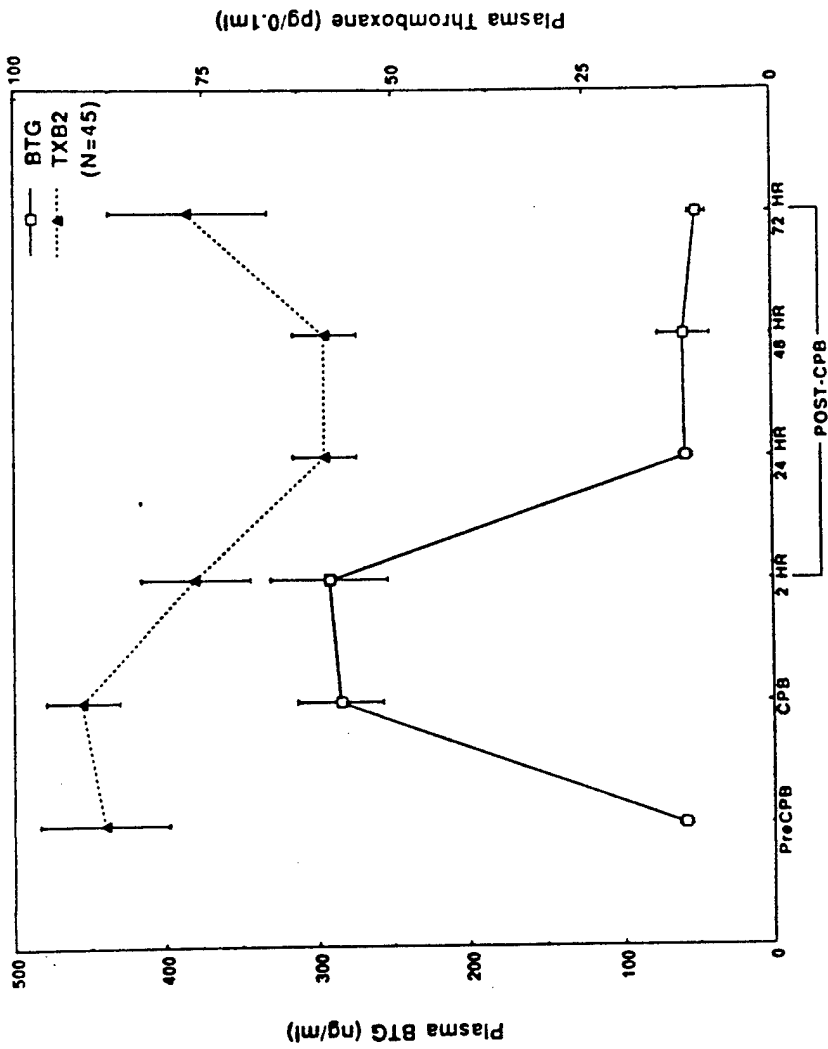
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