THEORETICAL AND PRACTICAL (CLINICAL) CONSIDERATIONS CONCERNING PROTEOLYTIC ENZYMES AND THEIR INHIBITORS WITH PARTICULAR REFERENCE TO CHANGES IN THE PLASMINOGEN-PLASMIN SYSTEM OBSERVED DURING ASSISTED CIRCULATION IN MAN

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THEORETICAL AND PRACTICAL (CLINICAL) CONSIDERATIONS CONCERNING PROTEOLYTIC ENZYMES AND THEIR INHIBITORS WITH PARTICULAR REFERENCE TO CHANGES IN THE PLASMINOGEN-PLASMIN SYSTEM OBSERVED DURING ASSISTED CIRCULATION IN MAN*

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Introduction

It is self-evident that progress cannot be made in our understanding of natural or induced phenomena without the availability of procedures that permit us to perform measurements. It seems also important that the reliability of the determinations be scrutinized from time to time in an attempt to establish whether and how a procedure would benefit from improvements.

Limitations in Methodology and Possible Errors in Interpretation

The relatively simple measurement of the plasminogen content of human plasma may serve as an example. Human plasminogen can be determined in several ways. In one method, inhibitors are removed by the precipitation of the inhibitor-free euglobulin from plasma at its isoelectric point (pH 5.2).[1] The inhibitor-containing supernatant is discarded, and the plasminogen-containing precipitate, dissolved in buffer, is activated with streptokinase.[2] The activity of the resultant plasmin is determined by one of three procedures: fibrinolysis, casein proteolysis, or hydrolysis of synthetic esters.

The precipitation step appears to be the weak link of this procedure. After precipitation; the recovery of proteins is inconsistent, a feature that limits the value of the method.

In another procedure, one based on the assumption that the inhibitor is destroyed following acidification,[3] the entire reaction sequence is carried out in the same test tube. Following 15 min incubation at pH 3.2†, the acidified plasma is neutralized, activated with streptokinase, and the degree of proteolysis determined.

Unfortunately, the acidification step does not destroy the inhibitor completely, nor is the degree of its destruction consistent (FIGURE 1). These findings suggest that more thought and effort are required to improve presently available methods for plasminogen determinations (FIGURE 2).

While on the subject of inhibitors of the plasminogen-plasmin system, it seems appropriate to emphasize the statement that the only accurate inhibitor determination available is for *antiplasmin*. Evidence suggests that the naturally occurring plasminogen activator differs from urokinase.[4] Hence, to equate changes in the concentration of plasma inhibitor for urokinase with alterations in the inhibitor for plasminogen activator of plasma is, in spite of its wide application, probably an error.

Assays for antiplasmin should account for two distinctly different antiplasmin

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† The pH of plasma mixed with an equal volume of 1/6 NHCl ranges from 3.1–3.3.

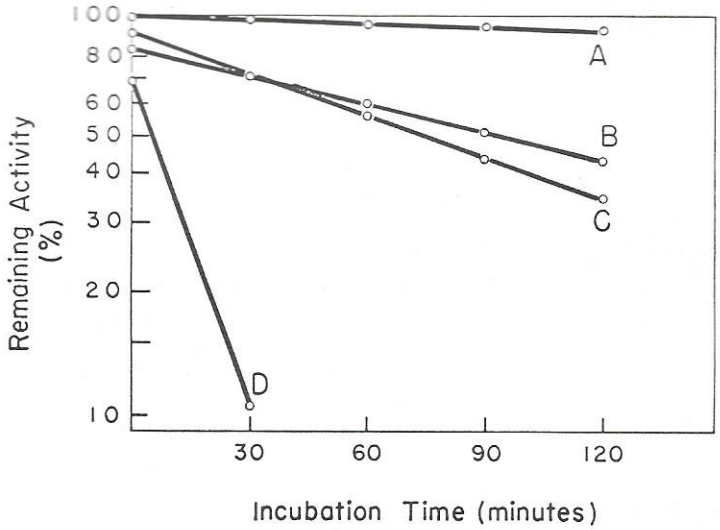

FIGURE 1. Antiplasmin determinations. Human plasmin (1.6 ml) and lysine HCl (1.6 ml; 4.4%) are incubated at 37° C with human plasma (0.8 ml), and the residual plasmin activity is determined by casein hydrolysis technique at 30, 60, 90, and 120 min on 0.3 ml aliquots of the incubation mixture. A. Determination on buffer, instead of plasma. B & C. Determinations on human plasma samples after incubation for 15 minutes with equal volumes of 1/6 N HCl followed by its neutralization with 1/6N NaOH.[3] D. Determinations on human plasma samples. The difference in value found between buffer and plasma samples at zero minutes represents the activity of the fast antiplasmin.

activities that have been observed in human plasma. The first, a fast-acting antiplasmin, is a competitive inhibitor; the second proceeds at a slow rate in what appears to be a stoichiometric reaction.[5] Determination of residual plasmin activity some time (e.g. 15 min) after plasmin addition to plasma[6] does injustice to this concept. Instead, the measurement of residual activity following the addition of plasmin to plasma at *zero* time and *two hours* later, as previously described by Norman and Hill [5] and by Shamish and Rimon,[7] allows for the separate determination of the two activities. In addition, this method requires the use of plasmin "stabilizers," e.g. lysine hydrochloride[7] or methyl amine,[5] in order to obtain meaningful and reproducible results. Variations in proteolytic activity of blood are reflected in changes of the fast-acting inhibitor only, leaving the slow inhibitor unchanged.

