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INFUSION OF AUTOLOGOUS LYSED PLASMA INTO THE BABOON: ASSESSMENT OF COAGULATION, PLATELET, AND PULMONARY FUNCTION

BY

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creating a fibrinolytic state in which plasmin was generated and fibrinogen and fibrin were cleaved. Aprotinin (1000 KIU/ml) was subsequently added to inhibit plasmin. This state resulted in a transient increase in activated partial thromboplastin time without a similar increase in thrombin time or prothrombin time, and a marked prolongation of the bleeding time lasting at least 24 but less than 48 hours. These changes may be attributed to the effects of FDP and D-dimer or, alternatively, to the decrease in fibrinogen. No untoward effects of this state were observed clinically. No change in pulmonary or renal function was seen.

ABSTRACT

Autotransfusion of shed blood, cardiopulmonary bypass and thrombolytic therapy are all conditions known to elicit a fibrinolytic state in man, with generation of plasmin and production of degradation products of fibrinogen and fibrin. The role of plasmin and of these degradation products in producing the coagulopathy, platelet dysfunction and pulmonary dysfunction sometimes seen in these states remains highly controversial.

Eight baboons were infused with 250 cc of autologous plasma treated with urokinase in doses of 3000 IU/ml or 5000 IU/ml, creating a fibrinolytic state in which plasmin was generated and fibrinogen and fibrin were cleaved. Aprotinin (1000 KIU/ml) was subsequently added to inhibit plasmin. This state resulted in a transient increase in activated partial thromboplastin time without a similar increase in thrombin time or prothrombin time, and a marked prolongation of the bleeding time lasting at least 24 but less than 48 hours. These changes may be attributed to the effects of FDP and D-Dimer or, alternatively, to the decrease in fibrinogen. No untoward effects of this state were observed clinically. No change in pulmonary or renal function was seen.

Autotransfusion of shed blood, cardiopulmonary bypass and thrombolytic therapy are all conditions known to elicit a fibrinolytic state in man, with generation of plasmin and production of degradation products of fibrinogen and fibrin. The role of plasmin and of these degradation products in producing the coagulopathy, platelet dysfunction and pulmonary dysfunction sometimes seen in these states remains highly controversial. It was the goal of this study to investigate the influence of fibrinogen and fibrin degradation products on hemostatic and pulmonary function in a healthy, normovolemic baboon model.

FIBRINOGEN, FIBRIN & CLOTTING

Fibrinogen is a 340 kilodalton molecule, 450 Angstrom in length. It is dimeric in structure, consisting of three pairs of polypeptide chains, referred to as the A(alpha), B(beta), and gamma chains (Figure 1). The N-terminal ends of these chains are linked to one another by disulfide bonds in a central region known as the N-terminal disulfide knot (NDSK) (Kwaan & Barlow, 1973; Marder & Budzyski, 1975; Budzynski & Marder, 1977; Doolittle, 1984).

The structure of fibrinogen was first proposed by Hall & Slayter in 1959 as an extended, multidomained molecule. Electron microscopy, amino acid sequencing and proteolytic studies have since confirmed such a model. As it is now conceived, the model of fibrinogen (Figure 2) encompasses two terminal domains, each approximately the size of an albumin molecule (63 kilodaltons). Each terminal domain is tethered by a three-stranded 'coiled-coil', 150 Angstrom in length, to a dimeric central domain (34 kilodaltons) containing the N-terminal region of each of the six chains. Several inter- and intrachain disulfide bonds are noted, most notably, three between the two dimeric halves and one between each A(alpha) and B(beta) chain. The C-terminal A(alpha) chain protrudes from the terminal domain for approximately two thirds of its entire length: this A(alpha) chain protuberance is highly exposed and subject to a number of proteases, most notably, plasmin (Doolittle, 1984; Francis & Marder, 1986).

Fibrinogen is regularly described in terms of its plasmic

degradation products, the so-called fibrinogen degradation products or FDP's. Under normal physiologic conditions, plasmin activity is restricted only to the fibrin clot by circulating antiplasmins. However, in thrombolytic therapy and in certain disease conditions, as well as for research purposes, plasmin lysis of fibrinogen may occur. The process of fibrinogen degradation proceeds in an asymmetric, three stage fashion (Figure 3) first proposed by Marder et al in 1967 (Marder et al, 1969a; Francis & Marder, 1986).

Stage I digestion involves the cleavage of multiple small peptides (ultimately, such unnamed peptides comprise 20% of all FDP's) from the fibrinogen molecule, most notably the C-terminal A(alpha) protuberance and a portion of the N-terminal B(beta) chain that includes fibrinopeptide B (Lahiri & Shainoff, 1973). This process yields fragment X, a 250 kilodalton molecule which remains physicochemically similar to fibrinogen and, albeit to a much lesser degree, clottable by thrombin (Kowalski, 1968; Marder et al, 1969a; Kwaan & Barlow, 1973; Niewiarowski et al, 1977; Marder & Budzynski, 1975).

Stage II digestion involves cleavage of one of the coiled-coils to yield a 150 kilodalton fragment consisting of the central domain tethered to one terminal domain (fragment Y) and one 80 kilodalton fragment consisting of the remaining terminal domain (fragment D). The subsequent cleavage of the remaining coiled-coil of fragment Y yields two additional fragments: a 45 kilodalton fragment consisting of the central domain (fragment E)

and a second fragment D. As such, Stage II digestion products include fragments X, Y, D and E.

Stage III digestion involves the cleavage of the remaining fragments X and Y so that only fragments D and E remain (Marder et al, 1969a; Marder & Budzynski, 1975; Budzynski & Marder, 1977). Fragment E contains the N-terminal regions of all six polypeptide chains and is, as such, analogous to the N-terminal disulfide knot (Budzynski & Marder, 1977; Olexa & Budzynski, 1980). Regardless of the rate of fibrinogen digestion, the same degradation products invariably appear in solution in exactly the same order (Marder et al, 1969a). As there is normal variation in the physicochemical properties of fibrinogen, so there is some variation in the structure and molecular weight of the FDP's (Kwaan & Barlow, 1973; Marder & Budzynski, 1975).

FIBRIN POLYMERIZATION - THE FIBRIN CLOT

The action of thrombin upon fibrinogen is to cleave specific arginyl-glycyl bonds, releasing fibrinopeptides A and B from the N-terminal A(alpha) and B(beta) chains (forming fibrin monomer) and exposing N-terminal regions of the resulting alpha and beta chains to serve as the principal contact sites for fibrin polymerization (Kowalski, 1968; Laudano & Doolittle, 1978). Initially, fibrin monomer aligns in a half-step, overlapping orientation with the central domain of one molecule aligned opposite to the terminal domains of two adjacent fibrin molecules (Figure 4). The result is a long, linear polymer

stabilized only by non-covalent interactions between central and terminal sites. Such a polymer is visible but soluble at low pH or high concentrations of urea (McKee et al, 1970; Gaffney, 1973; Doolittle, 1984). This so-called soluble fibrin is converted to urea insoluble, cross-linked fibrin by the formation of gamma chain cross-links, factor XIII catalyzed amide bonds between the epsilon-amino groups of lysine and gamma-carboxyamido groups of glutamine on adjacent C-terminal gamma chains (McKee et al, 1970; McDonagh et al, 1971; Gaffney, 1973; Doolittle, 1984).

Laudano and Doolittle (1978) propose an important role for the terminal domains (Figure 5) in the initial non-covalent stabilization of the non-gamma-cross-linked fibrin polymer. Noting that the N-terminal sequences of the thrombin exposed alpha and beta chains are respectively Glycine-Proline-Arginine (GPR) and Glycine-Histidine-Arginine (GHR), they synthesize tetrapeptide analogues of these sequences. They then observe that these analogues to bind to fibrinogen and fragment D with similar affinity constants, indicating specificity of binding. Moreover, they observe that analogues of GPR inhibit polymerization of fibrin monomer. (No such inhibition is seen by GHR analogues.) Such observations support the concept of reciprocal interactions between the central and terminal domains (specifically between the N-terminal alpha chain and the so-called GPR binding site) of fibrin monomer in early polymerization.

A large body of evidence supports the primacy of fibrinopeptide A (FPA) cleavage and the N-terminal alpha chain

over FPB cleavage and the N-terminal beta chain in early fibrin polymerization. The snake venoms Ancrod and Reptilase cleave only FPA from fibrinogen, yet result in linear polymerization. The venom of the species Agkistrodon contortrix hydrolyses FPB much more rapidly than FPA, yet no clotting occurs until sufficient FPA is cleaved (Kwaan & Barlow, 1973). Early products of plasmin activity, in which N-terminal B(beta) fragments (which include FPB) have been cleaved, remain thrombin clottable (Lahiri & Shainoff, 1973). Finally, the observations of Laudano and Doolittle (1978) that analogues of N-terminal alpha, but not beta chain, inhibit polymerization of fibrin monomer, leads to the conclusion the FPA cleavage is both necessary and sufficient for fibrin polymerization. FPB cleavage, alternatively, has been implicated in the lateral expansion of these linear fibrin strands, the so-called 'side-to-side' polymerization (Kwaan & Barlow, 1973).

Such a role for FPA and FPB cleavage is supported by the observations of Olexa and Budzynski (1980). They observe that while fragments X and Y and the N-terminal disulfide knot, after thrombin treatment, bind to both fibrinogen and fibrin monomer, binding is most avid to cross-linked fibrin; moreover, while fragment E from a plasmic digest of fibrinogen binds neither fibrinogen, fibrin monomer, nor cross-linked fibrin, fragment E derived from cross-linked fibrin in fact binds cross-linked fibrin. Such observations lead to the conclusion that there must exist four binding sites involved in fibrin polymerization: one

located on fragment D, not requiring thrombin activity; two on the NDSK, most likely activated by thrombin cleavage of FPA and FPB, and one available only on cross linked fibrin. Conceivably, this last region is stabilized by gamma cross-links to become available for binding to a thrombin activated central site. In sum, they propose the following sequence of events in fibrin polymerization (Figure 6): cleavage of FPA by thrombin exposes central binding sites complementary to the thrombin independent terminal sites. Fibrin strands align into a staggered, overlapping linear polymer which is quickly stabilized by C-terminal gamma chain cross-links. Cross-linking either reveals or stabilizes secondary thrombin-independent binding sites complementary to those revealed by cleavage of FPB. This association results in lateral association of adjacent linear fibrin polymers and the final fibrin product (Olexa & Budzynski, 1980).

In addition to the factor XIIIa mediated gamma chain cross-links, there is considerable evidence for extensive alpha chain polymerization in the final structure of cross-linked fibrin.

McKee et al (1970) are the first to propose alpha chain polymerization. Upon disulfide reduction and electrophoresis of soluble, non-cross-linked fibrin, they observe, as expected, alpha, beta and gamma monomer. Upon similar treatment of cross linked fibrin, in addition to the expected beta monomer and gamma dimer, alpha chain is found to be increasingly in the form of a high molecular weight polymer. After 90 minutes of incubation,

all alpha chain is in the form of alpha polymer. McKee et al conclude that in addition to rapid gamma chain cross-linking, slow but extensive alpha chain polymerization occurs and is directly related to the stability of the fibrin polymer. Such observations and conclusions are corroborated essentially in their entirety by McDonagh et al (1971) and by Gaffney and Brasher (1973). McDonagh et al propose that the alpha polymer has a molecular weight of at least 340 kilodaltons involving 5-6 alpha chains, or several units of fibrin monomer.

The consequence of ineffective or disrupted alpha chain polymerization can be shown in the work of Gaffney (1973) and Francis et al (1980). Gaffney, working with fibrinogen derived from patients on continuous streptokinase therapy, finds such fibrinogen to be similar to fragment X in clotting ability-slowly and incompletely clottable. Upon reduction and electrophoresis of such fibrinogen, low molecular weight alpha chain remnants are observed, no alpha polymer is seen. The implication is that without intact alpha chain polymerization, clotting is delayed and incomplete. Francis et al study the plasmin degradation products of a cross-linked fibrin clot. They observe that the major difference between the fibrin which remains in the clot and that which is released is the extent of intact alpha chain polymerization. In spite of extensive plasmic degradation of alpha chain, beta chain and gamma dimer, that fibrin which remains in the clot retains extensive alpha polymerization. Alternatively, that which remains in the clot but

is SDS washable (that is, maintained in clot only by non-covalent interactions) contains little alpha polymerization, and that which is freely liberated by plasmin consists of no alpha polymerization at all. This again suggests a critical role for the alpha chain in fibrin polymerization and clot formation.

FIBRINOLYSIS

The newly formed fibrin clot is temporary, and is removed by the fibrinolytic system, which coordinates the local digestion of fibrin at sites of deposition while avoiding systemic effects. The components of the fibrinolytic system include 1) plasminogen and plasmin; 2) plasminogen activators; and 3) inhibitors of plasmin and of plasminogen activators.

PLASMINOGEN AND PLASMIN

In 1941, Milstone demonstrated that the lysis of fibrin required the presence of an uncharacterized 'lytic factor' in serum. In 1944, this factor was found to be an enzyme precursor, converted to an active enzyme by derivatives of streptococci (now known as streptokinase). The enzyme precursor was named plasminogen, the enzyme plasmin (Collen, 1980). Plasminogen (Figure 7) is a single chain, 88 kilodalton glycoprotein. It contains 790 amino acids, 24 disulfide bridges and five homologous triple loop regions known as 'kringle' regions which appear to be lysine binding sites (LBS) and may have a significant role in the interaction of plasminogen and plasmin with fibrin (Collen, 1980; Francis & Marder, 1986).

Plasminogen exists in two forms, that with glutamate at its N-terminal position (glu-plasminogen) and its modified plasmic derivative, N-terminal-lysine plasminogen (lys-plasminogen). Lys-plasminogen is converted to plasmin via the cleavage of the arginine(560)-valine(561) bond, creating a two chain, disulfide

bonded enzyme. Glu-plasminogen is converted first to lys-plasminogen, then to plasmin, a process approximately twenty times slower than the conversion of lys-plasminogen alone (Collen, 1980; Pannell & Gurewich, 1987). Plasmin is an endopeptidase that hydrolyses arginine-lysine bonds, not only in fibrin but in factors V and VII, complement, ACTH, GH and glucagon (Francis & Marder, 1986). The active site of plasmin is on the B (C-terminal) chain and appears to involve histidine(602), aspartate(645) and serine(740). The lysine binding sites or 'kringle' regions are on the A (N-terminal) chain (Collen, 1980).

PLASMINOGEN ACTIVATORS

In 1947, Astrup and Perminn showed animal tissues to contain a substance which could activate plasminogen to plasmin, a substance now known as tissue plasminogen activator (tPA) (Collen, 1980). Plasminogen activators are, in general, serine proteases which hydrolyse a single arginine-valine bond (Lijnen et al, 1987). They may be characterized as intrinsic, extrinsic and exogenous. Intrinsic activators are normally present in plasma. They include Hageman factor, prekallikrein, and high molecular weight kininogen. Extrinsic activators originate in the tissues or vascular wall and are released into the blood stream. They include tissue plasminogen activator (tPA) and urokinase-type plasminogen activator (UK). These extrinsic activators are also commercially produced and, along with streptokinase, may be

infused for therapeutic purposes. They are then referred to as exogenous activators (Collen, 1980; Francis & Marder, 1986).

Tissue plasminogen activator may be extracted from several tissues as well as from post-operative and post-mortem blood. It is a two chain, disulfide linked, 60 kilodalton polypeptide. It binds avidly to fibrin and appears to have increased activity in the presence of fibrin. In fact, in the absence of fibrin it is a very poor plasminogen activator. This property of tPA localizes activation of plasminogen to sites of fibrin deposition and tends to minimize systemic plasminemia and fibrinogenolysis. tPA possesses kringle regions homologous to plasminogen which may mediate binding of tPA to fibrin (Collen, 1980; Matsuo et al, 1981; Francis & Marder, 1986).

Urokinase-type plasminogen activator (UK) was first demonstrated in urine in 1951 (Collen, 1980). It exists in several forms which may be isolated from urine, plasma or kidney cell tissue culture. Pro-urokinase or single chain urokinase (SC-UK - Figure 8) is a 55 kilodalton zymogen first isolated in 1981. In addition to its role as a proenzyme, it also possesses a small amidolytic activity, estimated at 0.1-0.5% that of its active enzyme form, two chain urokinase (TC-UK). Two-chain urokinase occurs in two forms, the high molecular weight form and its low molecular weight plasmic derivative. Both forms appear to have equivalent amidolytic activity. The generation of two chain urokinase is the result of plasmic cleavage of pro-urokinase at a single lysine-isoleucine bond, creating a two chain active enzyme

connected by a single disulfide bond. Clearly this mechanism of activation presents an opportunity for physiologic amplification (Figure 9), as the end product of urokinase action results in further urokinase activation. (Gurewich et al, 1984; Lijnen et al, 1986; Lijnen et al, 1987; Gurewich & Pannell, 1987; Pannell & Gurewich, 1987). Like tPA, urokinase has a catalytic center located on its C-terminal chain and a kringle region (Lijnen et al, 1987). Nevertheless, it is enzymatically distinct from tPA. Matsuo et al (1981) find that in both purified systems and in plasma, tPA is a far more efficient fibrinolytic agent than urokinase and has only minimal fibrinogenolytic properties. tPA seems to specifically activate fibrin bound plasminogen while urokinase activates both fibrin bound and free plasminogen, resulting in systemic plasminemia and fibrinogenolysis.

Streptokinase is a 47 kilodalton non-enzymatic protein produced by specific strains of beta-hemolytic streptococci. It forms a 1:1 complex with plasminogen which is then itself capable of plasminogen activation. Streptokinase, like urokinase, has no specific affinity for fibrin or fibrin bound plasminogen: its therapeutic use results in systemic plasminemia and fibrinogenolysis.

INHIBITORS OF PLASMIN AND OF PLASMINOGEN ACTIVATORS

The most significant inactivator of plasmin is the alpha(2)-antiplasmin. First identified in 1975, it is a 70 kilodalton glycoprotein which reacts with plasmin to form a stable 1:1

complex devoid of proteolytic activity. The kinetics of the plasmin-antiplasmin interaction involve a fast, reversible reaction, followed by a slow, irreversible reaction. The lysine binding sites of plasmin seem to play a central role in this interaction. When plasmin is bound to free lysine or to lysine residues of fibrin, it is much more slowly inactivated by antiplasmin. In addition, occupation of the active site of plasmin delays its inactivation. It is theorized that the active site of plasmin attacks a leucine-methionine bond in the C-terminal region of the antiplasmin, forming a strong, stable covalent bond between plasmic serine and antiplasmic leucine and inactivating the active site (Figure 10). In addition, alpha(2)-antiplasmin cross-links to fibrin alpha chain, delaying fibrin degradation (Collen, 1980; Francis & Marder, 1986). Alpha(2)-macroglobulin is a slowly reacting inhibitor of plasmin; it neutralizes excess plasmin only upon saturation of alpha(2)-antiplasmin.