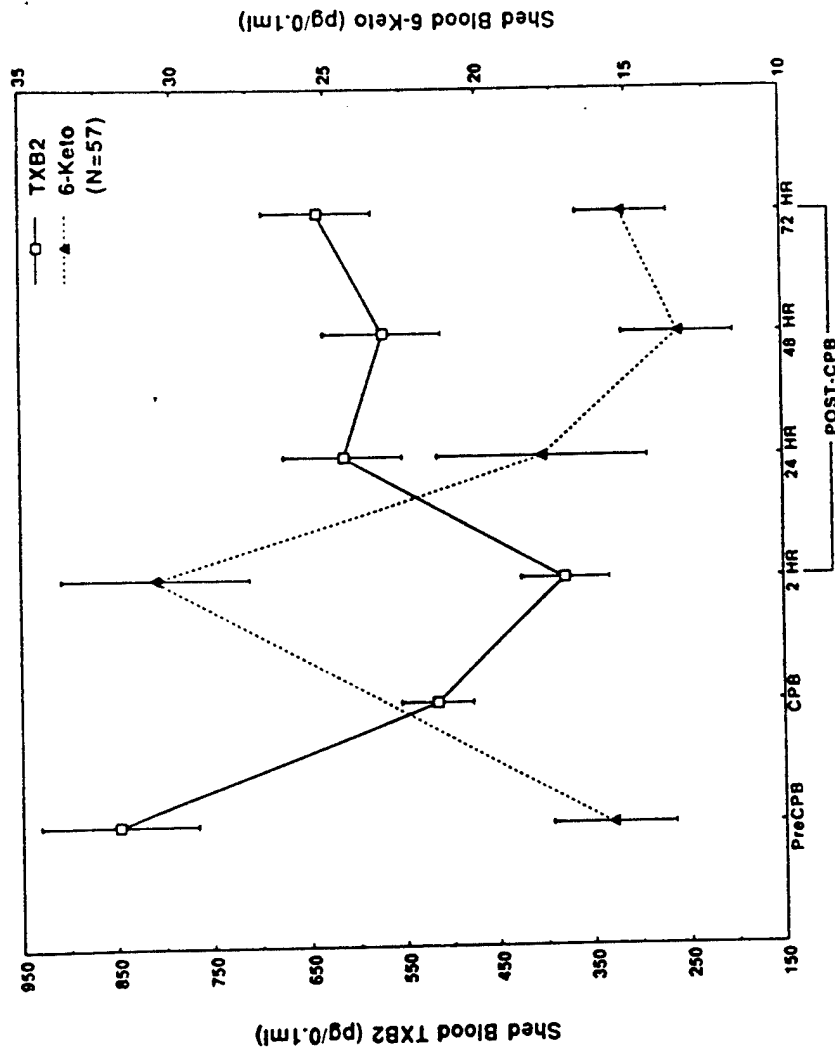
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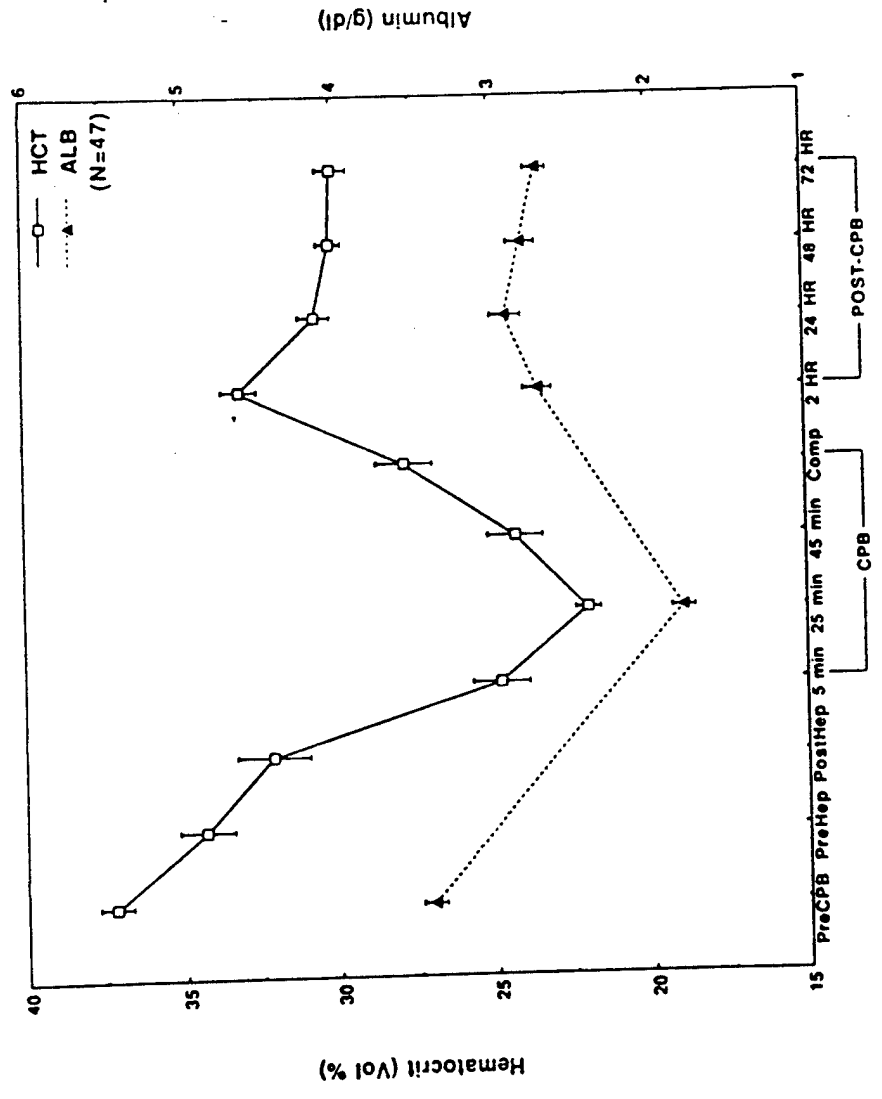