Another error, by no means uncommon, consists of the use of the euglobulin clot lysis time to assess the effectiveness of treatment of enhanced fibrinolysis with synthetic enzyme inhibitors.[8] This procedure is misleading, for it can be shown that both EACA[9] and Trasylol® are at best only partially precipitated in the euglobulin fraction.

The determination of the overall lytic activity of blood has relied heavily on the simple procedure of the plasma clot lysis time. It is interesting that the lysis time of whole plasma, obtained from patients during and after cardiopulmonary bypass, varies greatly with the amount of thrombin used to clot plasma. Contrary to what might be expected, in view of the lytic properties of thrombin,[10] it was found that the smaller the amount of thrombin used to clot plasma, the shorter the lysis time (TABLE 1). The circumstance surrounding this phenomenon and its

mechanism of action is as yet unexplained. With this in mind, technicians should record any deviation from the standard procedure to allow for accurate interpretation of data.

Relationship Between Cardiopulmonary Bypass and Shock

Changes in the plasminogen-plasmin system of blood have been studied extensively in a large numbr of clinical and experimental conditions. Of the latter, shock is an example of such conditions. In animals, shock is easily induced and reproduced, whether it is anaphylactic, endotoxemic, hypovolemic, or traumatic shock. Hence, they represent excellent experimental models. In contrast, this condition is less accessible to study in humans because preshock baseline values are rarely available.

Cardiopulmonary bypass, to support circulation during open-heart surgery, induces in its present form a shocklike state characterized by hypotension, cellular

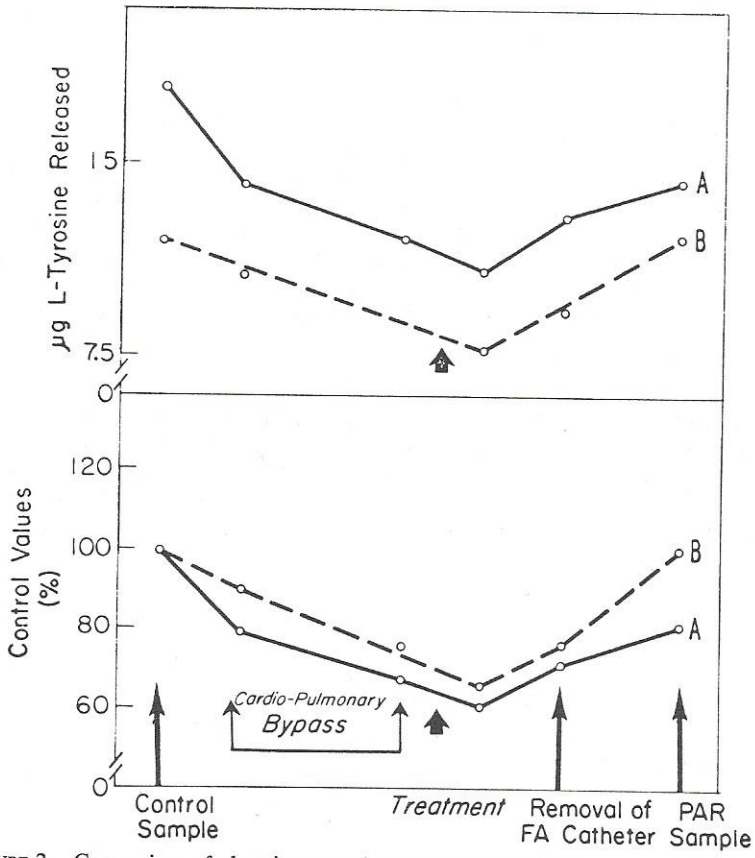

FIGURE 2. Comparison of plasminogen values at determined by two assay techniques. *Top half of graph*: Plasminogen recovery with technique A[3] is considerably larger than with technique B.[19] *Lower half of graph*: The effect of treatment with synthetic amiplasmin (Trasylol) persists longer in assay A than in B. Each value in these graphs represents an average of 20 determinations in 10 patients.

LYSIS TIME OF HUMAN PLASMA CLOTS (IN MINUTES)

Patient No.	Sample No.	No. of Units of Thrombin Used to Clot Sample 20	10	5	1
072664	1	72	20		
	4		26	2	
1046215	1	36	21		
	2	2	1		
1043761	1			30	23
	5			44	27
	6			50	36
095787	1			49	6
	5		23	19	
	6			33	23

hypoxia, and acidosis. During and following cardiopulmonary bypass, changes in the hemostatic mechanism of patients similar to those previously observed during shock in animals have been noted. Increased proteolytic activity, as well as hypercoagulability of blood, has been described in animals during and after shock.[11] Similar findings have been observed in man following cardiopulmonary bypass.[12] Depression of reticuloendothelial function and blood clearance have also been observed in experimental shock.[13] Findings are presented that blood clearance in man may also be defective during and after cardiopulmonary bypass, a feature thought to contribute to the development of bleeding.