Inhibitors of plasminogen activation remain essentially speculative. C1 inactivator, antithrombin III, and alpha(2)-macroglobulin are all thought to inhibit intrinsic activation. Inhibitors of extrinsic activation have never been isolated or identified, but have been postulated to be secreted by endothelial cells and or platelets. Non-inhibitory clearance mechanisms may play the most important role. Inhibitors of streptokinase include anti-streptococcal antibodies, most likely the result of previous streptococcal infection. Urokinase is

thought to be inhibited by alpha(2)-macroglobulin, alpha(1)-antitrypsin, antithrombin-III and alpha(2)-antiplasmin, although, again, non-inhibitory clearance mechanisms may be of most importance (Collen, 1980; Francis & Marder, 1986).

REGULATION OF FIBRINOLYSIS

During fibrin polymerization, plasminogen is adsorbed to fibrin by means of its lysine binding sites. Plasminogen activator, possessing high affinity for either fibrin or for fibrin bound plasminogen, activates plasminogen to plasmin. This plasmin is relatively resistant to alpha(2)-antiplasmin, given occupation of both its lysine binding and catalytic sites. Meanwhile, free plasminogen activator is either inhibited or has low affinity for free plasminogen, resulting in minimal systemic plasminemia. Moreover, that free plasmin which is generated is rapidly inhibited by antiplasmin, minimizing systemic fibrinogenolysis (Figure 11).

Evidence for this regulation includes the observation that occupation of lysine binding sites by amino acid analogues greatly increases plasminogen activation by urokinase; that tPA in the absence of clot has minimal activity; and that infusion of free plasmin results in only minimal fibrinolysis and fibrinogenolysis. Presumably, fibrin bound plasminogen is activated much more readily than its free form; moreover, free plasmin is inhibited and exerts minimal proteolytic effects. (Collen, 1980; Francis & Marder, 1986).

Another illustration of the regulation of fibrinolysis is the fibrinolytic activity of pro-urokinase. Pro-urokinase has been detected in normal serum, is stable, and may play a role in physiologic thrombolysis (Francis & Marder, 1986). Although pro-urokinase possesses only a fraction of the enzymatic activity of its active derivative, it appears both in vitro and in vivo to be a much more effective fibrinolytic agent than two-chain urokinase. The cause of this effect appears not to be any form of pro-urokinase to fibrin binding, but rather an increased susceptibility of fibrin bound plasminogen to the intrinsic activity of the proenzyme. While pro-urokinase is stable in plasma, free two-chain urokinase is complexed to plasma inhibitors; moreover, while fibrin bound plasmin is relatively resistant to alpha(2)-antiplasmin, that produced in plasma by free two-chain urokinase is rapidly inhibited. At concentrations of two- chain urokinase sufficient to saturate antiplasmin and effectively lyse clot, systemic plasminemia and fibrinogenolysis occurs (Gurewich et al, 1984). Lijnen et al (1986) observe that the presence of CNBr digested fibrinogen increases the intrinsic activity of free pro-urokinase. They propose that free pro-urokinase is complexed to a plasma inhibitor, whose inhibition is reversed in the presence of fibrin (Lijnen et al, 1986; 1987). This hypothesis seems unlikely. Gurewich et al (1984) observe that the addition of lysine to free plasminogen abolishes the 'fibrin specificity' of pro-urokinase, with nonspecific plasminemia and incomplete clot lysis in a pattern quite similar

to that seen for two chain urokinase. This seems to support the concept of a lysine mediated change in fibrin bound plasminogen that renders it more susceptible to pro-urokinase.

FIBRIN DEGRADATION PRODUCTS

Plasmic degradation of cross-linked fibrin occurs at a much slower rate than that of fibrinogen and results in significantly different derivatives. Francis et al (1980) propose four stages of fibrin digestion based on their in vitro observations of fibrinolysis. At first, proteolytic cleavages occur mainly in the N-terminal beta chain and along the gamma dimer. The alpha polymer remains intact so that all protein remains covalently bound to the fibrin matrix. Next, critical alpha polymer cleavage along the coiled coil region removes the covalent binding of several large derivatives within the clot. These derivatives, of molecular weight greater than 1000 kilodaltons, remain held in the fibrin matrix by non-covalent forces, but may be washed free by various methods. Following this, smaller derivatives of 195 to 800 kilodaltons are freely liberated into solution (Figure 12). Finally, these large complexes are degraded in solution into a 'terminal lysate' consisting mainly of fragment DD/E.

Fragment DD/E represents the so-called D-Dimer, two terminal domains with their gamma cross-linkage intact, found in covalent interaction with an adjacent fragment E. Although fragment DD/E forms the majority of the terminal lysate in vitro, it is rarely found in vivo. Once in plasma, the previously liberated large

fragments are protected from further degradation by circulating antiplasmins. Such larger fragments as DY, YY and DXD are logical extrapolations from what is known of the structure of the fibrin clot and are therefore expected to be seen (Francis et al, 1980a; Francis & Marder, 1986).

In fact, Francis et al (1980b) have isolated and purified several previously uncharacterized, large complexes released from degrading fibrin. In addition to fragment DD, they observe fragment Y and several variants of fragments D and E. Moreover, they isolate complexes whose molecular weight and protein structures are compatible with such fragments as DY, X, YY, XD and XY. The largest fragments are compatible with structures such as DXD, YXD, XX, YXY and XXD. It is clear to see the multiple permutations with which such complexes could be aligned to create even larger fibrin degradation products. (Francis et al 1980b).

FIBRINOGEN DEGRADATION PRODUCTS AND COAGULATION

The inhibitory effect of FDP on fibrin polymerization was first reported by Stockmorken in 1957 and by Niewiarowski and Kowalski in 1958 (Triantaphyllopoulos, 1959; Arnesen & Godal, 1973). Triantaphyllopoulos (1959) is the first to propose competitive inhibition of thrombin as a mechanism. He observes an increase in the thrombin clotting time of solutions of fibrinogen to which he adds what he calls the anticoagulant fraction of incubated fibrinogen (AFIF). Moreover, a Lineweaver-Burke plot of fibrinogen concentration vs. thrombin activity (Figure 13) shows AFIF to shift the curve in a competitively inhibitory fashion.

Alkjaersig et al (1962) dispute the assertion that FDP inhibits thrombin activity, insisting rather that FDP prolongs thrombin time through the inhibition of the polymerization stage of clot formation. They observe a prolongation of thrombin time in the presence of AFIF. However, no inhibition of thrombin esterolytic activity against the substrate benzylarginine methyl ester (BAMe), nor of thrombin enzymatic activity against fibrinogen is seen (Figure 14). Commenting on the pathogenesis of the impaired polymerization, they speculate that if inhibition were purely the result of diminished interaction between monomer units, then that clot which was formed would be structurally normal. However, in the presence of AFIF, clot structure is abnormal. Ultracentrifugal patterns of fibrin clotted in the presence of AFIF show several heterogenous components, rather than a single peak as is seen with fibrin alone. Perhaps FDP is

somehow incorporated into the clot structure, resulting in both delayed and defective polymerization. Ultracentrifugal examination of the AFIF indicates a maximal inhibitory effect secondary to a large, heat-precipitable, plasmin resistant product of fibrin digestion (most likely fragments D and/or E). Radioactive labelling studies with I(131) confirm not only the incorporation of AFIF into the defective clot, but that nearly 100% of the incorporated radioactivity consists of this large, plasmin resistant fragment. Such evidence supports the hypothesis that large products of fibrinogen degradation (later to be purified and characterized) somehow complex with fibrin monomer and inhibit the polymerization stage of clot formation.

This theory is refined by Kowalski (1968) in his conception that in addition to the presence of visible, precipitable, but defective clot formed by the incorporation of FDP into fibrin polymer, the vast majority of fibrin forms non-soluble complexes with FDP such as fragments X, Y and D. These unclottable complexes make fibrin polymerization and gelation impossible. Evidence for the existence of such "soluble fibrin" exists in the so-called paracoagulation test, in which several agents, including cold, low pH, and protamine cause precipitation of fibrin monomer. The successful paracoagulation of soluble fibrin monomer in a solution of fibrin and FDP strongly suggests inhibition of normal fibrin polymerization by FDP.

Latallo et al (1964) are the first to propose inhibition of both thrombin enzymatic activity and fibrin polymerization by

FDP. They initially observe that the prolongation of thrombin time by digests of fibrinogen is dependent on the duration of incubation, with maximal inhibition at early stages of digestion and a rapid drop-off and plateau of inhibition at later stages. This suggests two separate mechanisms of inhibition for such early and late FDP. Latallo et al observe that late FDP affect both thrombin clotting of fibrinogen and gelation of fibrin monomer to a similar degree. Early FDP prolong thrombin time markedly, but have only limited effects on the gelation of fibrin monomer (Figure 15). The implication is that early FDP affect the thrombin conversion of fibrinogen to fibrin, while late FDP inhibit fibrin polymerization. (Latallo et al, 1964; Kowalski et al, 1964). Such a conclusion is corroborated by the observation of Latallo et al that the quantity of small, trichloracetic acid (TCA) soluble fragments released from fibrinogen by thrombin is decreased in the presence of early FDP. Triantaphyllopoulos & Triantaphyllopoulos (1966) corroborate this evidence to an extent, but observe both early and late FDP to inhibit TCA-soluble fragment release by thrombin, implicating both early and late FDP in the inhibition of thrombin enzymatic activity.

Marder & Shulman (1969) are the first to correlate the anticoagulant activity of FDP with the presence or absence of specific degradation products (Figure 16). They find maximal prolongation of the thrombin time to be correlated to the time in plasmic digestion, stage II, when fragment Y concentration is

maximal. Using purified fragments, they find fragments X and Y to have marked anticoagulant activity, while fragments D and E have only slight effects. This corresponds well with previous reports of the relative effects of early and late FDP (Latallo et al, 1964; Kowalski et al, 1964; Kowalski, 1968).

Larrieu et al (1972), Arnesen & Godal (1973) and Arnesen (1974) limit their study to the effects of purified fragments D and E. Larrieu et al observe that while both D and E prolong the thrombin time, the inhibitory effect of fragment E may be overcome by additional thrombin. Moreover, in a fibrin polymerization system, fragment D alone is shown to have inhibitory effects (Figure 17), with a Lineweaver-Burke plot indicating competitive inhibition. Larrieu concludes that fragment D inhibits the polymerization of fibrin monomer, while fragment E is active as an inhibitor of thrombin enzymatic activity upon fibrinogen. Fragment Y, as the most potent anticoagulant, may possess both D-like and E-like anticoagulant properties (Larrieu et al, 1972).

Arnesen & Godal (1973) and Arnesen (1974) find fragments D and E to have only slight inhibitory activity in contrast to the far more potent stage II digestion products. Using N-terminal glycine release as an indicator of thrombin enzymatic activity, they observe fragment E alone to inhibit thrombin activity. Fragment D, while not inhibitory towards N-terminal glycine release, delays the time of gelation of fibrin monomer. As seen by Larrieu et al, increased concentrations of thrombin overcome

the fragment E mediated inhibition. Arnesen concludes that the very weak anticoagulant effects of fragments D and E are secondary to inhibition, respectively, of fibrin polymerization and thrombin enzymatic activity.

While much study has been devoted to the effects of fibrinogen degradation products (FDP) upon coagulation, very little study has been attempted on fibrin degradation products (fdp), which may play a significant role in fibrinolytic states.

Arnesen (1973) studies the effects of lysis products of fibrinogen, fibrin monomer, and fibrin polymer on thrombin time. He observes that while early degradation products of fibrin monomer and of fibrinogen prolong the thrombin time, products of fibrin clot degradation elevate thrombin time to a lesser degree than even the weak inhibitory effects of the late fibrinogen products D and E. In contrast, Dray-Attali & Larrieu (1977), examining the inhibitory effects of fragments D derived from fibrinogen, fibrin monomer, and cross-linked fibrin (D-Dimer), observe D-Dimer to be a much more potent inhibitor of thrombin time and of fibrin monomer polymerization. Given the generally weak effects of fragment D as an anticoagulant, the relevance of this observation remains in question.

FIBRINOGEN AND PLATELET AGGREGATION

The initial event in platelet aggregation is the encounter with a stimulus such as thrombin, ADP, epinephrine or connective tissue. The intact fibrinogen molecule appears to be an essential co-factor in this event. Washed platelets, or platelets from defibrinogenated plasma aggregate weakly or fail to aggregate in response to ADP, collagen, thrombin and epinephrine, a response which is reversed by the addition of fibrinogen (Niewiarowski et al, 1977; Bennett & Vilaire, 1979; Marguerie & Plow, 1983). During plasmic degradation in vitro, or during thrombolytic therapy, fibrinogen loses the ability to potentiate platelet aggregation in direct relationship to the extent of its digestion (Niewiarowski et al, 1977).

This aggregatory response appears to be mediated by a discrete receptor system not expressed by the resting platelet but exposed in response to such stimuli as ADP. The amount of fibrinogen bound seems directly related to the dose of agonist and of fibrinogen, and is saturable (Figure 18), indicating a specific receptor-ligand interaction (Niewiarowski et al, 1977; Bennett & Vilaire, 1979; Marguerie et al, 1980; Marguerie & Plow, 1983). The binding is initially reversible but quickly becomes irreversible. Marguerie et al (1980) observe that within 5 minutes of fibrinogen binding, only 50% of bound fibrinogen is dissociable; by 30 minutes, fibrinogen dissociation is negligible. The reversible step is the rate limiting step in the interaction, the Ka of which has been calculated to be from 8 X

10(-8) (Bennett & Vilaire, 1979) to 2 X 10(-6) (Marguerie & Plow, 1983). The interaction is divalent-ion dependent. Without Ca++, ADP induces a shape change, but no fibrinogen binding or platelet aggregation, Mg++ shows a similar, albeit reduced, effect (Marguerie et al, 1980; Marguerie & Plow, 1983). Exposure to collagen, thrombin and epinephrine stimulates binding of fibrinogen with a dose-response pattern, Ka, and divalent- ion dependence analogous to that of ADP, again supporting the concept of a specific fibrinogen receptor (Bennett & Vilaire, 1979; Marguerie & Plow, 1983). ADP appears to be an essential co-factor in fibrinogen binding. Marguerie and Plow (1983) observe that in the presence of apyrase and creatine phosphokinase (which eliminate ADP), fibrinogen binding is inhibited regardless of stimulus.

From such observations, the following sequence of events may be proposed for platelet fibrinogen binding (Figure 19): following a stimulus, the platelet releases ADP, causing exposure of previously unavailable fibrinogen receptors. This exposure requires the presence of Ca++ or Mg++, as well as the continuous presence of ADP. Reversible fibrinogen binding, independent of ADP but possibly requiring Ca++ or Mg++ then occurs, soon followed by irreversible binding. Either through direct bridging activity or through induction of electrostatic or physicochemical changes in the platelet membrane, the bound fibrinogen contributes in an essential way to platelet aggregation (Marguerie et al, 1980; Marguerie & Plow, 1983).

Investigation of the sites on the fibrinogen molecule directly responsible for binding to the platelet has been pursued via observation of the interaction of FDP with the fibrinogen receptor. Marguerie & Plow (1983) observe that while a 100 kilodalton fragment D inhibits the binding of fibrinogen to the platelet, an 80 kilodalton fragment, lacking only the C-terminal alpha chain, does not block such binding. Moreover, aggregated alpha chains support ADP induced platelet aggregation, while anti alpha chain Fab fragments inhibit fibrinogen binding. Nevertheless, multiple sites on the fibrinogen molecule may be involved (Niewiarowski et al, 1977).

Further evidence of the primary importance of fibrinogen in the aggregation of platelets may be seen in patients with congenital afibrinogenemia or Glanzmann's Thrombasthenia. Patients with congenital afibrinogenemia have extremely low levels of plasma fibrinogen. These patients have prolonged bleeding times, and their platelets fail to aggregate in response to ADP or epinephrine, a response that is reversed by the addition of fibrinogen (Weiss & Rogers, 1971; Marguerie et al, 1980, Marguerie & Plow, 1983). Platelets from patients with Glanzmann's Thrombasthenia change shape and participate in the release reaction, but fail to aggregate in response to a number of stimuli. (Weiss, 1967; Bennett & Vilaire, 1979; Marguerie & Plow, 1983). Bennett & Vilaire (1979) observe that these platelets fail to bind fibrinogen in response to ADP, and moreover, that they lack certain surface glycoproteins with a

molecular weight of 100-120 kilodaltons. The lack of these glycoproteins, no doubt the fibrinogen receptor, may explain the platelet defect in this disease.

Kowalski et al (1964a) are the first to report the inhibitory effects of FDP upon platelet aggregation. Adding FDP to platelet rich plasma, they note dose dependent inhibition of platelet aggregation and viscous metamorphosis in response to ADP, thrombin and connective tissue extract. In an animal model (1964b), Kowalski et al infuse streptokinase into dogs (Figure 20), noting that at the time fibrinogen is decreased to undetectable levels, bleeding time becomes prolonged, with frank oozing at catheter sites. This bleeding time extension spontaneously resolves within 90 minutes, long before correction of fibrinogen levels. Subsequent streptokinase infusion elicits bleeding time extension only in the presence of circulating fibrinogen. Infusion of early FDP (Figure 21) into the dog also causes an increased bleeding time and clinical oozing which normalizes within 30 minutes. These effects are not observed with late FDP.

Jerushalmy & Zucker (1965) similarly observe moderate inhibition of platelet aggregation in response to ADP and connective tissue in the presence of early (<60 minute digests) FDP, as well as inhibition of serotonin release in response to thrombin. Kopec et al (1966) observe FDP mediated inhibition of ADP release in response to thrombin and connective tissue. Jerushalmy and Zucker propose that given the known "antithrombic"

activity of FDP, it is possible that similar inhibition of thrombic activity on the platelet may be seen. Such a mechanism, however, fails to account for inhibitory effects against ADP and connective tissue. Kopec, et al, noting the role of FDP in reversing fibrinogen potentiation of platelet aggregation, propose a role for FDP as an antagonist in the fibrinogen-platelet interaction.

Contrary to these reports, Barnhart et al (1967) propose pro-aggregatory qualities for FDP. They observe infusion of FDP into the dog to cause transient thrombocytopenia, the extent and duration of which is directly related to the dose of FDP infused. Observing aggregated platelets in peripheral blood samples, and in vitro potentiation of platelet aggregation by FDP in whole blood, they propose such pro-aggregatory attributes. Barnhart et al appear to be alone in this hypothesis, as their observations have not been corroborated by others.

The question of which fraction of the FDP possesses maximal anti-platelet activity has been touched upon briefly in the preceding discussion and is considered with even more sophistication in other investigations.

Stachurska et al (1970) propose that the small, dialyzable fragments produced late in fibrinogen digestion are mainly responsible for its inhibitory activity (Figure 22). They observe that aggregation of platelets in platelet rich plasma (measured as time to visible gelation) by thrombin, ADP and epinephrine are all inhibited by FDP. This effect is diminished if not abolished

by dialysis of the digestion products. Moreover, the dialysate thus obtained shows inhibitory activity. Niewiarowski et al (1970) similarly find the inhibitor of platelet aggregation to be a low molecular weight, dialyzable peptide (Figure 23). However, unlike Stachurska et al, maximal antiaggregatory activity is seen early in digestion, at a time corresponding to the appearance of fragments X and Y in solution. This activity is again abolished by dialysis. Niewiarowski et al conclude that the small, inhibitory fragment is produced early in fibrinolysis. In a subsequent study, Niewiarowski et al (1971) appear somewhat more skeptical of their earlier results. Here, they observe both early and late FDP to inhibit the fibrinogen mediated enhancement of ADP induced platelet aggregation, as well as the expected elimination of this effect by dialysis. Nevertheless, these effects are weak, seen only at low concentrations of agonist.