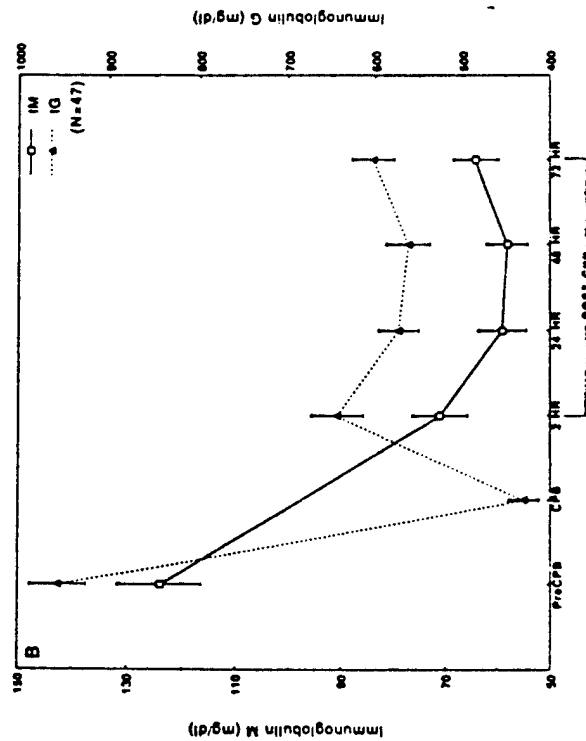
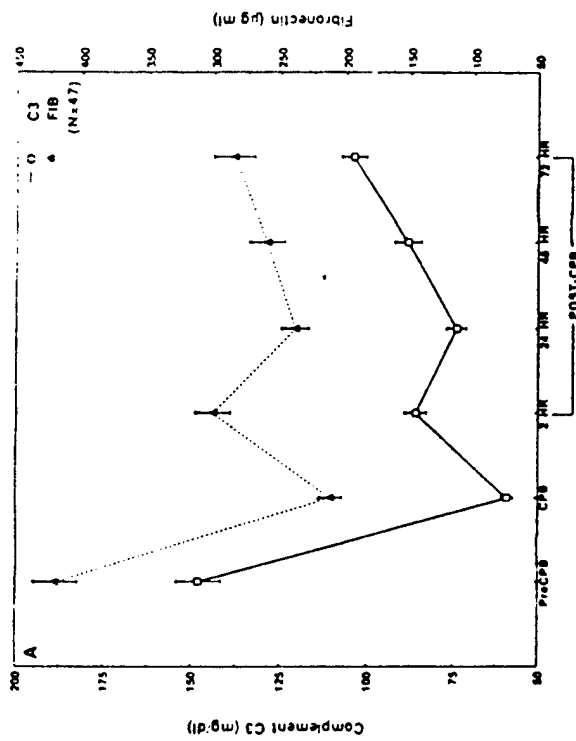