Mechanical Effect of Assisted Circulation on Blood

During cardiac bypass the blood is exposed to considerable trauma. The effect of the trauma on the cellular and plasmatic components of blood was experimentally determined by recirculating freshly drawn human blood through a pump oxygenator.[14] Destruction of significant numbers of platelets and erythrocytes was found. The first alteration could be determined directly. The second was inferred from the finding of a rise in plasma hemoglobin concentration.

TABLE 2

EFFECT OF PERFUSION ON BLOOD DURING IN VITRO STUDIES

Findings		Remarks
Platelet count	Decreased	The longer the perfusion, the more marked the decline in platelet number.
Fibrinogen concentration	Unaltered	
Plasma Hb concentration	Increased	The longer the perfusion, the greater the rise in plasma hemoglobin concentration.
Recalcification time	Shortened	Marked shortening occurs after 30–45 minutes of perfusion.
Thrombin generation time	Shortened	Marked shortening occurs after 30–45 minutes of perfusion.

As a result of cellular injuries, significant shortening of the recalcification and thrombin generation time became apparent during these experiments. The extent of the changes indicated the development of a state of marked hypercoagulability (TABLE 2). When cell-free plasma instead of whole blood was used for recirculation, no changes in recalcification time and thrombin generation time were present. It was concluded that the alteration of blood cells was related to the hypercoagulability changes observed in blood.[14]

Changes Observed During Cardiopulmonary Bypass

Response of Plasminogen-Plasmin System to Assisted Circulation. These in vitro experiments find their counterpart in patients undergoing open-heart surgery. In addition to evidence of blood-cell destruction, the extent of which increases as the time of bypass becomes longer,[14] a decline in fibrinogen and prothrombin complex is noted.[15] These changes are assumed to result from intravascular coagulation.

Shortly after the initiation of cardiopulmonary bypass, considerably plasminogen-activator activity is present in plasma. The degree of plasminogen- activator activity can be related to the duration of cardiac bypass.[16]

Maximum plasminogen-activator activity occurs at the termination of cardiac bypass. Initially, the increased plasminogen-activator activity was related to the formation of thrombin, when it was found that thrombin could induce a similar lytic response in experimental animals.[17] This concept is no longer tenable in view of the absence of plasminogen-activator activity following thrombin infusion into heparinized dogs.[18] During cardiopulmonary bypass the patient is adequately heparinized. This fact presumably excludes thrombin generation as a possible cause of increased lytic activity.

In addition to changes in plasminogen activator, circulating plasminogen concentrations decline. During cardiopulmonary bypass the maximum drop in plasminogen concentration reaches a mean value of 40.2% (S.D. ± 8.9%). (FIGURE 3.) This suggests the development of plasmin[16] in spite of lack of direct evidence for its activity.‡ Further evidence for its formation is provided by the finding of a decline of plasma antiplasmin levels.

The total antiplasmin activity of human plasma exceeds the potential plasmin activity several times.[19] There exists considerable controversy concerning the exact amount. We find that the fast-acting antiplasmin represents only 1/4–1/3 of the total antiplasmin activity of human plasma. Small as this fraction may seem, we observed that it will neutralize 1½–2 times the potential plasmin activity of plasma. In view of this finding, the conclusion that the human antiplasmin system has an extensive functional reserve capacity seems justified. The system shares this feature with the other antiproteinases of blood.[20]

During cardiopulmonary bypass, maximal decline of fast antiplasmin activity reaches a mean value of 34.3% (S.D. ± 4.8%). (FIGURE 3.) No significant changes are observed in the slow-acting antiplasmin. Thus it appears that the fast inhibitor, which serves to prevent the development of free plasmin activity, provides, through the extent of its decline, a useful indicator of the amount of plasmin neutralized.

Changes in the Character of the Lytic Activity. In these patients maximal lytic

‡ Occasionally lytic activity is observed following incubation of euglobulin and plasma samples on plates containing plasminogen-free fibrin. This has been thought to result from rapid activation of plasminogen with development of hyperplasminemia which temporarily overwhelms the antiplasmin system.

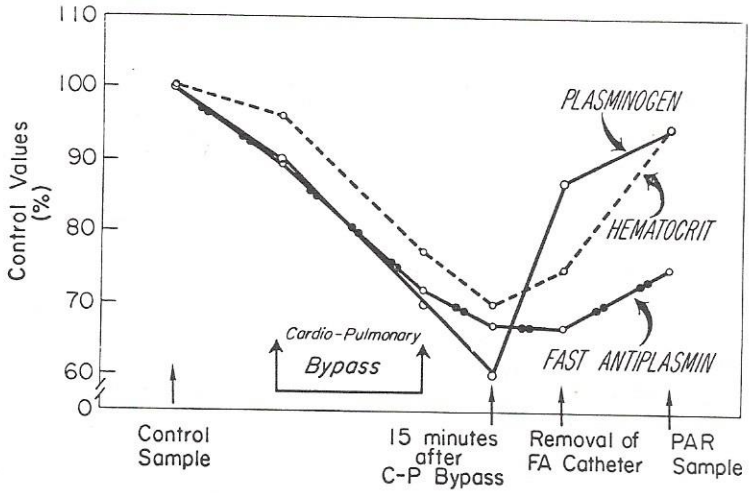

FIGURE 3. Plasminogen and antiplasmin concentrations (expressed in percent of pre-bypass value), observed during an after cardiopulmonary bypass in eight untreated patients. Maximal decline in plasminogen concentration reaches a mean value of 40.2% (S.D. = 8.9%), that of fast antiplasmin, 34.3% (S.D. = 4.8%). Plasminogen concentrations return to normal values shortly after cardiopulmonary bypass; antiplasmin concentrations remain reduced during the entire observation period.

activity is not infrequently associated with equal euglobulin and plasma clot lysis time values. At the same time, the euglobulin and plasma samples are noted to exert the same activity in the fibrin plate assay. This observation suggests the loss of antiplasmin. Determinations for antiplasmin activity fail, however, to substantiate this assumption.