Solum et al (1973) continue this line of skepticism, proposing that the effect of FDP on platelet aggregation is low and non-specific. They observe that intermediate size peptides from plasmic digests of fibrinogen inhibit platelet aggregation in response to ADP, thrombin, collagen and epinephrine in dose dependent fashion. However, the concentrations of FDP required for such inhibition exceed that expected in severe fibrinolytic states, or for that matter, in total plasma fibrinogenolysis. Moreover, Solum et al observe that products of degradation of human serum by trypsin, a wide range of low molecular weight peptides, show a similar dose dependent inhibition of ADP induced

platelet aggregation. As such, the anti-aggregatory effects of the small products of fibrinogen digestion are equalled by non-specific trypsin degradation products of similar size and charge. This calls into question the both specificity and clinical relevance of the role of FDP in impairment of platelet function.

FDP AND PULMONARY DYSFUNCTION

The adult respiratory distress syndrome (ARDS) is a syndrome of unknown etiology often seen in severely burned or traumatized patients. It is characterized by hypoxia, hyperventilation, hypocarbia, decreased lung compliance and multiple pathologic changes, including vascular congestion, interstitial edema, atelectasis, hypertrophy of alveolar lining cells and intra-alveolar hemorrhage and edema. Pulmonary microemboli and manifestations of disseminated intravascular coagulation-elevated levels of FDP, mainly fragment D - are fairly common findings in clinical studies of ARDS (Johnson et al, 1983, 1985; Luterman et al, 1977; Saldeen, 1982).

Luterman et al (1977) are the first to associate FDP with the hypoxia, platelet consumption, pulmonary interstitial edema, hemorrhage and capillary permeability consistent with the picture of ARDS. They infuse fragment D into rabbits, with resultant hyperventilation, hypoxemia, hypocarbia and thrombocytopenia (Figure 24). Infusion of thrombin results in similar findings, coincident with the advent of fibrinolysis. Furthermore, infusion of fragment D or of thrombin results in increased pulmonary capillary permeability to albumin and in pathologic changes including intravascular congestion, hemorrhage, interstitial edema, and hypertrophy of alveolar lining cells. They conclude that fragment D may play a significant role in the pathogenesis of ARDS.

Manwaring et al (1978) propose several mechanisms for the

pulmonary dysfunction induced by fragment D, including direct endothelial damage, intracapillary coagulation with secondary hypoxic or mechanical damage to the microvasculature and alterations in endothelial permeability secondary to the release of chemical mediators such as histamine or serotonin. Manwaring observes that infusion of fragment D into the rabbit results in tachypnea, hypocarbia and respiratory alkalosis (but no hypoxia) as well as thrombocytopenia. On pathologic examination, the lungs show focal evidence of increased extravascular fluid with dilated lymphatic channels, increased cellularity of alveolar septa, and increased extravasation of radiolabelled iodine. Rabbits given fragment E or albumin, or those treated with antihistamine before fragment D infusion show neither clinical pulmonary changes nor pathologic tissue changes. Manwaring proposes that complexes of fragment D and fibrin monomer may bring about platelet aggregation, with release of histamine and subsequent pulmonary pathology. It might be noted that no changes in platelet aggregation in vitro nor pathologic signs of microvascular aggregation are seen in this study.

Sueshi et al (1981) observe low molecular weight (15-25 K) FDP to increase vascular permeability in rabbit skin with extravasation of albumin, subendothelial deposits and endothelial gaps on electron microscopy. The response observed is similar to that seen in response to histamine, serotonin or bradykinin, and is abolished by antihistamines. Saldeen (1982) also observes endothelial gaps on electron microscopy and notes that agents

that counteract cellular contraction seem to inhibit the increase in vascular permeability induced by FDP.

Johnson et al (1983) infuse thrombin into sheep, causing pulmonary thromboembolization with an increase in pulmonary arterial pressure and pulmonary vascular resistance, a decrease in platelets and an increase in FDP. Lymph protein clearance, a marker for vascular permeability, is increased as well. When fibrinolysis is inhibited by tranexamic acid, thrombin infusion results in similar results, with the exception that FDP levels do not change. Moreover, lymph protein clearance remains stable in proportion to lymphatic flow, indicating that vascular permeability has not changed. Johnson et al conclude that plasminogen activation is necessary for the increase in lung vascular permeability that occurs after embolization. While recognizing that plasmin plays several roles, including activation of complement, the work supports the notion of FDP involvement in pulmonary vascular permeability changes. However, in a later study (1985), Johnson et al infuse FDP into an identical sheep model, noting no changes in pulmonary hemodynamics (Figure 25) nor in pulmonary vascular permeability. This results in some amount of skepticism as to the true role of FDP in pulmonary dysfunction. Johnson notes that the rabbits used in much of the literature have an extremely reactive pulmonary circulation, and are one of the few species in which histamine results in pulmonary edema. Studies of the role of peptide 6A (a five amino acid fibrinogen degradation product known to cause

release of histamine and thromboxane) in capillary permeability are contradictory (Kern et al, 1986; Saldeen, 1982). As such, the role of FDP in pulmonary dysfunction remains controversial.

OVERVIEW OF AUTOTRANSFUSION

Autotransfusion was first reported in 1818 in England, was first used in the U.S. in 1917 and has been used only sporadically ever since, usually in times when banked blood has been unavailable (Bennett et al, 1973; Bell, 1978).

Advantages of the technique include reduced risks of disease transmission, alloimmunization and hemolytic reaction. Cross matching is not necessary. In addition, the technique is readily available and inexpensive (Raines et al, 1976; Bell, 1978; Mattox, 1978; Noon, 1978; Schaff et al, 1978). In one study, autotransfusion reduced the rate of homologous transfusion in post-operative patients from 66 to 40 % (Hartz et al, 1988). In another, homologous blood requirements decreased by 50% (Schaff et al, 1978). Potential risks include microembolism of blood, fat or debris, air embolism, bacteremia, and dissemination of neoplastic cells, as well as the possibility of pulmonary insufficiency and coagulopathy (Bell, 1978).

Carty et al (1973) sample peritoneal blood from women undergoing surgery for ruptured ectopic pregnancy. Compared with venous samples from the same patients, the peritoneal blood is defibrinogenated, with significantly decreased plasminogen and greatly increased FDP. This is indicative of activation of plasminogen and generation of FDP from fibrinogen and fibrin in the peritoneal blood. Moore et al (1980) autotransfuse two times total intravascular volume into baboons, noting a DIC-like pattern with a transient decrease in fibrinogen and increase in

PT and PTT, and a more prolonged thrombocytopenia with impaired in vitro platelet function. Stillman et al (1976) reinfuse 3-12 liters of unwashed salvage blood into dogs, with resultant decrease in platelets and fibrinogen and some evidence of DIC. These changes seem unrelated to the extent of tissue contact of the shed blood. Dogs maximally reinfused die, while those that survive show rapid recovery of platelet and fibrinogen levels. Rakower et al (1973), employing autotransfusion on emergency trauma and vascular patients (minimum two liters) observe thrombocytopenia, hypofibrinogenemia and increases in PT, PTT and FDP with frank oozing from surgical sites not corrected by protamine or fresh frozen plasma. However, these observations are confounded by the concomitant use of homologous transfusion (minimum 5 liters) and systemic heparinization.

In contrast to these studies are several reports finding no increased risk resulting from autotransfusion. Napoli et al (1987) autotransfuse 25% of total blood volume collected from the pleural space of the dog. In spite of defibrinogenation and generation of FDP in the hemothorax blood, no hemostatic defects occur in the dog. There are only mild decreases in platelets and fibrinogen, negligible elevations in FDP, and no change in PT or PTT. Rakower et al (1974) autotransfuse into dogs two times the total blood volume from a surgically created tissue pouch. They observe platelet aggregates in the shed blood, as well as decreased platelet and fibrinogen levels in the dogs. PTT is increased (although this may be due to heparinization). In sum,

the hemostatic changes observed are not sufficient to cause coagulopathy. Bennett et al (1973) autotransfuse shed peritoneal blood into dogs at 4-9 times the total intravascular volume, observing no change in hemostatic factors.

Hartz et al (1988) autotransfuse post-operative shed mediastinal blood (average 800 ml) into cardiac patients. While the shed blood shows decreased platelets and fibrinogen and increased FPA and FDP, there are no in vivo changes in these hemostatic factors. Brenner et al (1973) autotransfuse an average of 1.5 liters of shed blood into 20 patients undergoing abdominal aortic reconstructive surgery. Compared to 20 patients using homologous transfusion, these patients experience no unusual bleeding complications or changes in PT, PTT or fibrinogen. Thrombocytopenia occurs in four of these twenty patients, but no bleeding complications occur. In addition, no increased risk of microaggregates or pulmonary dysfunction is seen. Raines et al (1976) observe thrombocytopenia in patients receiving more than 3.5 liters of autotransfusion, as well as in vitro impairment of activation and aggregation in platelets recovered from the shed blood of such patients. Nevertheless, these patients show no clinical impairment of platelet function. Moreover, there is no evidence for coagulopathy, microembolism or impairment of pulmonary function in any of these patients despite often massive autotransfusion. Bell (1978) and Schaff et al (1978) similarly show no increase in risk of coagulopathy, microembolism or infection in cardiac surgical patients receiving

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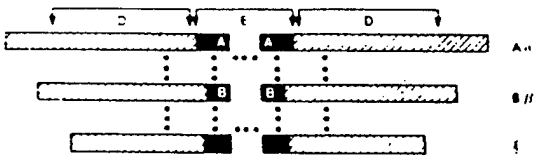
autotransfusion.

Despite some controversy, the risks of autotransfusion seem primarily hypothetical, with most animal and clinical work showing minimal adverse effects. Nevertheless, the role of FDP in coagulation, platelet function and pulmonary function remains in question. Its role in autotransfusion is complicated by shock, trauma and pathology, as well as the stresses of surgery and anesthesia. It is the goal of this study to examine the effects of FDP infusion in a healthy, non-traumatized, normovolemic baboon model, with full evaluation of coagulation profile, of platelet function (with special regard to bleeding time) and of pulmonary function.



V adapted from FURLAN & BECK, 1972
MARDER BUDZYNSKI & JAMES 1972
MILLS 1972
PIZZO SCHWARTZ HILL & McKEE, 1972

FIGURE 1

FROM BUDZYNSKI & MARDER (1977)

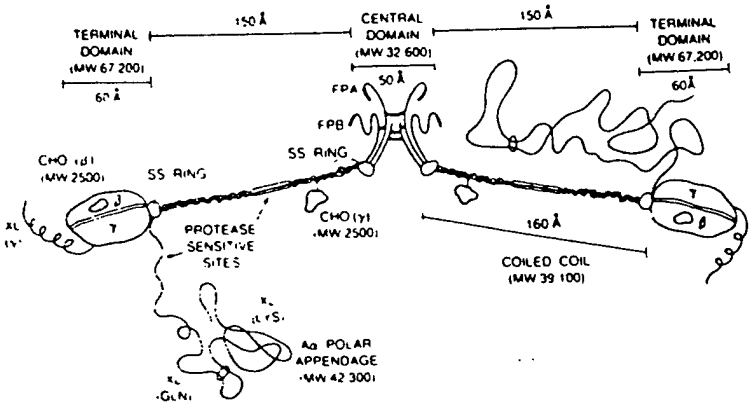


Figure 1 Schematic drawing of fibrinogen showing the central domain with amino terminals of all six chains, connecting coiled coils, the two distal domains, and the α-chain protuberances. The linear alignment of the three major domains and their noted dimensions reflect results obtained from electron microscopy (1). The lengths of the coiled coils (160 Å) are based on calculations for a helical structure 112 residues in length, and agree closely with EM results. Fibrinopeptides A and B are noted at the amino-terminal ends of the α and β chains. The two sets of polypeptide chains are held together by three disulfide bridges, two between the γ chains and one between the α chains. There are four carbohydrate clusters (CHO), each about M_r 2500 located on the γ chains near the central domain and on the β chains on each distal domain. Primary cross-linking sites (XL) are situated very near the carboxy termini of the γ chains. On the α chain, the two cross-linking glutamine acceptor sites (Gln) are about 200 residues from the five potential donor lysine sites (Lys). The overall molecular weight of this dimeric, 6-chained molecule is 340,000, and its overall length is approximately 450 Å. There are many regions of the molecule that are sensitive to plasmin, but the two specific sites noted here ("protease-sensitive sites") are of special interest: Cleavage at the proximal portion of the α-chain protuberance is critical in the conversion of fibrinogen to fragment X, which still retains the trinodular structure. Cleavage of all three chains in the central part of the coiled coils splits fragment X into Y + D and fragment Y into D + E. Fragments D and E correspond roughly to the terminal and central domains respectively, except that parts of the coiled coil between the protease-sensitive site and the proximal or distal disulfide ring are part of the degradation products (from 22).

FIGURE 2

FROM DOOLITTLE (1984)

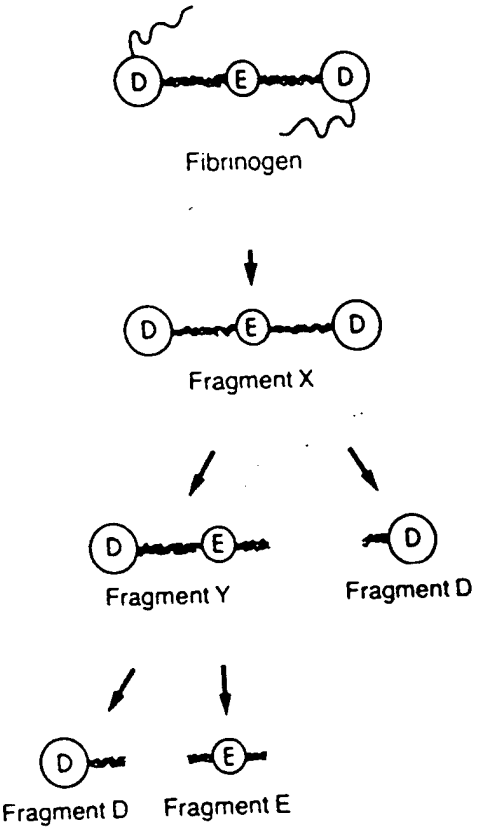


Figure 2 Asymmetric degradation of fibrinogen by plasmin. The principal structures are the three globular domains from which degradation Fragments D and E derive, the proposed α helical coiled coils connecting them, and the long Aα chain extensions from each of the D domain regions. Intermediate degradation Fragment X consists of all three domainal regions, but lacks the Aα chain extensions. Fragment Y consists of the central E domain with either of the terminal (Fragment D) domains connected by the coiled coil.

FIGURE 3

FROM FRANCIS & MARDER (1986)

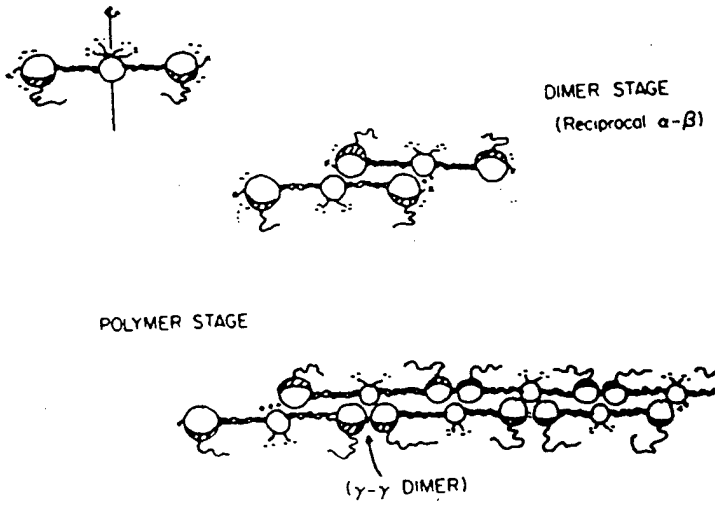


Figure 3 Initial polymerization events in fibrin formation. Removal of fibrinopeptides exposes sites on central domain that can then interact with complementary sites on distal domains of other molecules. An additional set of contacts (end-to-end) comes into play upon addition of third molecule (reprinted from 18).

FIGURE 4

FROM DOOLITTLE (1984)

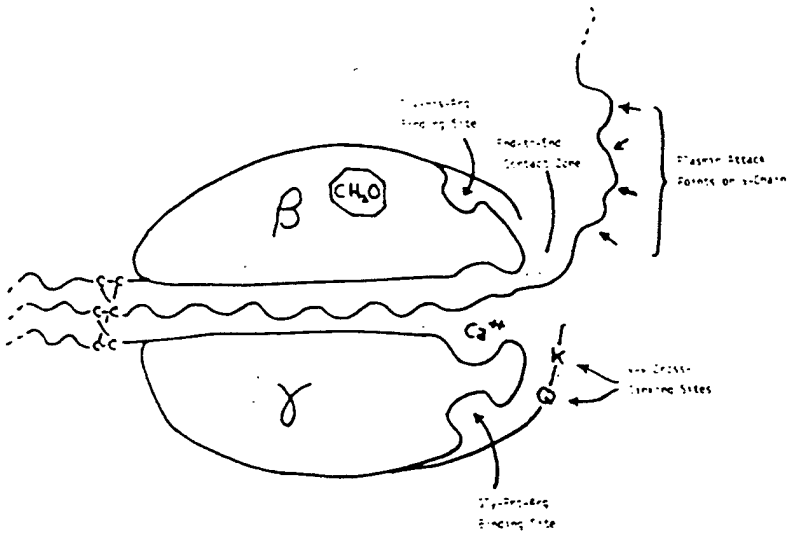


Figure 4 Schematic representation of distal domain of mammalian fibrinogen (from 44).