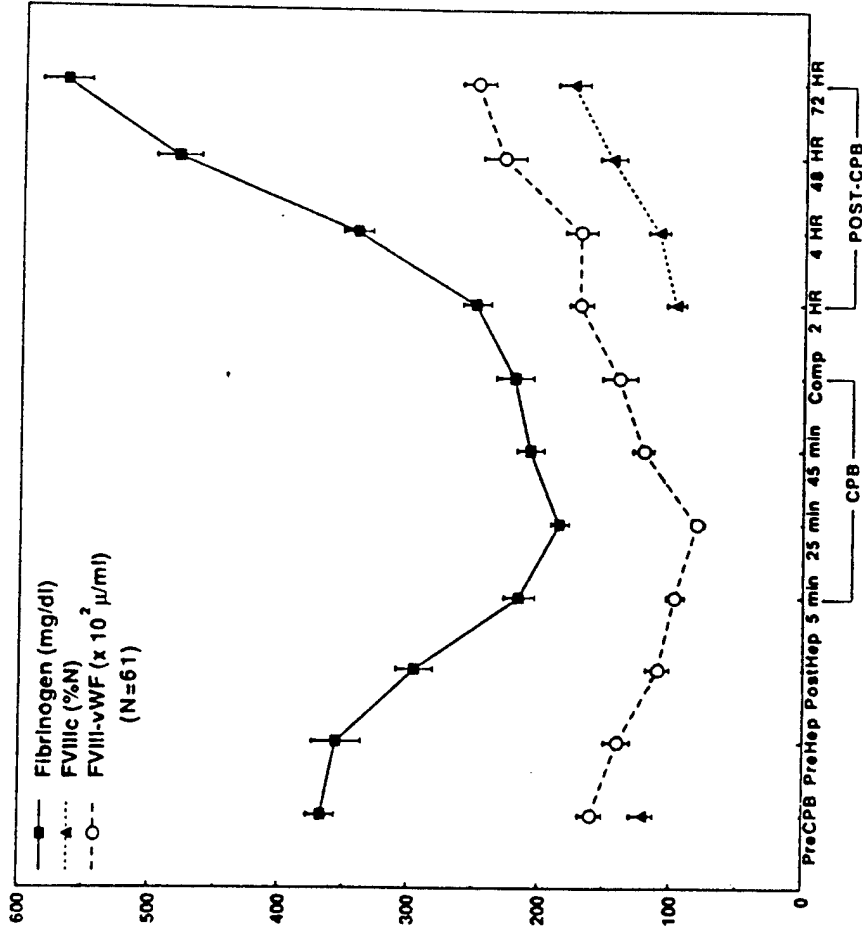
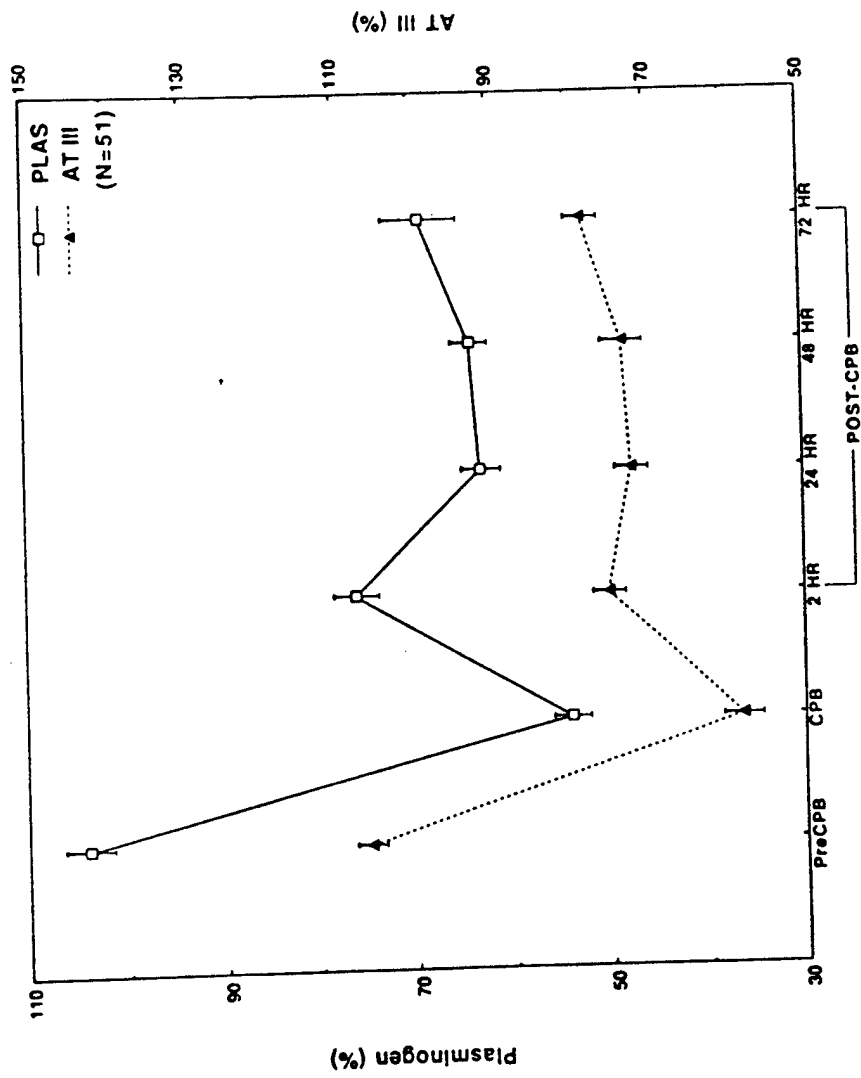


FIG. 8