This phenomenon has two striking features. The first is the prolonged duration of enhanced lytic activity. Frequently the plasma of these patients already exhibits considerable plasminogen-activator activity before bypass. The second concerns the degree of increased activity. This phenomenon becomes apparent in only those patients who develop a more than three- to five-fold increase in plasminogen-activator activity during cardiac bypass (FIGURE 4). This fact suggests that under these circumstances, inhibitor for plasminogen-activator activity rather than for plasmin has partially or completely been depleted. It is conceivable that these patients are those who will develop the hemorrhagic, fibrinolytic syndrome occasionally seen following surgery. Bleeding, however, is far less frequent than the described phenomenon.

Fibrinolysis and Fibrinogenolysis. Another feature deserving further comment concerns changes found in the plasma fibrinogen values. During a previous study we found that fibrinogen concentrations decline in the first five min of cardiac bypass, more than would be expected from hemodilution alone.[21] More recent studies confirm this finding. The rapid decline in fibrinogen concentration that takes place in the presence of adequate heparinization is similar to changes previously described in heparinized dogs following induction of shock.[11]

In contrast, no changes in fibrinogen concentration occur *during* bypass (FIGURE 5). End of bypass fibrinogen value was found to be 101 ± 5.4% of the early (five min) bypass fibrinogen concentration. Several conclusions can be drawn from this finding. First, in the presence of adequate heparinization, fibrin forma-

tion is absent during cardiopulmonary bypass. Second, fibrinogenolysis does not constitute a significant problem during assisted circulation. In the absence of fibrin formation or fibrinogen degradation, the quantity of fibrinogen proteolysis products formed is small and does not constitute a threat to the patient. Last, this finding, and our previous observation that fibrinogen concentrations remain unchanged during recirculation experiments with cell-free plasma,[14] indicate that this protein is not denatured during several hours of assisted circulation.

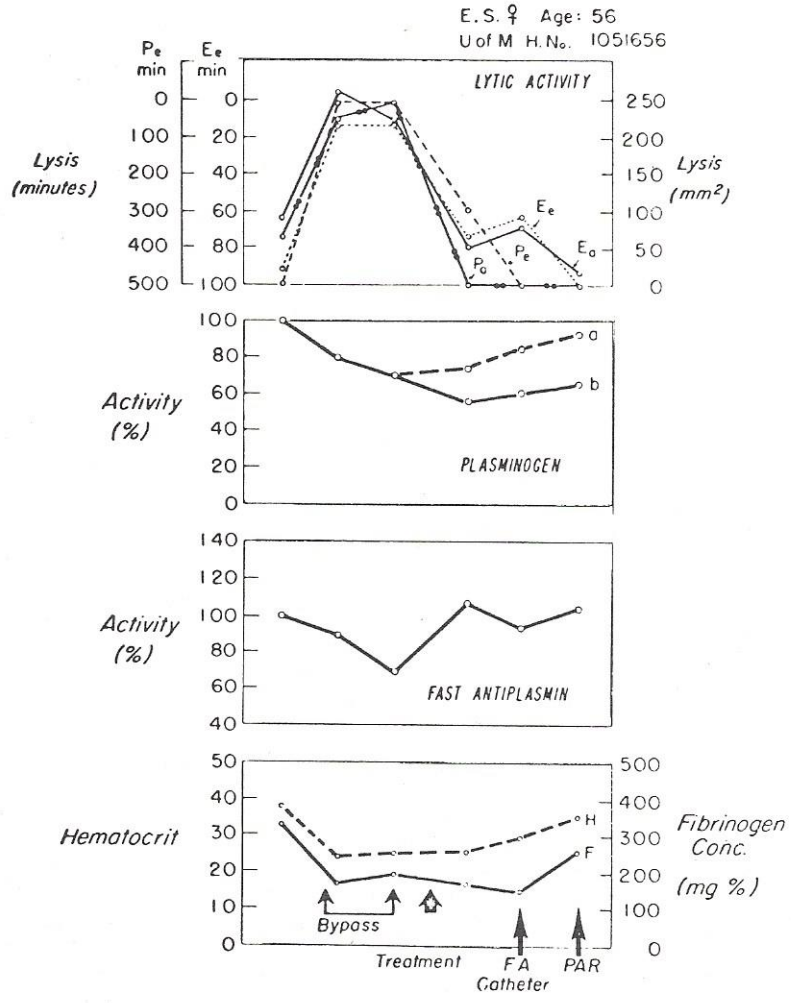

FIGURE 4. Overall changes in blood during and after cardiopulmonary bypass. *Lytic Activity*: During cardiopulmonary bypass, the lytic activity of euglobulin samples (Ee: euglob clot lysis time; Ea: lysis of euglobulin sample on fibrin plate) is equal to that exerted by plasma samples (Pe: plasma clot lysis time; Pa: lysis of plasma sample on fibrin plate). *Plasminogen*: Determination A[19] is less affected by Trasylol than determination B.[3] This finding indicates that Trasylol is probably not precipitated in the euglobulin fraction. *Antiplasmin*: Fast antiplasmin activity value returns to normal after Trasylol administration. *Fibrinogen*: Note the decline in fibrinogen concentration after the administration of protamine.