FIGURE 5

FROM DOOLITTLE (1984)

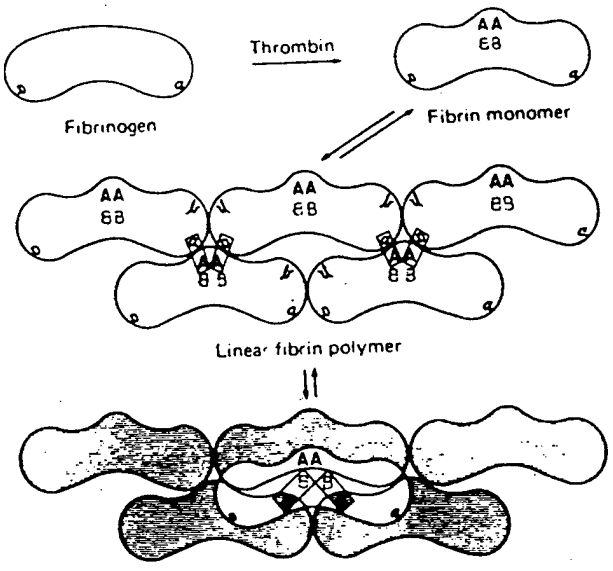


FIG. 2. Model for human fibrin polymerization. Fibrinogen is a bivalent molecule depicted as a flexible banana model (29, 30) with an available polymerization site on the fragment D domain of the molecule "a". Upon cleavage of fibrinopeptides A and B by thrombin, fibrin monomer is formed and a three-nodule form (31, 32) is accentuated in this figure. By this reaction two sets of binding sites ("A" and "B") on the NH_2-terminal domain of the molecule become available. Polymerization sites depicted by broken letters are on the opposite side of the molecule from sites referred to by solid letters. The "A" sites are complementary to the "a" sites on the fragment D domain. The binding of these sites induces linear polymerization of the molecules. The linear polymerization of fibrin monomers results in the formation of a new bivalent polymerization site "bb." The crosslink bonds between two γ chains of the neighboring fibrin monomer molecules either may participate in the binding site or may stabilize the sites on aligned fibrin monomer molecules. The "b" site is complementary to the thrombin-activated site on the NH_2-terminal domain of fibrin monomer "B." The two "B" sites on the second (upper) layer of fibrin bind to the "bb" sites on the first (lower, shaded) layer. Meanwhile, the alignment of the fibrin monomer molecules on the second (upper) layer results in the formation of "bb" binding sites that will enable the addition of a third layer of fibrin. Therefore, the binding of "A" to "a" sites promotes linear polymerization of the fibrin monomer molecules as well as fibrin strand branching, whereas the binding of "BB" to "bb" sites allows lateral aggregation. The interaction of these four sites represents a major mechanism for fibrin polymerization; however, other interactions such as those responsible for the ordered precipitation of fibrinogen by protamine sulfate may also play a role.

FIGURE 6

FROM OLEXA & BUDZYNSKI (1980)

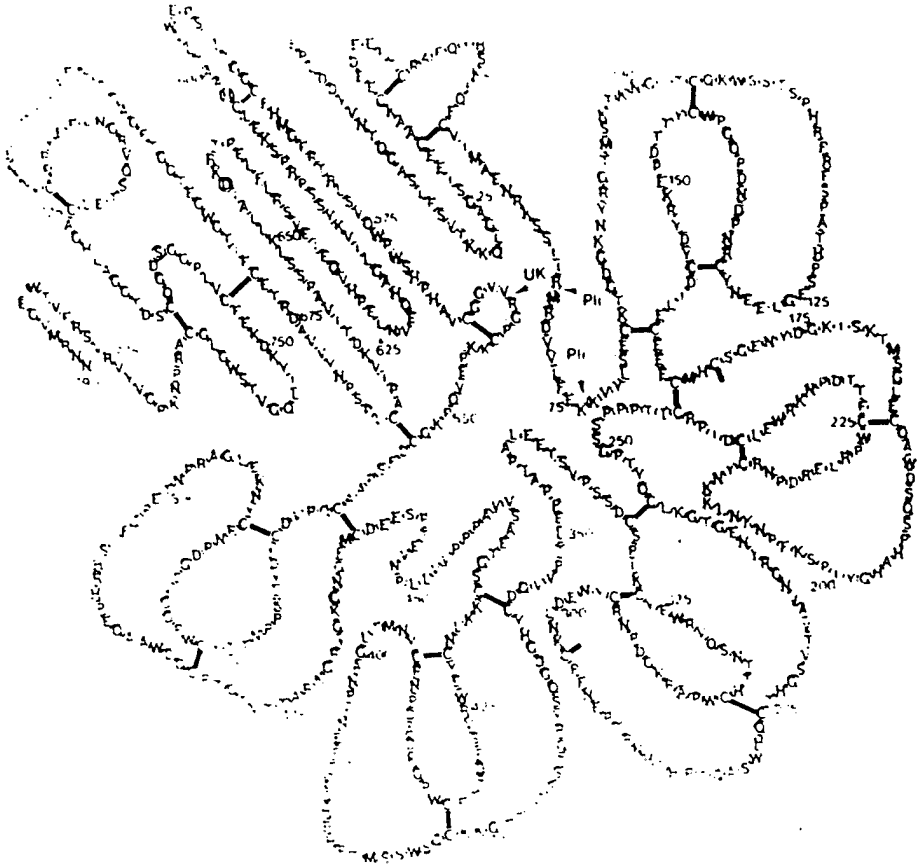


FIGURE 7

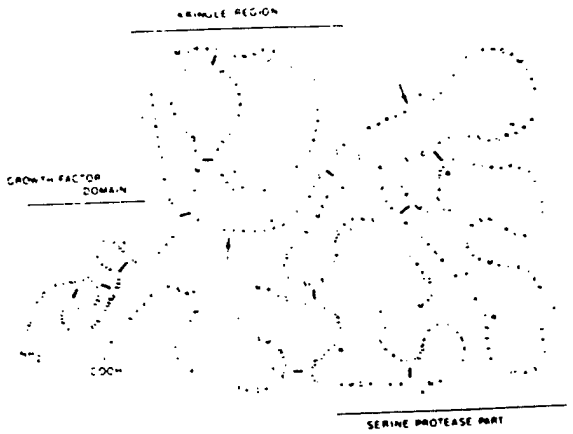


FIG. 1. Schematic representation of the primary structure of Mr 54,000 single-chain urokinase-type plasminogen activator (scu-PA). High Mr (54,000) two-chain urokinase-type plasminogen activator (tcu-PA) is obtained by hydrolysis of the Lys_{158}-Ile_{159} peptide bond, and subsequent hydrolysis of the Lys_{135}-Lys_{136} peptide bond yields low Mr (33,000) tcu-PA. Low Mr (32,000) scu-PA is generated by specific hydrolysis of the Glu_{143}-Leu_{144} peptide bond. The active site residues His_{204}, Asp_{255}, and Ser_{356} are indicated by an asterix. (Adapted with permission from Holmes et al[18] and Gunzler et al.[21])

FIGURE 8

FROM LIJNEN ET AL (1987)

Plasminogen Activation by Sc-UK-Primary and Secondary Reactions

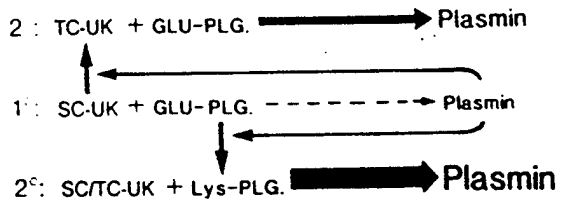


FIG. 4. Illustration of the primary (1°) reaction of single-chain pro-UK (sc-UK) with Glu-plasminogen and the secondary (2°) reactions related to tc-UK and Lys-plasminogen formation, which provide positive feedback and amplify plasmin generation.

FIGURE 9

FROM GUREWICH & PANNELL (1987)

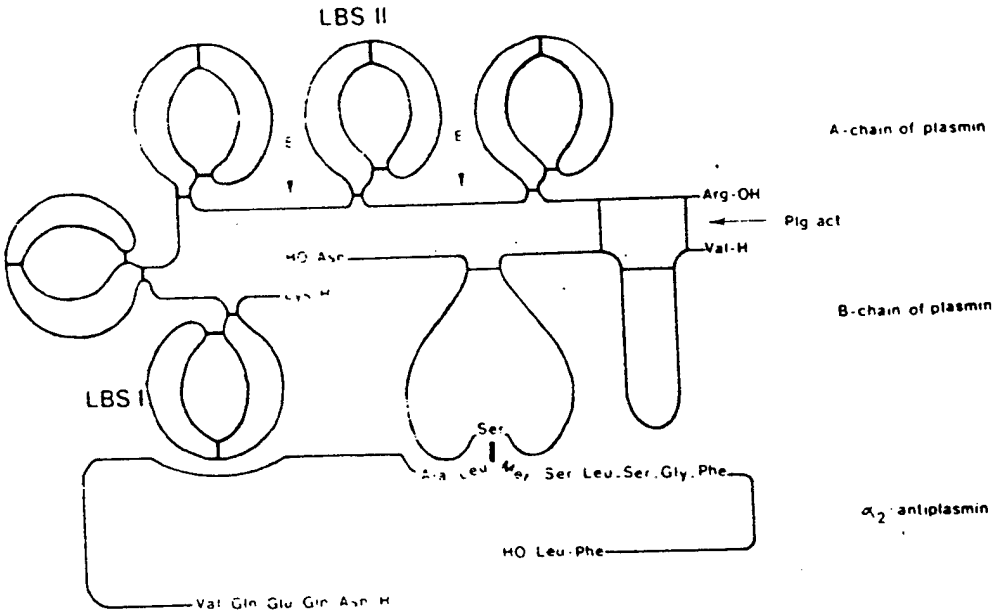


FIGURE 10

FROM COLLEN (1980)

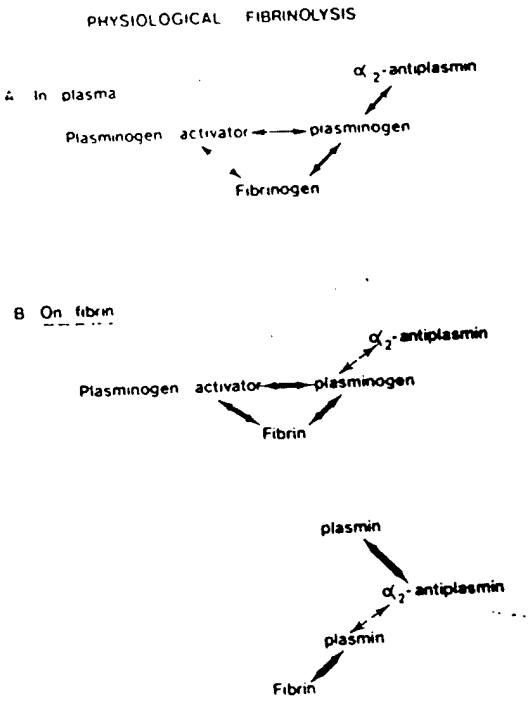


Fig. 4. Schematic representation of the interactions between fibrinogen, plasminogen, α_2-antiplasmin and plasminogen activator. The size of the arrows is roughly proportional to the affinity between the different components.

FIGURE 11

FROM COLLEN (1980)

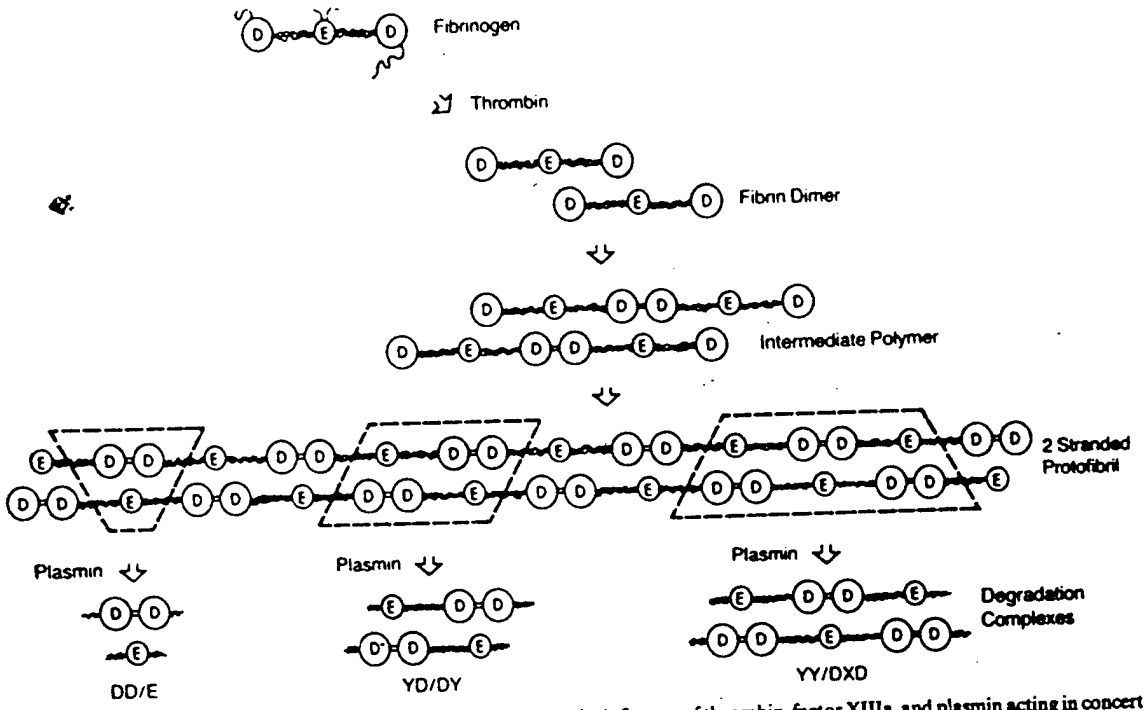


Figure 3 Fibrin polymerization, cross-linking, and degradation showing the influence of thrombin, factor XIIIa, and plasmin acting in concert on the structure of cross-linked fibrin degradation products. After thrombin liberation of the fibrinopeptides, two fibrin monomers form a half-overlap fibrin dimer as the initial step of polymerization. Additional monomers are added to each end by a similar half-overlap process to form an intermediate polymer and then a protofibril. Factor XIIIa catalyzes the formation of cross-links between γ chains of contiguous terminal domains. Plasmic degradation of a long two-stranded protofibril results in the series of noncovalently bound complexes (*bottom*), the smallest of which is DD/E. The presence of fragments larger than DD (such as DY) attached noncovalently to liberated complementary regions of another fibrin strand provides the basis for this scheme of ever-larger cross-linked fibrin degradation complexes. (For convenience, the α chain extensions are shown only for the intact fibrinogen molecule.)

FIGURE 12

FROM FRANCIS & MARDER (1986)

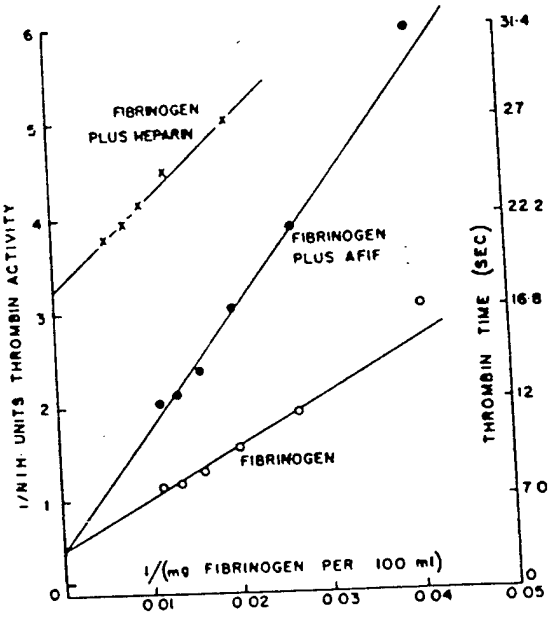


FIG. 3. Lineweaver-Burk plot of the effect of fibrinogen concentration on the thrombin activity of plain fibrinogen, fibrinogen mixed with AFIF and fibrinogen mixed with heparin.

FIGURE 13

FROM TRIANTAPHYLLOPOULOS (1959)

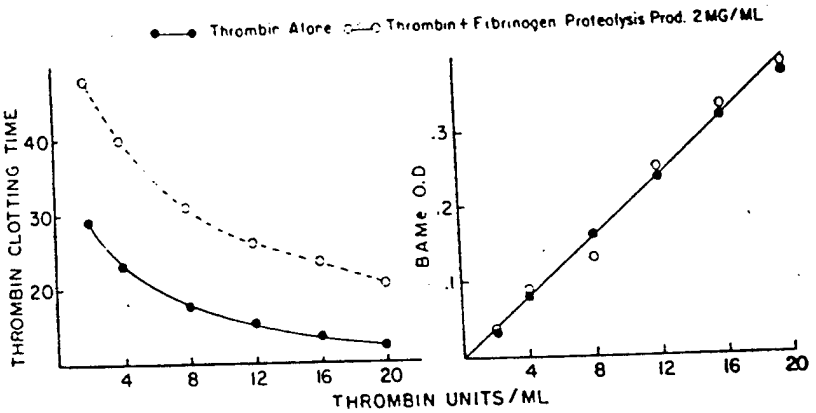


FIG. 1. (LEFT) THROMBIN CLOTTING TIMES (ORDINATE) AS A FUNCTION OF THROMBIN CONCENTRATION (ABSCISSA) FOR A NORMAL PLASMA AND FOR THE SAME PLASMA CONTAINING FIBRINOGEN PROTEOLYSIS PRODUCTS (2 MG PER ML). (RIGHT) BAMe ESTERASE ACTIVITY (ORDINATE) AS A FUNCTION OF THROMBIN CONCENTRATION (ABSCISSA) IN THE PRESENCE AND IN THE ABSENCE OF FIBRINOGEN PROTEOLYSIS PRODUCTS (2 MG PER ML). Despite the striking increase in thrombin clotting time that results from the presence in plasma of fibrinogen proteolysis products (left-hand panel), the enzymatic actions of thrombin on BAMe were not inhibited by these products (right-hand panel).

FIGURE 14

FROM ALKJAERSIG ET AL (1962)

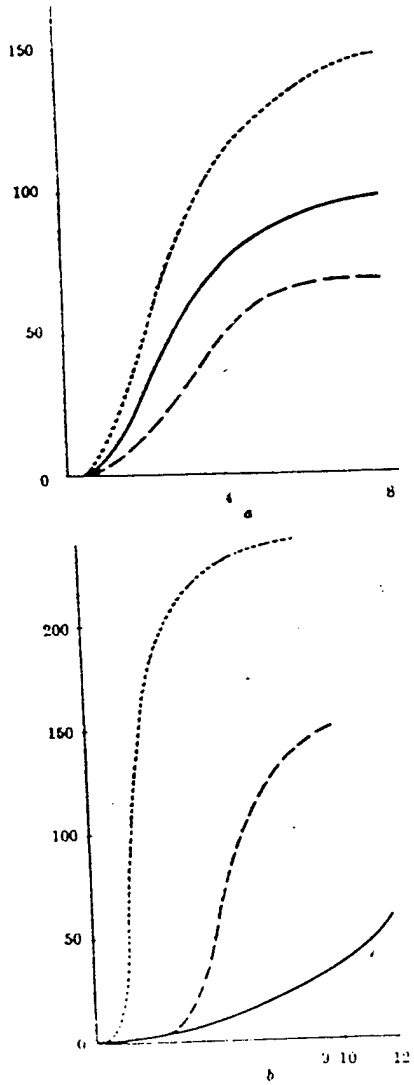


Fig. 2. Influence of 'early' and 'late' FDP on optical density changes during clotting of fibrin monomer (*a*) and fibrinogen with thrombin (*b*). Ordinates give absorbance at 350 mμ and abscissæ time (min). Final concentrations of fibrin monomer and fibrinogen were 0·45 mg/ml. and 0·55 mg/ml. respectively; concentrations of both 'early' and 'late' FDP were 0·12 mg/ml. Reaction *p*H = 7·5, phosphate buffer molarity 0·05; temperature 20° C. For experiments with fibrinogen 0·02 N.I.H. u./ml. of thrombin was used. Readings were performed every 15 sec. Upper dotted line—control (without FDP), solid line—with 'early' FDP, and broken line—with 'late' FDP

FIGURE 15

FROM LATALLO ET AL (1964)

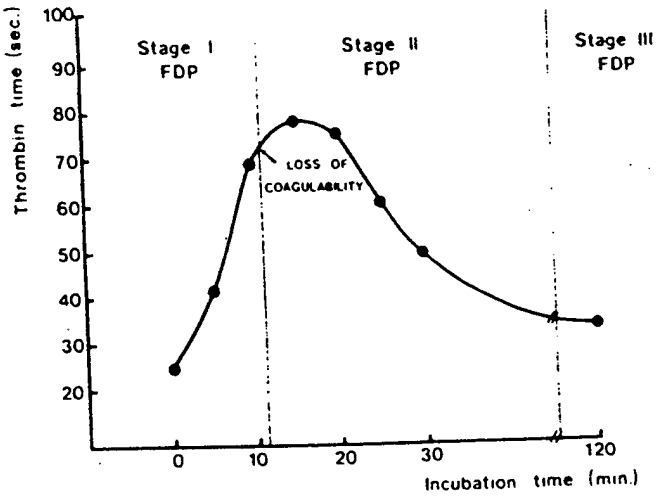


Figure 1. The influence on the thrombin clotting time of mixtures of FDP from the different fibrinogenolytic stages (according to Marder et al 1969) (cfr. 'Experimentals').