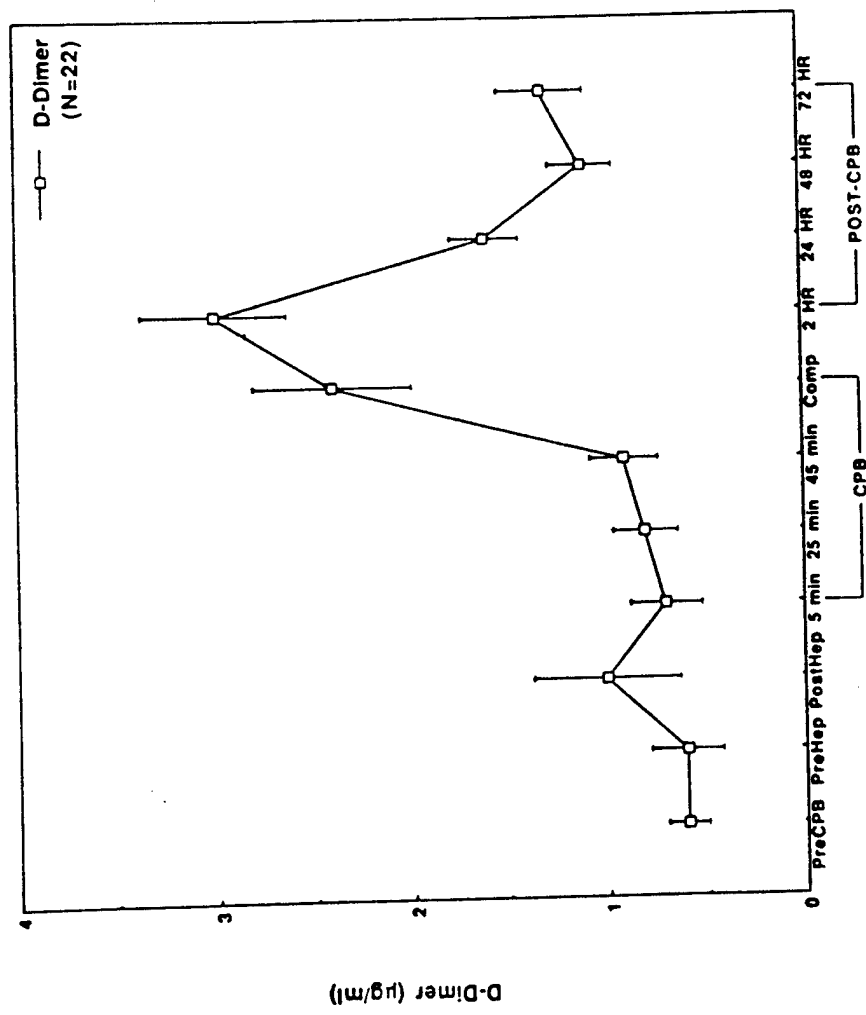


Fig 10

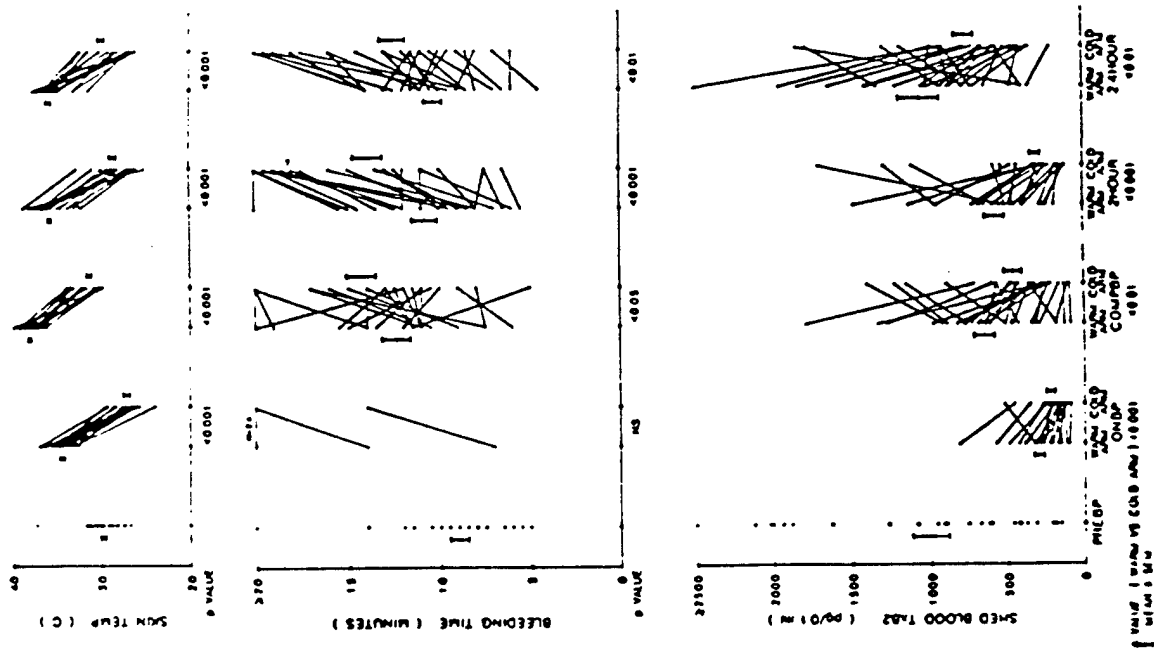


FIG. 11