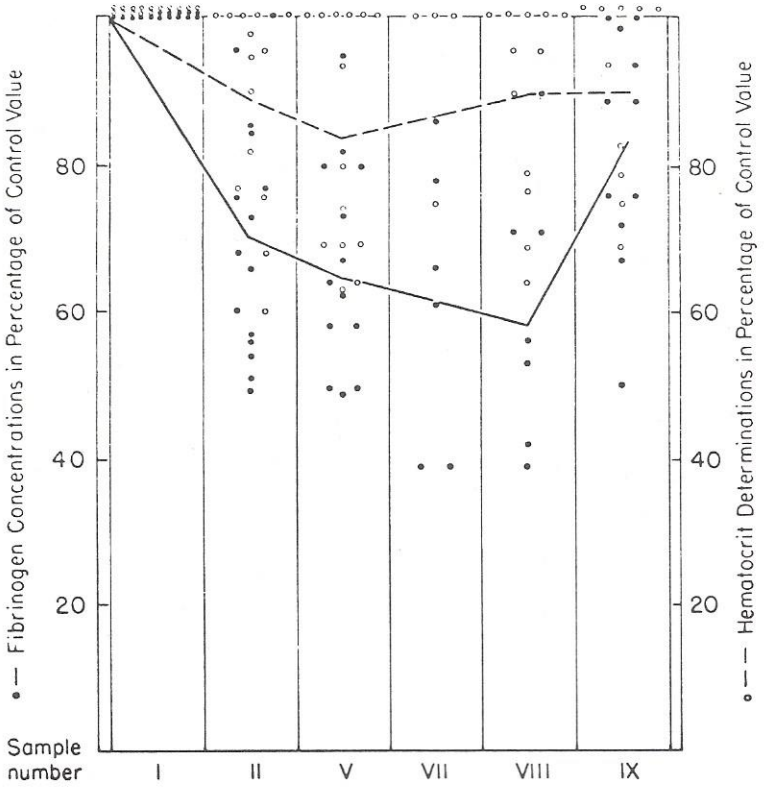

FIGURE 5. Changes in fibrinogen concentration during and after cardiopulmonary bypass. Fibrinogen concentrations decline in the first five min of cardiac bypass more than would be expected from hemodilution alone (Sample I, before bypass; Sample II, five min after start of cardiopulmonary bypass). No changes occur during cardiopulmonary bypass (Sample V, end of bypass sample). Marked decline in fibrinogen occurs after protamine administration (Sample VII is obtained shortly after protamine administration).

Changes Observed after Cardiopulmonary Bypass

Development of Hypercoagulability of Blood. In contrast to the absence of change during cardiopulmonary bypass, marked decline in fibrinogen occurs after protamine administration.[22] This decline has been ascribed to the immediate loss of heparin anticoagulation. Therefore, a permissive defibrination, as found in clotting, may occur. In addition, fibrinogen, precipitation has been noted after the use of protamine and polybrene.[21] The question whether heparin neutralization is necessary seems justified,[23] in view of the short half-life of heparin.[24] In a group of patients not subjected to heparin neutralization, fibrinogen values 15 min after cardiopulmonary bypass were 153 ± 51%. The value observed in a protamine-treated group of patients was 66 ± 22% (FIGURE 6). This difference is significant.

Protamine administration does not seem to affect the fibrinogen concentration of patients undergoing short cardiopulmonary bypass for correction of simple defects. The fall in fibrinogen value following the administration of protamine,

however, may reach dramatic values in a few isolated patients. Following replacement of an aortic valve in one patient, an 85% drop in fibrinogen concentration was found in blood samples collected 15 min apart.

Under normal conditions, rapid clearance of coagulants from blood occurs.[25] Thus, heparin neutralization is not expected to cause significant difficulties. Because defibrination develops, a defect in the clearance function is implied; this is currently being evaluated in our laboratory.

Development of Hypofibrinolytic State. Following bypass, proteolytic activity rapidly disappears. *Hypofibrinolysis* develops, as demonstrated by greatly prolonged euglobulin and plasma clot lysis times (FIGURE 7). Several changes occur that account for this phenomenon. Plasminogen, fibrinogen, and antiplasmin levels of plasma are reduced. The more pronounced change, however, is observed in plasminogen-activator activity. While this is occasionally present before surgery, it always completely disappears postoperatively. This particular response occurs so consistently that it permits the determination of the in vivo half-life of the activator released during assisted circulation.