FIGURE 16

FROM ARNESEN & GODAL (1973)

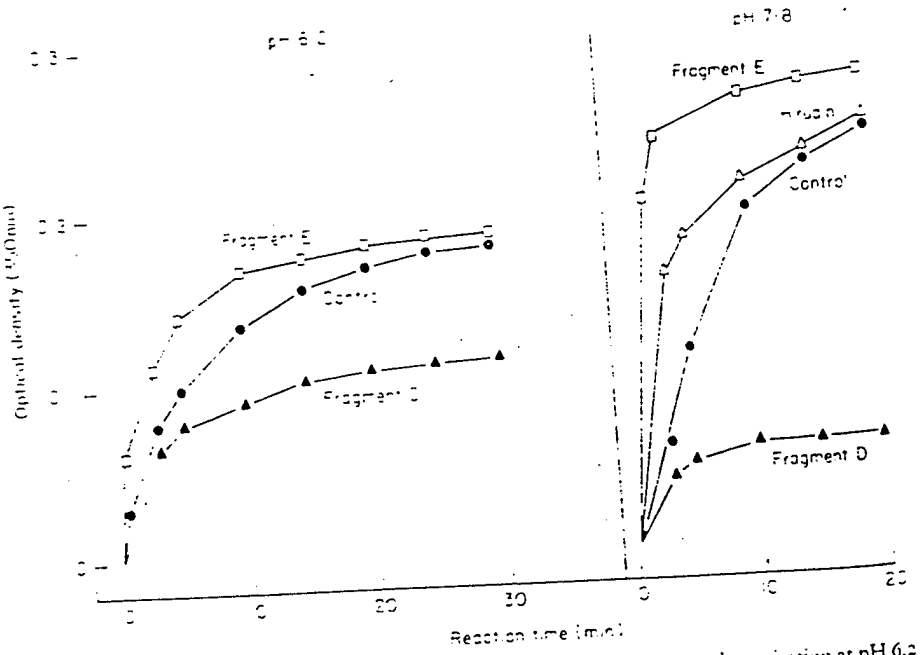


Fig 7. Comparative effect of fragment D and fragment E on fibrin monomer polymerization at pH 6.2 and of fragments D and E and hirudin at pH 7.8. Final protein concentration of both fragments D and E was 0.6 mg/ml; that of hirudin, 0.6 u/ml.

FIGURE 17

FROM LARRIEU ET AL (1972)

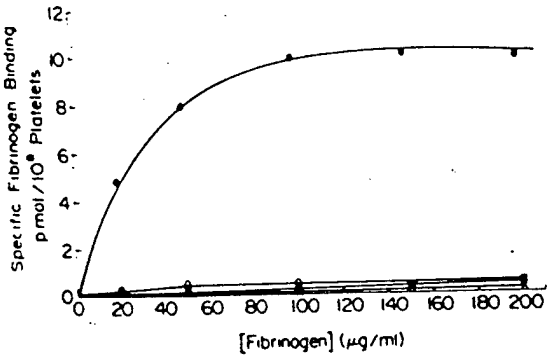


FIGURE 10 Comparison of ADP-stimulated fibrinogen binding to normal and thrombasthenic platelets. Gel-filtered platelets from one normal subject and from three patients with Glanzmann's thrombasthenia were incubated with increasing concentrations of ^{125}I-fibrinogen, 0.5 mM $CaCl_2$, and 10 μM ADP for 3 min at 37°C. Specific fibrinogen binding was determined as described in Fig. 2. Each point represents the mean of triplicate determinations. ●, normal subject; ▲, patient 1; ▼, patient 2; ■, patient 3.

FIGURE 18

FROM BENNETT & VILAIRE (1973)

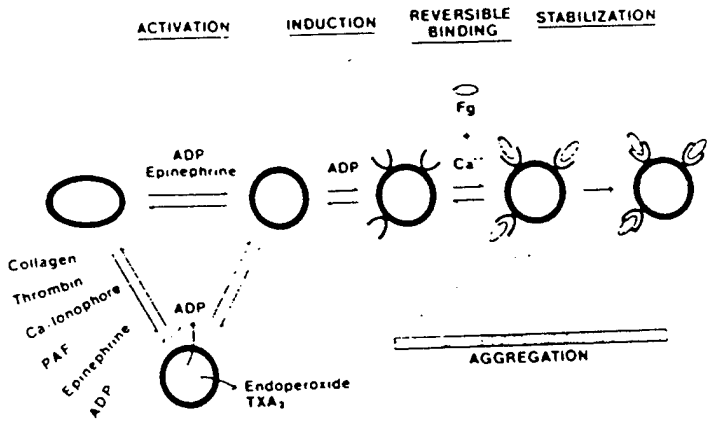


FIGURE 3. Schematic representation of the fibrinogen-dependent pathway of platelet aggregation. In this model sequence, it is hypothesized that aggregation of platelets by a variety of stimuli is dependent upon and concomitant with the binding of fibrinogen to its platelet receptor. This binding occurs in the presence of calcium (or magnesium) ions. Induction of this platelet receptor requires activation of the cell, and this activation can proceed through the secretion of platelet components such as ADP or prostaglandin derivatives. These components are themselves potent aggregating agents which can act synergistically or individually on other circulating platelets. ADP and epinephrine are unique inducers, since it has been possible to show the binding of fibrinogen with these stimuli in the absence of detectable secretion of serotonin. With all stimuli it was possible to demonstrate the association of fibrinogen with the platelets and it is postulated that this interaction regulates platelet aggregation.

FIGURE 19

FROM MARGUERIE & PLOW (1983)

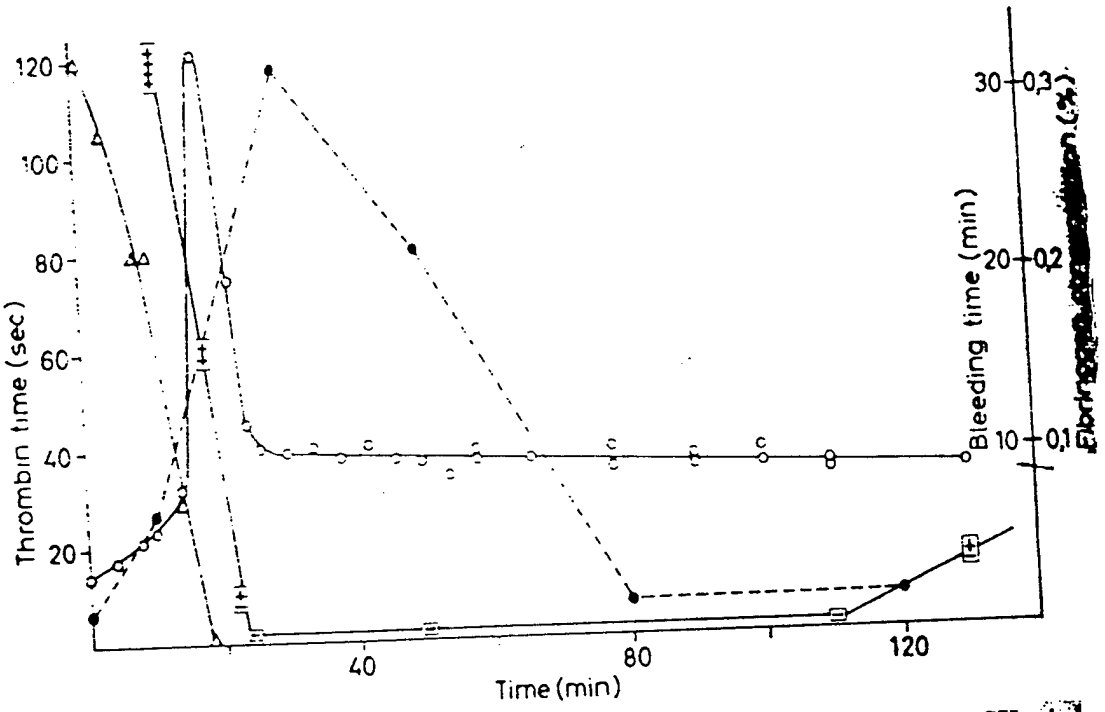
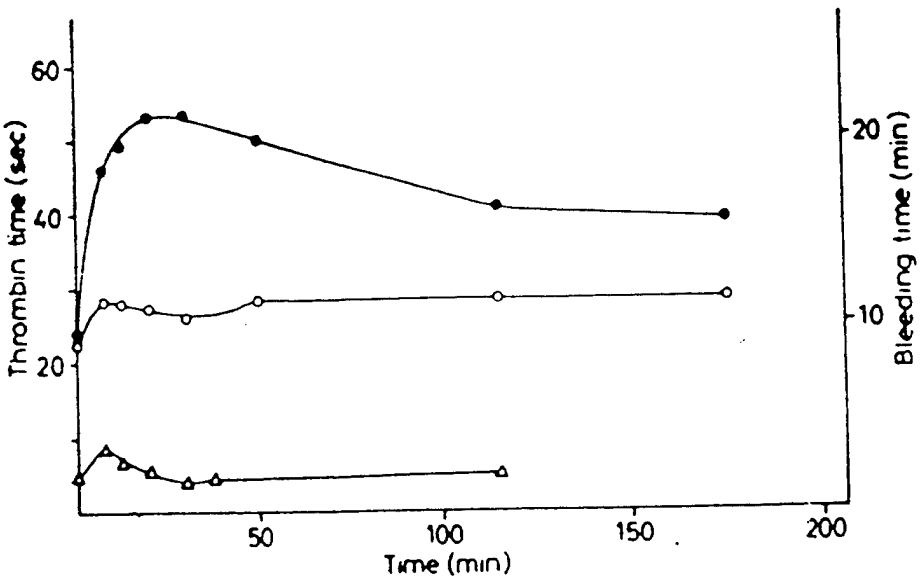


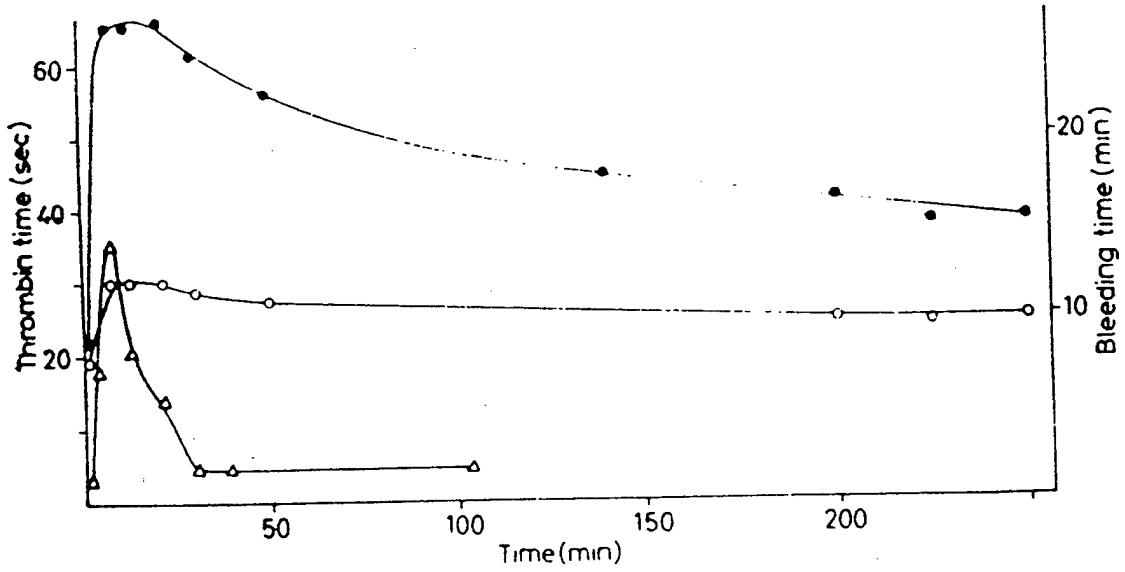
Fig. 2. Effect of a single dose of SKPl injected into a dog. 15 ml of SKPl (2500 SK u/ml, 5 mg of Pl/ml) was injected intravenously into a dog of 10 kg of weight. ○ thrombin time (method [illegible]), ▲ fibrinogen concentration, + and − positive and negative paracoagulation ● bleeding time.

FIGURE 20

FROM KOWALSKI ET AL (1964)



Effect of late FDP injection into untreated dog. 60 ml of 5% solution of late FDP was injected intravenously into a dog of 11 kg of weight. ○ thrombin time (method 1a); ● thrombin time (method 1f); △ bleeding time.



Effect of early FDP injection into untreated dog. 60 ml of 5% solution of early FDP was injected intravenously into a dog of 11 kg of weight. ○ thrombin time (method 1a); ● thrombin time (method 1f); △ bleeding time.

FIGURE 21

FROM KOWALSKI ET AL (1964)

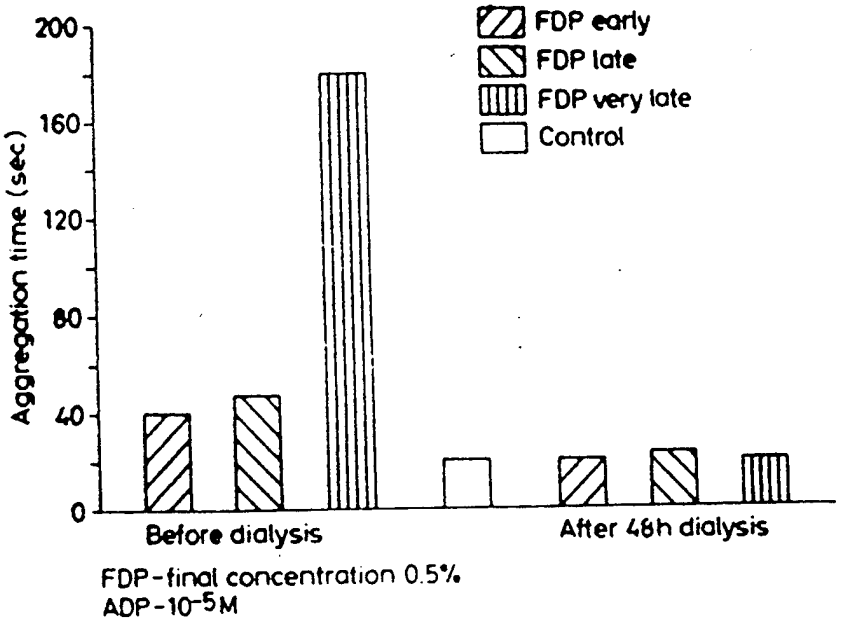


Fig. 2. Influence of FDP formed at various stages of proteolysis on platelet aggregation induced by ADP in PRP (platelet rich plasma). Effect of dialysis on activity of FDP.

FIGURE 22

FROM STACHURSKA ET AL (1970)

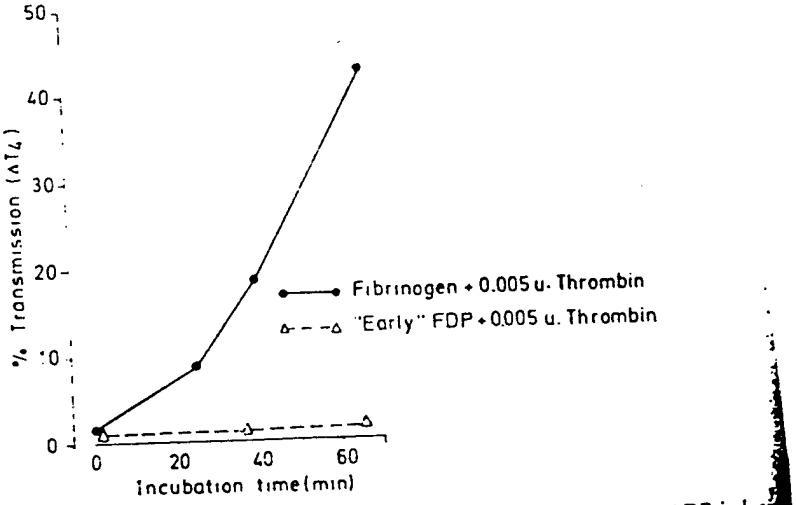


Fig. 5. The effect of fibrinogen and of early FDP preincubated with thrombin on ADP-induced platelet aggregation.

FIGURE 23

FROM NIEWIAROWSKI ET AL (1970)

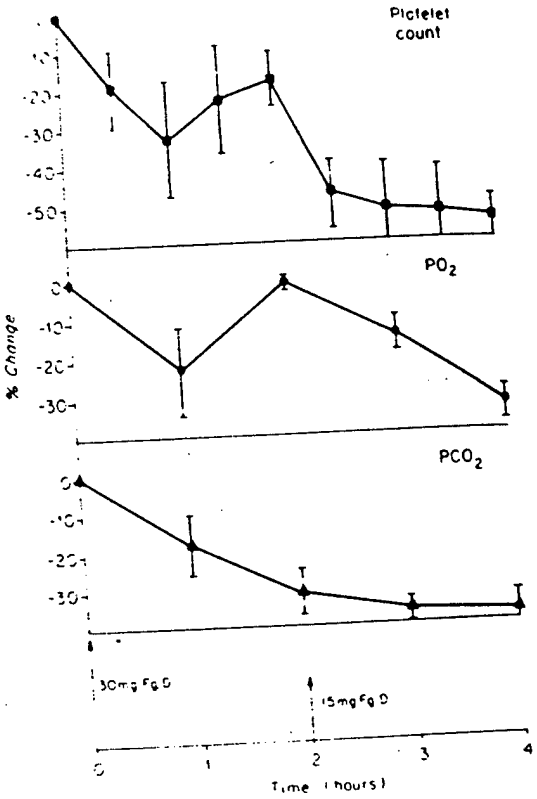


Fig. 7. Sequential changes of platelet concentrations, arterial Po_2, arterial Pco_2, and serum split product concentrations in fragment D infused animals.