Tierciles of Total Blood Loss During the Initial 4 Hour Period Post-CPB

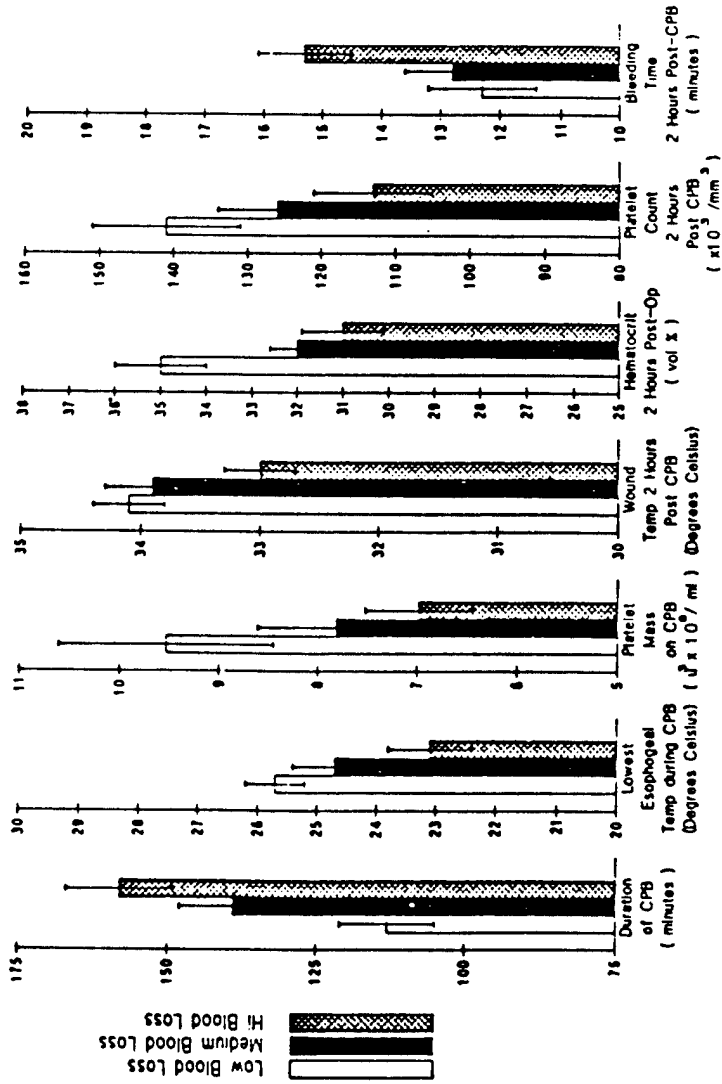


Fig. 12