In Vivo Half-Life Values for Plasminogen Activator Released During Cardiopulmonary Bypass. The in vivo half-life value for plasminogen-activator activity following short cardiopulmonary bypass procedures for repair of atrial septal defects in five patients who exhibited maximal plasminogen-activator activity at the end of bypass was 13.4 ± 6.2 minutes. This value became prolonged after more extended bypass. The mean value for 50 patients undergoing cardiopulmonary bypass for correction of a variety of defects was 42 minutes. It was not unusual, however, to find a plasma half-life value as long as 75 minutes. An example of such a prolonged value observed in a patient operated upon for correction of a tetralogy of Fallot, is shown in FIGURE 8.

The fate of plasminogen activator is not clear. Recently Holemans has shown

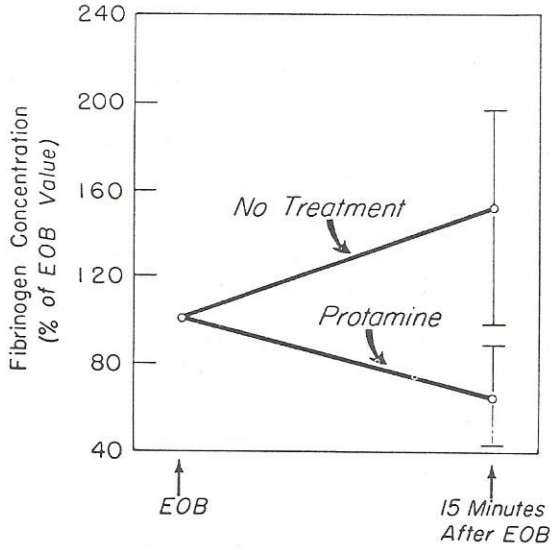

FIGURE 6. Effect of heparin neutralization on fibrinogen values. After protamine administration, fibrinogen concentration is 66 ± 22% of end of bypass value; in untreated patients this value is 153 ± 51%. Each group consists of 8 patients.

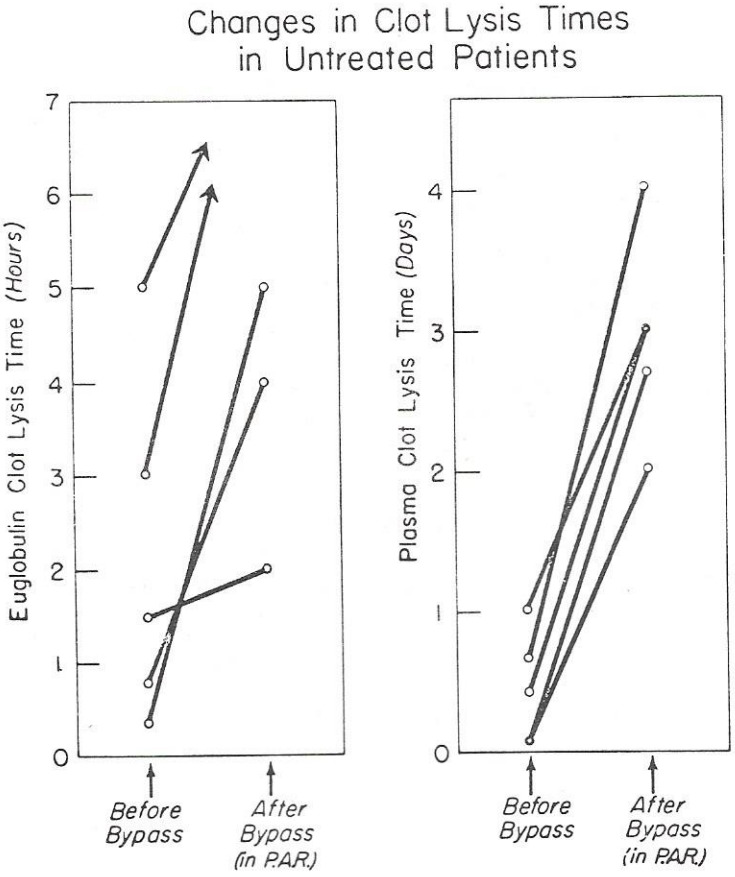

FIGURE 7. Development of hypofibrinolysis after cardiopulmonary bypass. Euglobulin and plasma clot lysis times become greatly prolonged after cardiopulmonary bypass. None of these patients received synthetic antiplasmins.

that it is not excreted in the urine.[4] Clearance by the reticuloendothelial system has been postulated.[26] Sherry and coworkers found half-life value of 12–13 min for the activator released following injection of nicotinic acid in human volunteers.[26] This value is the same as that found after short cardiopulmonary bypass procedures. One cannot escape the impression that the activator activities observed following these different stimuli may be identical.

Prolonged half-life values, a feature of considerable clinical interest, can then be ascribed to impairment of reticuloendothelial function, a feature previously observed during experimental shock.[13]

Hypofibrinolysis and Hypercoagulability. A point in question is whether the hypofibrinolysis, observed several hours after polybrene administration, is also associated with a hypercoagulability state. Occasionally, thrombin time determinations provide values shorter than those observed preoperatively. This difference is especially pronounced in patients receiving synthetic inhibitors. These changes are consistent with either hypofibrinolysis, hypercoagulability, or both.