FIGURE 24

FROM LUTERMAN ET AL (1977)

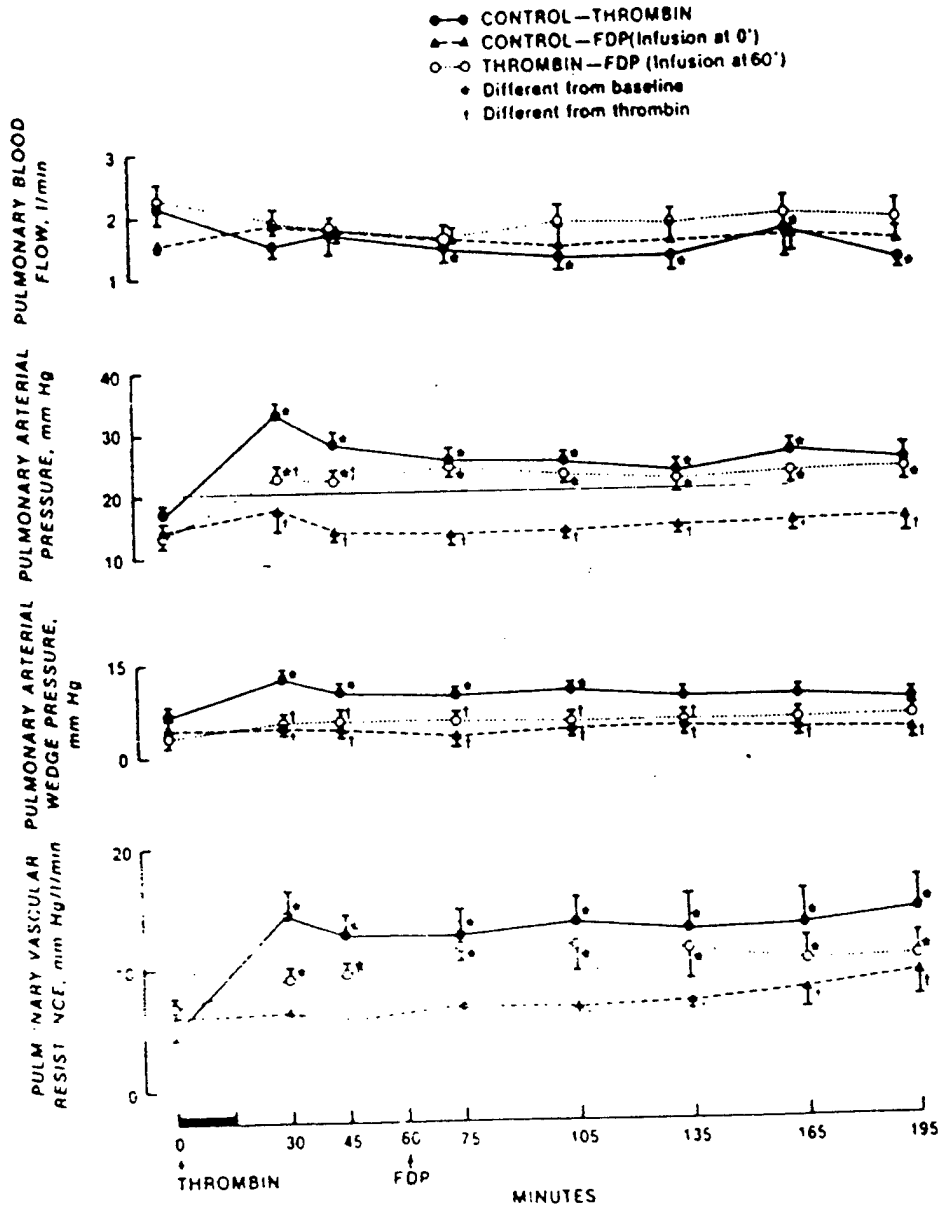


FIGURE 25

FROM JOHNSON ET AL (1985)

MATERIALS AND METHODS

Eight healthy male baboons of the species Papio cynocephalus or Papio anubis weighing 25-35 kg were sedated with a 200 mg intramuscular injection of Ketamine HCl. A 16 G intravenous catheter was placed in the right femoral vein. The baboons were anesthetized with Sodium Pentobarbital (130 mg), intubated with a 6.5 cuffed endotracheal tube and ventilated on room air at a rate of 10 and a tidal volume of 10cc/kg. Repeated small doses of pentobarbital were used throughout the study period to maintain depth of anesthesia, determined as that required to abolish the lid reflex. Ventilatory settings were adjusted to maintain arterial CO2 between 30 and 40 mmHg and subsequently remained unchanged for the duration of the study.

EKG leads were placed on the chest for monitoring of heart rate and rhythm. The right or left femoral artery was catheterized with a 20 G intravenous catheter for determination of arterial blood pressure and sampling of arterial blood. The left femoral vein was cannulated with a pulmonary arterial catheter introducer and an 8F Swan-Ganz Thermodilution Catheter (American Edwards; Irvine, CA) was placed into the left femoral vein and floated into the pulmonary artery for monitoring of central venous pressure, pulmonary arterial pressure, pulmonary arterial wedge pressure, cardiac output and core body temperature and for sampling of mixed venous blood. Core body temperature was maintained between 36.5 and 37.5 degrees Celsius using a warming

blanket and lamps.

A catheter was placed in the urinary bladder for monitoring of urine output and collection of urine samples. Infusion of normal saline via the 16 G catheter was adjusted to maintain a minimum urine flow of 1 cc/kg/hr.

All venous blood samples were withdrawn from the 16 G catheter.

Heart rate, arterial pressure, central venous pressure, pulmonary arterial pressure, pulmonary arterial wedge pressure, cardiac output, core temperature and urine output were measured at half hour intervals and at sampling times, determined to be baseline, pre-serum infusion, 10, 30, 60 minutes and 4 hours post plasma infusion. Cardiac output was determined in triplicate using the thermodilution technique on a Cardiac Output Computer 9520 (Edwards Laboratories; Santa Ana, CA).

Arterial blood gasses were measured hourly and at sampling times. Mixed venous blood gasses were measured at sampling times. End-expiratory and inspiratory pO2 and pCO2 were measured at sampling times. pH, pO2 and pCO2 were measured on a Stat Profile 4 (Nova Biomedical; Waltham, MA). Hemoglobin, %O2 Hb and % CO Hb were measured on a Co-Oximeter 282 (Instrumentation Laboratories; Lexington, MA).

Pulmonary vascular resistance was calculated from measurements of cardiac output and mean pulmonary arterial pressure. Respiratory shunt was calculated from measurements of hemoglobin concentration, hemoglobin saturation (arterial and venous), oxygen partial pressure (arterial and venous) and carboxyhemoglobin concentration (arterial). Dead space was calculated from measurements of arterial and end-expiratory carbon dioxide partial pressure.

After obtaining baseline measurements, 250 ml of whole blood was withdrawn in 50 ml increments from the intravenous catheter and transferred into a non-anticoagulated transfer pack on wet ice. The whole blood was centrifuged at 3700 RPM for 10 minutes at 4 degrees C, the plasma expressed into a separate transfer pack also on ice and the remaining red blood cells returned to the animal through a 170u transfusion filter and the intravenous catheter. Five minutes after reinfusion, the process was repeated for another 250 ml of whole blood, creating a pool of approximately 250 ml of plasma.

The plasma was weighed to approximate volume, treated with Urokinase (Abbokinase; Abbott Laboratories; North Chicago, IL) at either 3000 or 5000 IU/ml (determined at random) and incubated at 37 degrees celsius to generate plasmin and lyse fibrinogen. After 30 minutes, plasmin was inactivated with Aprotinin (Sigma Chemical Co.; St. Louis, MO) at 1000 KIU/ml and the newly lysed plasma was reinfused through a 170u transfusion filter and the intravenous catheter.

Randomization

Eight index cards, four with "3000 IU/ml" and four with "5000 IU/ml" were made at the outset of the study. After the baboon for each study was chosen, an index card would be drawn at random to determine the dose of urokinase to be used in that study.

HEMATOLOGIC MEASUREMENTS

Hematocrit, hemoglobin concentration, red blood cell count (RBC), white blood cell count (WBC) and platelet count were measured in whole blood collected into K(3)EDTA anticoagulant. Mean platelet volume was measured in platelet rich plasma obtained from K(3)EDTA whole blood. Platelet mass was calculated as the product of the mean platelet volume and platelet count.

Hematocrit was measured using the microhematocrit technique. Hemoglobin concentration was measured using the Coulter Hemoglobinometer (Coulter Electronics, Hialeah, FL). RBC and WBC was determined in a Coulter Counter with a 100u aperture (Coulter Electronics; Hialeah, FL). Platelet count was performed manually by phase microscopy. Mean platelet volume was measured using a linear scale on a Coulter ZBI counter with an H4 channelyzer attachment and a 50/60 aperture (Coulter Electronics; Hialeah, FL).

COAGULATION MEASUREMENTS

Thrombin time, (TT), prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, D-Dimer, alpha(2)-antiplasmin and anti-thrombin III were measured in plasma obtained from whole blood collected into NaCitrate anticoagulant. Fibrinogen degradation products (FDP) was measured in serum obtained from whole blood collected into a commercially available tube containing thrombin and epsilon aminocaproic acid (Wellcome

Diagnostics; Dartforth, UK). Plasma samples were stored at -80 degrees Celsius prior to analysis.

TT, PT and aPTT were determined using an automated instrument (Coag-U-Mate) and commercially available activating reagents (General Diagnostics; Morris Plains, NJ). Fibrinogen concentration was measured in a clotting time assay using the Coag-U-Mate (General Diagnostics; Morris Plains, NJ). D-Dimer was determined by ELISA (American Bioproducts; Parsippany, NJ), using the technique of Rylatt et al (1983). Alpha(2)-antiplasmin was measured in a chromogenic assay using the substrate CBS 33.08 (American Bioproducts; Parsippany, NJ) and the technique of Gallimore et al (1979). Anti-thrombin III was measured in a chromogenic assay using the substrate CBS 34.47 (American Bioproducts; Parsippany, NJ), and the technique of Odegard et al (1975). FDP was measured by latex bead agglutination (Thrombo-Wellcotest; Wellcome Diagnostics; Dartford, UK), using the technique of Arocha-Pinango (1972).

MEASUREMENT OF PLATELET FUNCTION

Bleeding time was determined in duplicate using the Simplate II bleeding time device (General Diagnostics; Durham, NC) and the technique of Mielke et al (1969). Skin temperature was measured within 5 millimeters of the bleeding time site using a Mon-A-Therm 6500 (Mon-A-Therm Inc.; St. Louis, MO).

Plasma thromboxane B2 (TxB2), the stable breakdown product of thromboxane A2 was measured in plasma obtained from whole blood collected into a heparin (1000 u/ml) coated tube containing 50 lambda of ibuprofen (1.9mg/ml). Shed blood TxB2 was measured in plasma obtained from shed blood collected at the bleeding time site into a 1 ml heparin coated syringe containing 20 lambda of ibuprofen. von Willebrand factor (vWF) was measured in plasma obtained from K(3)EDTA whole blood. Beta thromboglobulin (BTG) was measured in plasma obtained from whole blood collected into a commercially available tube containing 134 mM EDTA and 15 mM theophylline (Amersham International; Arlington Heights, IL). Plasma samples were stored at -80 degrees Celsius prior to analysis.

TxB2 was measured by radioimmune assay (New England Nuclear Corp.; Boston, MA), using the technique of Moncada et al (1978). vWF was measured by ELISA (American Bioproducts; Parsippany, NJ), using the technique of Cejka (1982). BTG was measured by radioimmune assay (Amersham International; Arlington Heights, IL), using the technique of Ludlam and Cash (1976).

PULMONARY MEASUREMENTS

C3a was measured in plasma obtained from whole blood collected in K(3)EDTA anticoagulant. C3a was measured by radioimmune assay (Amersham International; Arlington Heights, IL), using the technique of Chenoweth and Hugli (1980).

RENAL MEASUREMENTS

Blood urea nitrogen (BUN) and creatinine was measured in serum obtained from whole blood collected into a preservative free tube collected at baseline and four hours. Samples were stored at -80 degrees Celsius prior to analysis.

BUN and creatinine were determined in a chromogenic assay using commercially available substrates (Roche Diagnostics; Nutley, NJ).

FOLLOW-UP MEASUREMENTS

Twenty four and forty eight hours post serum infusion, the animal was sedated with Ketamine HCl (200 mg). An 18 G intravenous catheter was inserted into either femoral vein and the animal was anesthetized with Sodium Pentobarbital. Additional boluses were administered so as to abolish the lid reflex and maintain adequate depth of anesthesia.

A template bleeding time was performed, skin temperature was determined and shed blood was collected as described previously for measurement of TxB2.

Whole blood was collected from the intravenous catheter as described above for measurements of hematocrit, hemoglobin concentration, red blood cell count, white blood cell count, platelet count, mean platelet volume, thrombin time, prothrombin time, activated partial thromboplastin time, fibrinogen, D-Dimer, alpha(2)-antiplasmin, anti-thrombin III, fibrinogen degradation products, thromboxane B2, von Willebrand factor, beta thromboglobulin, blood urea nitrogen and creatinine.

STATISTICS

Comparison of sample means was performed by use of the two-tailed, paired Student's t-test. Correlations were plotted and analyzed on the Hewlett-Packard 9845B (Hewlett-Packard Co.; Sunnyvale, CA).

RESULTS

OPERATING ROOM MONITORING (Table 1)

Heart rate and cardiac output were not significantly changed over the anesthetic course. Mean arterial pressure increased significantly from 117 mmHg to 129 mmHg ($p<0.01$) after plasma infusion, remaining at such an elevated level for the remainder of the procedure. Mean pulmonary arterial pressure decreased from 12 mmHg to 11 mmHg from baseline to pre-infusion ($p<0.05$) but returned to near-baseline values at 1 hour and remained as such for the remainder of the procedure. Urine output was doubled from baseline at pre-infusion ($p<0.05$) and remained elevated, with maximal values at 1 hour, for the remainder of the procedure. At only one time was urine output less than 1 cc/kg/hr (0 cc from 30 to 60 minutes in Study #6). pH, paO2 and paCO2 were stable throughout the anesthetic course.

ROUTINE HEMATOLOGIC MEASUREMENTS (Table 2)

Hematocrit, hemoglobin concentration and red blood cell count all increased slightly from baseline to pre-infusion, hematocrit significantly so ($p<0.05$). All three levels decreased dramatically following plasma infusion ($p<0.01$) at which point they stabilized for the remainder of the anesthetic course. From 4 hours to 24 hours, all three measurements decreased approximately 20% ($p<0.01$), with an additional slight, non-significant decrease at 48 hours. White blood cell count increased significantly from 13.68 x10*6/ul baseline to 20.54

x10*6/ul at pre-infusion ($p<0.05$) and remained at such an elevated level up to 4 hours, at which it decreased to 14.92 x10*6/ul ($p<0.01$), with another significant decrease ($p<0.05$) to 10.0 x10*6/ul at 24 hours. Platelet count decreased greatly following infusion ($p<0.05$), remaining at such a decreased level through 48 hours. On only one occasion did platelet count decrease below 100 x10*3/ul in any baboon at any time (98 x10*3/ul at 24 hours in Study #8). Mean platelet volume remained stable throughout the anesthetic course, increasing significantly from 6.34 u*3 to 7.14 u*3 ($p<0.01$) only at 24 hours. Platelet mass remained stable through the course of the study and through 48 hours of follow up.

ASSESSMENT OF COAGULATION FACTORS (Table 3)

While thrombin time (TT) and prothrombin time (PT) were not significantly changed over the study, the activated partial thromboplastin time (aPTT) increased significantly from 36.5 seconds at pre-infusion to 40.4 seconds at 30 minutes ($p<0.01$). Fibrinogen decreased from 109 mg/dl to a minimum of 78 mg/dl at 10 minutes ($p<0.01$). It remained significantly decreased from pre-infusion values throughout early follow-up, then increased dramatically to a supernormal value of 206 mg/dl at 24 hours and 278 mg/dl at 48 hours. Antiplasmin was stable early in follow-up, but decreased significantly from 67.6 % of control at pre-infusion to 43.6% of control at 4 hours ($p<0.05$) before returning to pre-infusion values at 24 and 48 hours. Antithrombin

III was stable throughout the study course.

FIBRINOLYSIS MATERIAL (Tables 3 & 4)

Following plasma infusion, levels of fibrinogen degradation products (FDP) increased from close to zero at pre-infusion to elevated levels of 165 ug/ml ($p<0.01$) at 10 minutes that persisted through 4 hours. At 24 hours, FDP levels of 26 ug/ml were greatly decreased from the previous day, but remained significantly elevated ($p<0.05$) over pre-infusion levels. Only at 48 hours did FDP levels return to baseline. D-Dimer levels followed a similar pattern as FDP, with large and significant increases ($p<0.01$) from 10 minutes through 4 hours and smaller but still significant increases at 24 hours. Unlike FDP, D-Dimer levels remained significantly increased ($p<0.01$) at 48 hours.

When stratified by dose of urokinase (Table 4), the levels of FDP and D-Dimer tend to rise and fall in parallel to the cumulative results. FDP levels at 60 minutes and 4 hours are much higher in the 3000 IU/ml group than in the 5000 IU/ml group, but these data contain so much variance as to render the resulting differences non-significant. D-Dimer levels are consistently higher in the 5000 IU/ml group than in the 3000 IU/ml group. However, the differences are small and non-significant (Table 4).

Similarly, alpha(2)-antiplasmin levels tend to follow the trend established in the cumulative results. Early in follow-up, levels tend to be lower in the high-dose group, especially so at 60 minutes (29.8 vs. 54.3% of control). Nevertheless, these

results are non-significant. Later in follow-up, at 4 and 24 hours, results are lower in the low-dose group, but again non-significantly so.

ASSESSMENT OF PLATELET FUNCTION (Table 5)

Bleeding time almost doubled from pre to post plasma infusion, from 148 seconds pre-infusion to a maximum value of 284 seconds at 10 minutes ($p<0.01$). Bleeding time remained similarly elevated at the $p<0.05$ or $p<0.01$ level through 60 minutes, decreasing to 240 seconds ($p<0.01$) at 4 hours and 216 seconds ($p<0.05$) at 24 hours. At 48 hours, bleeding time was elevated at 201 seconds, but this elevation was non-significant. Skin temperature was stable throughout the anesthetic course, but was increased significantly at 24 hours ($p<0.01$). As mentioned previously, platelet count decreased significantly from 240 x10*3 pre-infusion to 197 x10*3 post infusion ($p<0.05$), remaining decreased throughout the follow up period. Mean platelet volume and platelet mass did not significantly differ from pre-infusion through 48 hours. Plasma thromboxane B2 (TxB2) decreased significantly from 189 pg/0.1 ml to 120 pg/0.1 ml at 10 min ($p<0.05$), remained stable throughout the procedure and then decreased significantly ($p<0.01$) once again to 54 ng/0.1 ml at 24 hours. Shed blood TxB2 remained stable at pre-infusion levels throughout the study, decreasing significantly from pre-infusion only at 24 hours ($p<0.01$). von Willebrand factor (vWF) and beta thromboglobulin (BTG) were not significantly changed throughout

the study and follow-up periods.

CORRELATION ANALYSES (Tables 6 & 7)

Correlations of PT with FDP, D-Dimer and fibrinogen were all significant at $p<0.01$ (Table 6). The strongest correlation was found to be between PT and FDP ($r=0.68$), though correlations with D-Dimer ($r=0.42$) and fibrinogen ($r=-0.55$) were also very strong. Correlation of aPTT was strongest with D-Dimer ($r=0.47$, $p<0.01$), although it was also moderately strong with FDP ($r=0.38$, $p<0.05$) and fibrinogen ($r=-0.37$, $p<0.05$). Correlation of TT with fibrinogen was moderately strong ($r=-0.41$, $p<0.01$), but with FDP and D-Dimer were non-significant at $p<0.05$.

Correlations between bleeding time (Table 7) and platelet count, platelet volume, skin temperature, plasma TxB2, shed blood TxB2, vWF and FDP were all non-significant at $p<0.05$. Correlation between bleeding time and D-Dimer was strongest at $r=0.33$ ($p<0.01$) although that between bleeding time and fibrinogen was also moderately strong at $r=-0.29$ ($p<0.05$).

Correlations between bleeding time and the logarithm of FDP and D-Dimer proved to be moderately strong at $r=0.45$ and $p<0.01$ for each.

ASSESSMENT OF RESPIRATORY FUNCTION (Table 8)

As mentioned previously, paO2 and paCO2 did not change significantly over the course of observation. Respiratory shunt increased from 17.476% to 24.732% at 10 minutes, but this

increase was not significant. Pulmonary vascular resistance (PVR) and pulmonary dead space (Vd/Vt) also did not show statistically significant change.

C3a levels were moderately increased from 63ng/ml pre-infusion to 77 ng/ml at 10 minutes. However, this change was not significant.

Correlations of PVR, Qs/Qt and Vd/Vt with FDP, D-Dimer and C3a were all non-significant at $p<0.05$ (Table 9).

ASSESSMENT OF RENAL FUNCTION (Table 10)

Serum BUN and creatinine were not significantly changed over the course of the procedure and through 48 hours of follow-up. As mentioned previously, urine output was maintained if not increased after plasma infusion. Urine output dropped below 1 cc/kg/hr only once in any of the eight experimental animals.

TABLE 1 - OPERATING ROOM ON-LINE MONITORING

	Baseline	Pre-Infusion	1 Hour	2 Hour	3 Hour	4 Hour
Heart Rate (beats/min)						
Mean:	122	115	123	122	126	126
SD:	13	7	10	11	12	11
n:	8	8	8	8	8	8
Mean Arterial Pressure (mm Hg)						
Mean:	121	117	129+	129	128	130
SD:	17	14	18	19	20	21
n:	8	8	8	8	8	8
Mean Pulmonary Arterial Pressure (mm Hg)						
Mean:	12	11*	14*	13	14	13
SD:	5	4	5	5	5	5
n:	8	8	8	8	8	8
Pulmonary Artery Wedge Pressure (mm Hg)						
Mean:	4	4	5	5	6	5
SD:	3	2	3	3	3	4
n:	7	8	8	8	8	8
Cardiac Output (l/min)						
Mean:	3.42	3.20	3.45	3.45	3.34	3.47
SD:	0.46	0.81	0.40	0.64	0.37	0.65
n:	7	7	8	8	8	8
Urine Output (ml/30 min)						
Mean:	31	66*	125	82	43	45
SD:	24	26	80	59	27	26
n:	8	8	8	8	8	8
Arterial pH						
Mean:	7.49	7.47	7.47	7.47	7.48	7.48
SD:	0.03	0.02	0.03	0.02	0.03	0.03
n:	8	8	8	8	8	8

TABLE 1 - OPERATING ROOM ON-LINE MONITORING (CON'T)

	Baseline	Pre-Infusion	1 Hour	2 Hour	3 Hour	4 Hour
Arterial pO2 (mm Hg)						
Mean:	91.3	91.4	90.8	90.0	88.4	91.6
SD:	13.3	17.6	15.6	13.6	14.7	14.6
n:	8	8	8	8	8	8
Arterial pCO2 (mm Hg)						
Mean:	32.9	32.1	31.7	31.1	30.6	30.8
SD:	2.9	1.9	3.4	2.5	2.4	2.5
n:	8	8	8	8	8	7

*$p < 0.05$ as compared to previous measurement time.
+$p < 0.01$ as compared to previous measurement time.

TABLE 2 - ROUTINE HEMATOLOGIC MEASUREMENTS

	Base-line	Pre-Infusion	10	30	60	4 HR	24 HR	48 HR
Hematocrit (V%)								
Mean:	42.0	44.1*	38.0+	39.0	39.7	39.7	32.4+	31.0
SD:	2.4	2.5	3.6	2.9	3.2	2.7	4.3	4.3
n:	8	8	8	8	8	8	8	6
Hemoglobin (g/dl)								
Mean:	13.7	14.2	12.2+	12.6	12.8	12.7	10.7+	9.9
SD:	0.6	0.7	1.0	0.6	0.8	0.7	1.0	1.1
n:	8	8	8	8	8	8	8	6
RBC ($X10^6$/ul)								
Mean:	5.40	5.55	4.70+	4.88*	4.98	5.04	4.20+	3.92
SD:	0.30	0.28	0.41	0.36	0.44	0.25	0.40	0.53
n:	7	8	8	8	8	8	8	6
WBC ($X10^3$/ul)								
Mean:	13.7	20.5*	16.9	17.3	18.9	14.9+	10.1*	9.2
SD:	4.3	5.5	2.1	2.2	5.2	3.9	2.8	2.4
n:	8	8	8	8	8	8	8	6
Platelet Count ($X10^3$/ul)								
Mean:	257	240	197*	207	202	184	166	183
SD:	81	74	78	82	77	67	73	81
n:	8	8	8	8	8	8	8	6
Platelet Volume (u^3)								
Mean:	6.30	6.28	6.20	6.58	6.09	6.34	7.14+	6.93
SD:	1.43	1.31	1.22	1.31	1.28	1.25	1.35	1.53
n:	7	7	7	7	7	7	7	5
Platelet Mass (u^3X10^3/ul)								
Mean:	15.53	14.86	12.26	14.22	12.24	11.95	11.99	13.29
SD:	4.10	6.09	3.88	5.08	4.08	4.20	4.52	4.25
n:	7	7	7	7	7	7	7	5

*$p < 0.05$ as compared to previous measurement time
+$p < 0.01$ as compared to previous measurement time

TABLE 3 - ASSESSMENT OF COAGULATION FACTORS

	Baseline	Pre-Infusion	10	30	60	4 HR	24 HR	48 HR
PT (sec)								
Mean:	11.6	11.7	13.6	13.1	13.2	12.2	10.9	9.9
SD:	1.4	0.5	3.0	2.1	2.6	1.6	0.8	0.7
n:	8	8	8	8	8	8	8	6
aPTT (sec)								
Mean:	35.9	36.5	40.5	40.4+	38.0	35.6	36.8	34.2
SD:	9.2	1.9	2.7	3.2	3.4	2.9	3.3	2.3
n:	6	6	6	6	6	6	6	5
TT (sec)								
Mean:	10.9	15.2	17.6	17.1	16.3	12.1	9.7*	9.9*
SD:	1.7	7.1	9.0	8.9	8.0	3.0	1.4	0.7
n:	7	7	7	7	7	6	5	4
Fibrinogen (mg/dl)								
Mean:	148	109	78+	78*	82+	94*	206+	278+
SD:	21	13	13	13	13	17	36	49
n:	8	8	7	7	7	8	8	6
Alpha (2) antiplasmin (IU/ml)								
Mean:	66.1	67.6	70.6	56.8	42.0	43.6+	69.3	81.7
SD:	18.1	13.5	15.4	16.1	17.1	10.1	18.0	39.2
n:	7	7	8	8	8	8	8	5
Anti-thrombin III (% of control)								
Mean:	96.4	78.3	80.7	83.8	83.6	87.0	81.6	97.0
SD:	8.5	11.0	14.5	13.0	11.5	7.8	8.9	11.4
n:	6	8	8	8	8	8	8	6

TABLE 3 - ASSESSMENT OF COAGULATION FACTORS (CON'T)

	Baseline	Pre-Infusion	10	30	60	4 HR	24 HR	48 HR
FDP (ug/ml)								
Mean:	3	4	165+	170+	280	170*	26*	6
SD:	7	7	106	100	412	197	26	9
n:	8	8	8	8	8	8	8	5
D-Dimer (ug/ml)								
Mean:	0.20	0.38	17.49+	16.08+	17.43+	14.63+	7.19+	2.10+
SD:	0.09	0.22	7.48	5.31	5.63	6.89	5.25	1.07
n:	7	8	8	8	8	8	8	6

*$p < 0.05$ as compared to pre-infusion measurement time
+$p < 0.01$ as compared to pre-infusion measurement time

TABLE 4 - FDP, D-DIMER AND ALPHA (2) ANTIPLASMIN AT HIGH (5000 IU/ML) AND LOW (3000 IU/ML) DOSE UROKINASE

	Baseline	Pre-Infusion	10	30	60	4 HR	24 HR	48 HR
FDP (low dose)								
Mean:	5	5	160	160	400	210	25	7
SD:	10	10	113	113	588	287	37	12
n:	4	4	4	4	4	4	4	3
FDP (high dose)								
Mean:	0	3	170	180	160	130	28	5
SD:	0	5	115	101	113	60	15	7
n:	4	4	4	4	4	4	4	2
D-Dimer (low dose)								
Mean:	0.22	0.36	13.49	13.56	15.69	13.25	7.06	2.10
SD:	0.11	0.28	8.45	5.30	6.72	7.86	7.83	1.37
n:	4	4	4	4	4	4	4	4
D-Dimer (high dose)								
Mean:	0.19	0.41	21.50	18.60	19.18	16.00	7.33	2.09
SD:	0.04	0.19	4.02	4.57	4.55	6.63	1.70	0.27
n:	4	4	4	4	4	4	4	2
Alpha (2) antiplasmin (low dose)								
Mean:	68.5	65.7	74.5	60.5	54.3	41.9	64.9	89.9
SD:	11.1	9.0	6.4	17.8	15.0	13.0	21.9	40.1
n:	3	3	4	4	4	4	4	4
Alpha (2) antiplasmin (high dose)								
Mean:	64.3	69.0	66.8	53.0	29.8	45.3	73.6	49.0
SD:	23.7	17.4	21.8	15.9	7.2	8.0	15.1	----
n:	4	4	4	4	4	4	4	1

*$p < 0.05$ as compared to low dose measurement at same measurement time
+$p < 0.01$ as compared to low dose measurement at same measurement time

TABLE 5 - ASSESSMENT OF PLATELET FUNCTION

	Baseline	Pre-Infusion	10	30	60	4 HR	24 HR	48 HR
Bleeding time (sec)								
Mean:	140	148	284+	279*	283+	240+	216*	201
SD:	28	22	99	117	100	53	58	71
n:	8	8	8	8	8	8	8	6
Skin temperature (C)								
Mean:	31.5	32.0	31.7	31.3	31.2	31.6	33.7*	33.2
SD:	1.5	1.5	1.4	1.5	1.7	1.7	0.7	1.4
n:	8	8	8	8	8	8	8	6
Platelet count ($X10^3$/ul)								
Mean:	258	240	197*	207	202	184*	166	183
SD:	81	74	78	82	77	67	73	81
n:	8	8	8	8	8	8	8	6
Platelet volume (u^3)								
Mean:	6.30	6.28	6.20	6.58	6.09	6.34	7.14	6.93
SD:	1.43	1.31	1.22	1.31	1.28	1.25	1.35	1.53
n:	7	7	7	7	7	7	7	5
Platelet mass (u^3X10^5/ul)								
Mean:	15.53	14.86	12.26	14.22	12.24	11.95	11.99	13.29
SD:	4.10	6.09	3.88	5.07	4.08	4.20	4.52	4.25
n:	7	7	7	7	7	7	7	5
Plasma TxB_2 (pg/0.1 ml)								
Mean:	124	189	120*	125	118	124	54+	33
SD:	29	88	46	53	34	107	41	9
n:	6	7	7	7	7	7	5	3
Shed blood TxB_2 (pg/0.1 ml)								
Mean:	1175	1433	1059	900	1065	877	451+	372
SD:	531	712	590	408	420	320	218	181
n:	7	7	6	6	7	7	7	3

TABLE 5 - ASSESSMENT OF PLATELET FUNCTION (CON'T)

	Baseline	Pre-Infusion	10	30	60	4 HR	24 HR	48 HR
VWF								
(% of control)								
Mean:	220	160	198	200	240	215	234	226
SD:	114	64	87	116	137	153	108	132
n:	8	8	8	8	8	8	8	6
BTG								
(ng/ml)								
Mean:	139	125	78	66	47	29	10	22
SD:	72	50	61	31	19	11	2	2
n:	3	3	3	3	3	3	2	2

*$p < 0.05$ as compared to pre-infusion measurement time
+$p < 0.01$ as compared to pre-infusion measurement time

TABLE 6 - CORRELATION COEFFICIENTS BETWEEN PT, aPTT, TT, AND FDP, D-DIMER, FIBRINOGEN

	r	p	n
PT and			
FDP	0.68	< 0.01	61
D-Dimer	0.42	< 0.01	61
Fibrinogen	-0.55	< 0.01	60
aPTT			
FDP	0.38	< 0.05	46
D-Dimer	0.47	< 0.01	46
Fibrinogen	-0.37	< 0.05	45
TT			
FDP	0.20	NS	54
D-Dimer	0.15	NS	55
Fibrinogen	-0.41	< 0.01	50

TABLE 7 CORRELATION COEFFICIENTS BETWEEN BLEEDING TIME AND OTHER PLATELET RELATED MEASUREMENTS

	r	p	n
Bleeding time and			
Platelet count	0.02	NS	62
Platelet volume	-0.05	NS	54
Platelet mass	-0.02	NS	54
Skin temperature	-0.03	NS	62
Shed blood TxB_2	-0.10	NS	50
Plasma TxB_2	-0.25	NS	50
VWF	-0.03	NS	62
Fibrinogen	-0.29	< 0.05	60
FDP	0.14	NS	61
D-Dimer	0.33	< 0.01	61
log FDP	0.45	< 0.01	61
log D-Dimer	0.45	< 0.01	61

TABLE 8 - ASSESSMENT OF RESPIRATORY FUNCTION

	Baseline	Pre-Infusion	10	30	60	4 HR
Arterial pO_2 (mmHg)						
Mean:	92.2	91.4	88.6	91.6	90.8	91.6
SD:	12.9	17.6	17.2	14.7	15.6	14.6
n:	8	8	8	8	8	8
Arterial pCO_2 (mmHg)						
Mean:	33.2	32.1	32.5	31.7	31.7	30.8
SD:	2.8	1.9	2.4	2.6	3.4	2.5
n:	8	8	8	8	8	7
PVR (mmHg/l/min)						
Mean:	0.12	0.12	0.14	0.14	0.14	0.15
SD:	0.03	0.04	0.06	0.05	0.04	0.06
n:	8	7	8	8	8	8
$QS_{/QT}$ (%)						
Mean:	20.59	17.48	24.73	15.47	17.59	15.78
SD:	10.85	6.90	12.19	5.23	8.32	5.18
n:	8	8	8	8	8	8
$VD_{/VT}$ (%)						
Mean:	27.59	31.00	32.11	34.01	27.39	31.87
SD:	9.46	8.89	8.82	14.04	8.73	8.99
n:	8	8	8	8	8	7
C3a (ng/dl)						
Mean:	96	63	77	75	64	50
SD:	78	65	55	53	63	48
n:	7	7	7	7	7	7

*$p < 0.05$ as compared to pre-infusion measurement time
+$p < 0.01$ as compared to pre-infusion measurement time

TABLE 9 - CORRELATION COEFFICIENTS BETWEEN PULMONARY VASCULAR RESISTANCE (PVR), RESPIRATORY SHUNT, DEAD SPACE, AND FDP, D-DIMER, C3A

	r	p	n
PVR and			
FDP	0.24	NS	46
D-Dimer	0.14	NS	46
C3a	0.23	NS	40
Respiratory shunt and			
FDP	0.13	NS	48
D-Dimer	0.10	NS	47
C3a	-0.01	NS	42
Dead Space and			
FDP	-0.13	NS	47
D-Dimer	0.06	NS	46
C3a	-0.11	NS	41

TABLE 10 - ASSESSMENT OF RENAL FUNCTION

	Baseline	4 HR	24 HR	48 HR
BUN (mg/dl)				
Mean:	13.0	10.7	13.4	12.1
SD:	4.0	3.4	9.3	9.5
n:	7	7	7	6
Creatinine (mg/dl)				
Mean:	1.17	1.30	1.65	1.59
SD:	0.17	0.63	1.04	0.77
n:	7	7	7	6

*$p < 0.05$ as compared to baseline measurement time
+$p < 0.01$ as compared to basleine measurement time

DISCUSSION

OPERATING ROOM AND HEMATOLOGIC DATA

The animals were hemodynamically stable throughout the course of the procedure, with heart rate and cardiac output essentially unchanged over 4 hours. Mean arterial and pulmonary arterial pressures remained stable as well, with changes only around the time of infusion, most likely due to the infusion of lysed plasma in an amount equal to one sixth the intravascular volume. The increase in urine output even prior to lysed plasma infusion may be attributed to the volume increase associated with the two courses of hemorrhage and transfusion, as well as with the eventual serum infusion. As urine output is a more sensitive marker of central volume status than arterial pressure, it might be expected that this marker would increase in response to increased intravascular volume (perhaps via atrial natriuretic factor) slightly before mean arterial and pulmonary arterial pressure. Arterial pH, pO2, and pCO2 all remained stable throughout the procedure.

The changes in hematocrit, hemoglobin concentration and red blood cell count resulted from dilution of the intravascular volume. During infusion, the intravascular space was diluted by one sixth of its volume. From 4 to 24 hours, third space volume gains were redistributed into the intravascular space. In each situation, the three measurements decreased significantly. The dramatic and significant increase in white blood cell count resulted most likely from mobilization of the marginated pool of

leukocytes from the marrow in response to the stress of anesthesia, instrumentation and hemorrhage. As the procedure continued into the follow up period, the WBC returned to baseline value. The decrease in platelet count again resulted from dilutional changes in the intravascular space although there may in addition have been consumption. The fact that the platelet count did not decrease in parallel with the hematocrit, hemoglobin concentration and RBC attests to the release of sequestered platelets from the spleen and the production of young platelets in the bone marrow. The increase in mean platelet volume at 24 hours attests to this new platelet production. Platelet mass, a reflection of platelet number and volume, remained unchanged throughout.

PLATELET FUNCTION

Platelet function is defined essentially by adhesion and aggregation. Adhesion is mediated by binding of von Willebrand factor between platelet glycoprotein Ib and exposed subendothelial components. Platelet to platelet aggregation is mediated primarily by fibrinogen via platelet glycoprotein IIbIIIa (Michelson, Unpublished manuscript). Irreversible aggregation is accompanied by the so-called release reaction, with release of substances such as beta thromboglobulin and platelet factor 4 from alpha granules, ADP and serotonin from dense granules and thromboxane A2 from the platelet membrane.