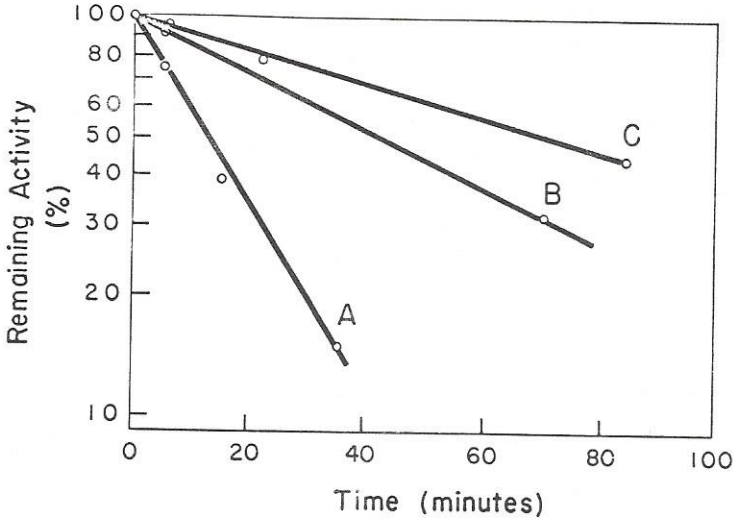

FIGURE 8. In vivo turnover rates of plasminogen activator following cardiopulmonary bypass. A represents the decay curve of plasminogen-activator activity in plasma following short cardiopulmonary bypass procedures for the repair of atrial septal defects in five patients who exhibited maximal plasminogen-activator activity at the end of cardiopulmonary bypass. In vivo half life : 13.4 ± 6.2 minutes. B represents a similar curve for 50 patients undergoing assisted circulation for a variety of defects. In vivo t ½ : 42 minutes. C represents the in vivo plasminogen activator disappearance curve in a patient operated upon for correction of a tetralogy of Fallot. In vivo t ½ : 72 minutes.

Effect of Treatment on Observed Changes

Earlier, several allusions were made regarding various types of treatment instituted to prevent the development of clotting or bleeding problems. Heparin is used during cardiopulmonary bypass to prevent the clotting of blood. Antiproteolytic agents are used prophylactically at the end of bypass to compensate for loss of inhibitor during bypass and to inhibit excessive fibrinolysis. After bypass, heparin action is neutralized to promote normal coagulation as required for adequate surgical hemostasis. Consequently, the use of protamine to neutralize heparin has found wide acceptance.

Effect of Protamine. The coagulation-promoting effect of protamine administration may assume dangerous proportions. This is apparent from the drop of the plasma fibrinogen level observed after its use. In addition, thrombocytopenia and deficiency of factor VIII have been reported. Following rapid injection, systemic reactions included hypotension and tachycardia, followed by bradycardia and changes in the respiratory rate.[27] A question at issue is whether heparin neutralization is necessary. The results of Castaneda's study[23] would suggest that it is not.

Several features of the hypercoagulability state that develops following the injection of protamine sulfate are of particular interest. This state is frequently associated with increased plasminogen-activator activity. Both hypercoagulability of blood and increased plasminogen-activator activity observed at this time may result from a defect in blood clearance. The situation favoring the rapid lysis of fibrin formed during the coagulation of fibrinogen has been created. If large quantities of fibrin are formed, the possibility exists that, as a result of fibrinolysis,

large amounts of fibrinogen-degradation products are also formed. These breakdown products exert marked anticoagulant activity. Indeed, testing the blood sometimes reveals it to be unclottable. An oozing develops that gradually or rapidly assumes catastrophic proportions. This bleeding fails to respond to treatment—including massive transfusions. Only rarely can enough freshly drawn blood be obtained to prevent the development of shock. Not infrequently the patient exsanguinates.

There are exceptions to this course. In a number of instances we have found that incoagulable blood becomes clottable in the thrombin time assay upon the addition of protamine sulphate. Whether this phenomenon represents paracoagulation,[28] or merely inadequate heparin neutralization from rapid breakdown of protamine sulphate (a phenomenon recently described by Frick),[29] is presently not known. Under these circumstances it has been our practice to administer protamine sulphate in a dose of 50 mg after the clotting time has been determined and found to be greatly prolonged. We proceed with administration of protamine at regular intervals as long as it continues to shorten the clotting time.§ This regimen has been of benefit in a number of cases. These include patients in whom heparin neutralization was *not* carried out at the end of cardiopulmonary bypass.

Thus, we are left with the question of when to use protamine and when to abstain from using it. It would seem that the regimen that includes neutralization of heparin with small divided doses of protamine, *only* when bleeding poses a problem, has merits that far outweigh the routine use of this drug.

Effect of Antiproteolytic Agents. Antiproteolytic agents have gained wide use after cardiopulmonary bypass.[9] The concentrations of epsilon amino caproic acid required for the neutralization of plasmin are higher than for plasminogen activator.[30] Following its use. the hypofibrinolytic state develops more rapidly.

Patients who receive no treatment (neither protamine nor inhibitor) continue to have low levels of fast-acting antiplasmin after surgery (FIGURE 9). In contrast, their plasminogen concentrations may reach preoperative control values in relatively short periods of time, especially after short bypass (FIGURE 3). The reason for the apparent difference in reappearance rates between plasminogen and antiplasmin is presently unknown.