A highly significant prolongation of the bleeding time was observed in our baboon model following the infusion of the autologous lysed plasma. Factors thought to play a role in bleeding time prolongation, and which may be observed in order to investigate the nature of the platelet defect here include: platelet number and volume; skin temperature and shed blood thromboxane B2; plasmin; beta thromboglobulin and plasma thromboxane B2; von Willebrand factor and platelet glycoprotein Ib; and fibrinogen, FDP and D-Dimer. In addition, the Aprotinin used for plasmin neutralization may play a role in modifying platelet function.

Deficiencies in platelet number and/or platelet volume may contribute to bleeding time prolongation. The combined effects of these two variables is often expressed as the platelet mass, the product of platelet number and platelet volume. However, this

conception is somewhat simplistic. For example, bleeding time is correlated with platelet number only when such number is below 100K. At or above 100K, platelet number is sufficient so as to not prolong bleeding time (Harker and Slichter, 1972). Thompson et al (1982) note platelet size to be directly related to the aggregatory response to ADP, to serotonin uptake and to serotonin release in response to thrombin. However, Martin et al (1983) note that the effect of platelet volume predominates over that of platelet number. They observe that stimulated large platelets produce proportionately more TxA2 per unit of volume that control platelets. Through somewhat complex mathematical models, they predict that much larger changes in platelet number than platelet volume are required for identical changes in bleeding time.

In our model, platelet number decreased from pre-infusion to post-infusion times, most likely a dilution effect of the large (250 cc) lysed plasma infusion. Nevertheless, at only one time did platelet count decrease below the 100 X 10*3/ul thought to be necessary for a normal bleeding time, and in that one case, no effect on bleeding time was observed. Mean platelet volume increased slightly (non-significantly) over the study, if anything, serving to reduce bleeding time. Platelet mass was unchanged. Platelet number, volume and mass were not significantly correlated with bleeding time. These factors likely played no role in the platelet dysfunction observed.

Thromboxane A2 is a potent platelet aggregatory agent. It is synthesized in the platelet and acts in part by augmenting

exposure of platelet glycoprotein IIbIIIa and by inducing release of ADP and serotonin from the dense granules (Coller, 1990).

Valeri et al (1987) investigate the relationship of bleeding time to local skin temperature and to shed blood thromboxane B2 levels in a baboon model. They observe bleeding time to be inversely related to both skin temperature and thromboxane B2 (the stable breakdown product of TxA2) levels at the bleeding time site. Moreover, both bleeding time and TxB2 return to normal following restoration of normal skin temperature. They conclude that lowering of local skin temperature prolongs bleeding time by reversibly inhibiting platelet production of thromboxane A2, inhibiting platelet aggregation at the local level. Valeri et al (Unpublished manuscript), observe a similar effect in cardiopulmonary bypass patients, with local cooling resulting in decreased shed blood TxB2 and increased bleeding time. Interestingly, aspirinated patients exhibit reduced shed blood thromboxane and increased bleeding time regardless of skin temperature. As aspirin inhibits thromboxane synthesis, this finding supports the notion that the skin temperature effect is manifested through its effect on thromboxane A2 production.

In our model, skin temperature remained stable throughout the study course, increasing significantly only at 24 hours. Curiously, it is at 24 hours that shed blood TxB2 levels are significantly decreased. This decrease in TxA2 production at the site of incision may play a role in the bleeding time prolongation seen at 24 hours, although the extent of this role

is unclear. At no time were skin temperature or shed blood TxB2 significantly correlated with bleeding time.

The role of plasmin in platelet dysfunction and bleeding time extension is controversial. Gimple et al (1989) observe a prolongation of bleeding time in patients treated with tPA for myocardial infarction. This prolongation returns to normal in 4 hours and is not correlated with fibrinogen or FDP level.

Plasmin has been implicated, alternatively, with the non-specific activation of platelets and release of alpha granule contents, with cleavage of von Willebrand factor and with cleavage of platelet glycoprotein Ib. Each hypothesis has as its ultimate result the impaired function of platelets and the prolongation of bleeding time.

Niewiarowski et al (1973) observe plasmin to cause reversible aggregation of washed platelets, accompanied by release of adenine nucleotides, serotonin and fibrinogen. These platelets are subsequently less responsive to aggregation by thrombin or collagen. Pre-treatment of these platelets with inhibitors of the release reaction such as Persantin causes them to retain their post-plasmin sensitivity to thrombin and collagen. This argues for a role for plasmin in the non-specific activation and degranulation of platelets.

Beta thromboglobulin, found in the platelet alpha granule, is found in plasma as a result of degranulation during platelet activation and the release reaction. Increased BTG levels, as is seen in extracorporeal bypass, is indicative of non-specific

platelet activation. It is hypothesized that such activated platelets are impaired in their subsequent function (Czer et al, 1987; Valeri et al, 1992; Michelson, unpublished manuscript). Elevated plasma TxB2 levels similarly indicate non-specific activation of platelets (Valeri et al, 1992). Addonizio et al (1980) perfuse blood through a membrane oxygenator in vitro, observing non-specific platelet activation with release of TxA2 and alpha granule contents. Davies et al (1980) observe increases in plasma TxB2 early in cardiopulmonary bypass in coronary bypass patients. Harker et al (1980) observe temporary platelet dysfunction, as evidenced by an increased bleeding time, in association with depletion of alpha granules and increased plasma BTG in coronary bypass patients. Valeri et al (1992) observe large increases in plasma BTG and TxB2 in a series of 37 patients on cardiopulmonary bypass. This is corroborated by Pumphrey and Dawes (1983) in their work with cardiopulmonary bypass and prosthetic valve patients. Alternatively, Zilla et al (1989) observe no significant increase in BTG or TxB2 in their group of 18 bypass patients.

von Willebrand factor is a glycoprotein ligand which normally exists in the subendothelium. vWF mediates platelet adhesion to damaged vascular surfaces via platelet glycoprotein Ib (GP Ib), although it may also interact non-specifically with platelet glycoprotein IIbIIIA (GP IIbIIIa), normally through to mediate platelet aggregation (Coller, 1990). Deficiencies in vWF

or its glycoprotein receptor result in bleeding diatheses. Harker and Slichter (1972) note that patients with von Willebrand disease, a congenital deficiency of vWF, have prolonged bleeding times which are corrected with vWF. Henririksson and Nilsson (1979) observe partial degradation of vWF by plasmin in vitro. Federici et al (1988) note proteolytic fragments of vWF in the serum of patients undergoing thrombolytic therapy for up to 72 hours following infusion. They hypothesize a role for plasmin in this cleavage and in the subsequent bleeding diatheses of these patients.

Platelet glycoprotein Ib, as mentioned previously, mediates platelet adhesion to damaged subendothelium via its specific interaction with von Willebrand factor (Coller, 1990). Czer et al (1987) observe desmopressin, which promotes vWF release from storage sites, to improve bleeding times in cardiopulmonary bypass patients. They argue that the increased vWF levels may compensate for a defect in GP Ib occurring during bypass, perhaps secondary to generation of plasmin. Plasmin has been shown to cleave GP Ib in vitro. Adelman et al (1985) treat platelets with plasmin and show the loss of a 180 kilodalton protein consistent with platelet GP Ib. Simultaneously, a 135 kilodalton protein consistent with glycocalicin, a proteolytic product of GP Ib, appears in the surrounding medium. They correlate the loss of this protein to the loss of in vitro platelet aggregation in response to ristocetin, a vWF dependent process, and conclude the protein cleaved by plasmin to be GP Ib.

Nevertheless, internal stores may rapidly replace GP Ib on the platelet surface (Michelson et al, 1988; Michelson et al 1991). Michelson et al, using the murine monoclonal antibody 6D1, observe intraplatelet stores of GP Ib four times that observed on the platelet surface, making it one of the most abundant platelet proteins. They imply that rapid restoration of the membrane GP Ib occurs in a large proportion of platelets whose surface glycoproteins have been cleaved.

The role of Aprotinin in experimentally preserving platelet function and shortening bleeding time during cardiopulmonary bypass lends further support to the role of plasmin in the transient platelet dysfunction. van Oeveren et al (1987) observe bypass patients treated with Aprotinin to exhibit lower plasma TxB2 levels, indicating reduced non-specific platelet activation. Bleeding times were not prolonged. Royston et al (1987) note an 80% reduction in blood loss in bypass patients treated with Aprotinin. Both van Oeveren and Royston suggest a dual role for Aprotinin. A known inhibitor of plasmin, it may either protect platelets from non-specific activation or it may protect GP Ib from plasmin cleavage (Neither give much credence to inhibition of fibrinogenolysis). Regardless of its exact mechanism of action, the role of Aprotinin in protecting platelets seems to indicate a role for plasmin in the transient platelet dysfunction seen during cardiopulmonary bypass, fibrinolytic therapy and salvage blood autotransfusion.

An important question is whether plasmin is introduced into

our system as part of the contents of the autologous plasma transfusion. As discussed above, the presence of plasmin in vivo may play a large role in the impairment in platelet function seen in the baboons. According to van Oeveren et al (1987), 150 Kallikrein Inhibitor Units (KIU) per ml is sufficient to inhibit in vivo the plasmin generated during cardiopulmonary bypass. In our own, preliminary studies, we observed 1000 KIU/ml, a greatly increased amount, to be required for inhibition of all plasmin generated in vitro. Nevertheless, in spite of the addition of this concentration of Aprotinin to the lysed plasma, the data support the notion that plasmin was indeed either infused or generated intravascularly post-infusion.

First and foremost of this data is the rapid decrease in fibrinogen following infusion. Dilution may account for 15-20% of the decrease; the remainder in all likelihood results from cleavage. As anti-thrombin III levels remain stable, thrombin is not likely to be the agent of this cleavage. Alpha(2)-antiplasmin, alternatively, decrease dramatically and significantly, indicating the presence of large quantities of plasmin intravascularly.

It might be asked that if plasmin is active intravascularly, why then do fibrinogen levels not decrease or FDP levels not increase more rapidly? Most likely, fibrinogen (an acute phase reactant) is rapidly synthesized and FDP rapidly cleared, comprising initially a steady state until, some time after 4 hours, plasmin is neutralized and fibrinogen synthesis/

FDP clearance may predominate.

Nevertheless, if plasmin is present in the baboon, its role in the platelet dysfunction is still unclear. Plasma BTG and TxB2 were increased prior to serum infusion (likely secondary to the stress of instrumentation, hemorrhage and red blood cell transfusion - although errors in sample collection may have resulted in artifactually high early BTG levels) and returned to normal following infusion, indicating absence of non-specific platelet activation by plasmin. von Willebrand factor levels were unchanged throughout the study. The role of plasmin in platelet glycoprotein Ib (GP Ib) levels is unclear. Additional studies using the monoclonal antibody 6D1 (Coller et al, 1983) may eventually be used in our laboratory to investigate if plasmin is indeed cleaving GP Ib in our model. However, even if GP Ib is cleaved, von Willebrand factor may still interact non-specifically with platelet glycoprotein IIbIIIa (Coller, 1990), obviating or reducing the effect of this loss. In addition, the doubling of bleeding time in our model is seen at the earliest post-infusion measurement, 10 minutes, at a time when alpha(2)-antiplasmin levels are almost identical to pre-infusion. Antiplasmin levels decreased at 30 and 60 minutes, significantly so only at 4 hours, accompanied by no corresponding increase in bleeding time. This 'uncoupling' of bleeding time and antiplasmin calls into question the role of plasmin in our model.

Another factor in the thrombocytopathy following serum infusion is the rapid and highly significant decrease in plasma

fibrinogen following infusion. Fibrinogen mediates platelet to platelet aggregation through its interaction with glycoprotein IIbIIIa. Fibrinogen levels decreased from 109 mg/dl to 78 mg/dl immediately following infusion and remained significantly decreased through 4 hours of follow up. While much higher than the levels of 20-50 mg/dl seen in patients with the platelet defect seen in congenital afibrinogenemia (Weiss & Rogers, 1970), these levels are still below the 100-200 mg/dl thought to be required for optimal platelet function in humans (Bennett & Vilaire, 1979). Moreover, the correlation between bleeding time and fibrinogen is stronger than that between bleeding time and FDP, significant at $p<0.05$. However, at 24 hours, the bleeding time prolongation persists fully (206 seconds, $p<0.05$) in the face of supernormal fibrinogen levels (211 mg/dl), calling into question the causal relationship between bleeding time and fibrinogen.

Fibrinogen degradation products have been discussed in the literature extensively (and controversially) with regard to their effect on in vitro and in vivo platelet function. In Phase I of the thrombolysis in myocardial infarction (TIMI) trial, Rao et al (1988) observe fibrinogenolysis in patients infused with streptokinase or tPA. They find both major and minor bleeding complications to be associated not with fibrinogen but with FDP. Unfortunately, they do not mention bleeding time or any specific test of platelet function in these patients.

In our model, FDP levels rise dramatically and

significantly post infusion and persist through 24 hours of follow-up. However, correlation between bleeding time and FDP is non-significant at $p<0.05$. This certainly calls into question the relationship between FDP and the thrombocytopathy. However, alternatively, if the FDP effect is, as postulated, a competitive inhibition, then the relationship may be non-linear at such high levels as are seen here and may not be effectively captured by linear correlation analysis. Correlation analysis between log FDP and bleeding time in fact reveals a moderately strong correlation ($r=0.45$, $p<0.01$).

Alternatively, D-Dimer has rarely been discussed as a determinant of platelet dysfunction. Post infusion, D-Dimer levels rise almost in parallel to those of FDP. Moreover, correlation between bleeding time and D-Dimer is moderately strong ($r=0.33$, $p<0.05$). If the D-Dimer effect is similar to that proposed for FDP, it is possible that strength of the observed correlation is due to the relatively low D-Dimer levels observed, confining the correlation line to the 'steep', possibly more linear part of the relationship. Indeed, correlation analysis between log D-Dimer and bleeding time reveals an increased correlation coefficient ($r=0.45$, $p<0.01$), but not nearly the increase seen for FDP.

COAGULATION

As discussed previously, fibrinogen degradation products (FDP) have long been attributed for coagulopathy in fibrinolytic states. Inhibition of the action of thrombin on fibrinogen, and interference with the gelation of formed fibrin monomer have long been proposed as the pathologic mechanisms.

In our model, thrombin time and prothrombin time were not significantly prolonged over the course the procedure. The large standard deviation for thrombin time resulted from one animal (Study #1) having an elevated thrombin time (>30 seconds) at baseline and throughout the study. Activated partial thromboplastin time, alternatively, was significantly prolonged at 30 minutes. This extension was associated with both dramatically decreased levels of fibrinogen and increased levels of FDP and D-Dimer. Correlation analysis show fibrinogen ($r=-0.37$, $p<0.05$), FDP ($r=0.38$, $r<0.05$) and D-Dimer ($r=0.47$, $p<0.01$) to all be moderately correlated with aPTT, with fairly small differences between the three. As such, it is difficult to separate out the differential effects of these variables. Interestingly, prothrombin time is strongly correlated with FDP ($r=0.68$, $p<0.01$) and fibrinogen ($r=-0.55$, $p<0.01$) and moderately correlated with D-Dimer ($r=0.42$, $p<0.01$). Again, the differential effects of these variables are difficult to separate. The correlation of thrombin time with fibrinogen ($r=-0.41$, $p<0.01$) but not with FDP or D-Dimer supports the idea that decreased plasma fibrinogen may predominate in producing the coagulopathy

observed.

Thrombin time seems most closely linked to the plasma fibrinogen level. Even before infusion of lysed plasma, thrombin time rises from 10.9 seconds to 15.2 seconds as the plasma fibrinogen level falls from 148 mg/dl to 109 mg/dl. PT and aPTT do not change during this time, indicating less of a dependence on fibrinogen levels.

A study in which autologous FDP and D-Dimer could be infused without causing in vivo fibrinogenolysis, as was originally intended, would be ideal for separating out these variables.

An important question is that of whether the two doses of urokinase used to lyse plasma had different effects upon the blood elements thought to affect coagulation, platelets and pulmonary function. As seen in Table 4, there were no significant differences in FDP, D-Dimer and alpha(2) antiplasmin in the high and low dose groups. Nevertheless, with only four animals in each group, the "power" of such a comparison is quite low. That is to say, the chance a beta error, the chance of finding "no difference" when in fact there is a difference between two groups is fairly high. Increased numbers may result in either a significant difference, or a more meaningful statement of "no difference". Nevertheless, in that each baboon was randomized to receive high or low dose urokinase, and (more importantly) in that each animal served as its own control, with statistical analysis run only on paired samples, the results should not be biased on the basis of dose difference.

PULMONARY FUNCTION

Elevations of plasma C3a, often used as a sign of activation of the complement cascade, have been associated with pulmonary dysfunction in both cardiopulmonary bypass and renal dialysis patients. Moore et al (1988) observe C3a levels to increase for at least two hours following cardiopulmonary bypass. Moreover, these levels are significantly higher in patients requiring mechanical ventilation for longer than 24 hours than in patients without respiratory complications. Craddock et al (1977) note elevations of C3a in renal dialysis patients, associated with a syndrome of pulmonary dysfunction including pulmonary engorgement and sequestration of granulocytes.

Alternatively, there is less evidence for C3a elevation secondary to autotransfusion of shed blood. Bengtson et al (Unpublished manuscript) see elevated C3a in the shed blood of 12 total hip replacement patients, but no increase in plasma C3a upon reinfusion of this shed blood. Similarly, in our model, no significant increases in plasma C3a were observed, despite the generation of C3a in the lysed plasma (205 +/- 56 ng/dl; n=7). Similarly, no significant changes were seen in arterial pO2, pCO2, pulmonary vascular resistance, respiratory shunt or dead space following serum infusion.

RENAL FUNCTION

No changes in BUN and creatinine levels were seen over the course of the procedure or through 48 hours of follow up. Urine output increased over the study, secondary mainly to the dramatic shifts of intravascular volume involved in the two courses of hemorrhage and transfusion and the 250cc plasma infusion. Given the sometimes rapid shifts occurring in intravascular volume, it is difficult therefore to assess the effects of FDP, D-Dimer or plasminemia on urine output. The lack of change in BUN and creatinine provides limited evidence for an absence of effect on renal function.

CONCLUSION

Eight baboons were infused with 250 cc of autologous serum treated with urokinase in doses of 3000 IU/ml or 5000 IU/ml, creating a fibrinolytic state in which plasmin was generated and fibrinogen and fibrin was cleaved. This state resulted in a transient increase in activated partial thromboplastin time without a similar increase in thrombin time or prothrombin time, and a marked prolongation of the bleeding time lasting at least 24 but less than 48 hours. These changes may attributed to the effects of FDP and D-Dimer or, alternatively, to the decrease in fibrinogen. No clinical evidence of coagulopathy or bleeding disorder (e.g. conjunctival or mucosal petechiae, bleeding from catheter sites or mucosa) was observed in any of the study animals. No change in pulmonary or renal function was seen.

Further studies will be necessary to separate out the roles of plasmin, fibrinogen, FDP and D-Dimer in the transient coagulopathy and thrombocytopathy observed.

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