The activity of the fast inhibitor in patients who received one injection of Trasylol¶ immediately after cardiopulmonary bypass returned rapidly to preoperative levels (FIGURE 6). In addition, plasma clot lysis times became prolonged after cardiopulmonary bypass in 87.5% of the Trasylol-treated group versus 25% of the untreated patients.

When plasminogen-activator activity remains demonstrable for longer periods of time, the use of synthetic antiproteinases may be beneficial in preventing hemorrhage, especially in those patients in whom the administration of protamine is followed by a decline in fibrinogen concentration. It is questionable, however, if its use is required in patients in whom heparin activity is not neutralized.

In view of the known affinity of plasminogen activity for fibrin, it might be expected that patients who do not receive protamine do not develop the previously described vicious cycle. Thus, in the absence of fibrin formation, fibrinolysis does not develop. Since fibrinogenolysis and the formation of fibrinogen-degradation products do not appear to constitute a significant problem in the heparinized

§ Clotting times should be determined because, following the administration of protamine sulfate in excessive quantities, a coagulation defect is produced that may persist for a considerable length of time.

¶ Dose in adults: 250,000 KI Units; in children: 125,000 KI Units.

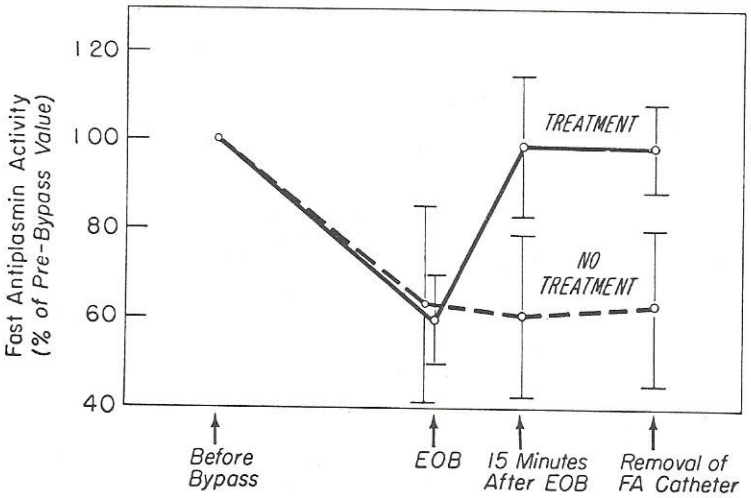

FIGURE 9. Effect of cardiopulmonary bypass on fast antiplasmin activity. In untreated patients, antiplasmin activity remains depressed. In contrast, its activity returns rapidly to normal values following the administration of Trasylol, shortly after the end of bypass (E. O. B.).

patient, the use of proteolytic enzyme inhibitors may not be needed in those patients where heparin neutralization is not instituted. The advantage of this approach lies in the prevention of extensive intrathoracic clot formation and thrombosis.[31] If protamine is required sometime after cardiopulmonary bypass, small doses of the drug and transfusion of freshly drawn blood have controlled hemorrhage without the need of additional proteinase inhibitors.

DISCUSSION AND CONCLUSIONS

This study, which is an extension of previous studies of the effect of cardiopulmonary bypass on the clotting mechanism of blood, was initiated to investigate the fate of the natural antiplasmins during assisted circulation.

The results of this study indicate that the fast antiplasmin serves the important function of preventing the development of plasmin activity. This function is well preserved, since no free plasmin can be demonstrated during or after cardiopulmonary bypass. Blood content of plasmin inhibitor provides, through the extent of its decline, a useful indicator of the amount of plasmin neutralized.

Although the fast antiplasmin represents only a fraction of the total antiplasmin activity of human plasma, we find that it can neutralize one and one-half to two times the activity that would result if all available plasminogen were to be activated. In view of the affinity of the proteolytic enzyme plasmin for a number of plasma proteins (e.g. fibrinogen, complement, factor V, VIII, et cetera), the action of the fast antiplasmin is indeed significant in preserving hemostasis.

In contrast, plasminogen activator is quite harmless in the absence of fibrin formation. In addition, its activity in blood is short lived. Hence, problems in hemostasis are anticipated only if fibrin formation is allowed to proceed. Then lysis of fibrin can occur through activation of plasminogen that is associated with fibrin. These considerations suggest that, during or after cardiopulmonary bypass,

conditions associated with the formation of fibrin may pose substantial hazards to the patient. In this regard the potential threat of protamine neutralization, previously noted, has been confirmed by this study.

While normally coagulant and lytic activities are rapidly cleared from the blood, evidence is presented in this study for the occasional interference with their normal clearance following cardiopulmonary bypass. Thus, following short and uncomplicated cardiopulmonary bypass procedures, fibrinogen concentrations hardly change after protamine neutralization. After longer and more complex procedures, however, a considerable drop in fibrinogen value is not uncommon. These findings suggest that during cardiopulmonary bypass the clearance of coagulants from blood is defective. Similar defects are also noted in the clearance of plasminogen activator. Normal half-life for plasminogen activator is 13 minutes. Abnormal values of 70 min are by no means rare after bypass. The significance of these findings with regard to heparin neutralization has been discussed.

Several allusions were made regarding possible causes of the noted changes. Currently we believe that both surface activation,[32] as a result of trauma to blood, and shock may play a significant etiological role.

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