Problems in Hemostasis During Open-Heart Surgery: *

I. On the Release of Plasminogen Activator

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THE HEMORRHAGIC syndrome in patients operated upon for correction of cardiac defects with the aid of a pump oxygenator constitutes a significant problem. Incoagulable blood and the generalized oozing which suddenly develops in an initially dry surgical field have been universally observed in occasional patients.

The coagulation mechanism of 50 patients was studied in order to evaluate changes which do occur during and after cardiac bypass. In this paper the effect of extracorporeal circulation on the release of plasminogen activator will be evaluated. Plasminogen activator is a substance (or substances) required to convert the inactive plasma protein plasminogen into the active enzyme plasmin. Plasmin is a proteolytic enzyme which is capable of lysing fibrin and other coagulation factors. Plasminogen activator activity is measured by the lysis produced in a test system in which substrate fibrinogen and plasminogen are in abundance.

In the past, several investigators have found an activation of the fibrinolytic enzyme system during extracorporeal circulation (Osborn *et al.*,[9] von Kaulla *et al.*,[12] Nillson *et al.*[8]) In one patient severe postoperative hemorrhage was ascribed to the observed plasminogen activation by von Kaulla and Swan.[12] These investigators noted that the degree of activation was related to factors such as efficiency of perfusion and degree of postoperative acidosis.

The fact that plasminogen activator is as a rule released during extracorporeal circulation could be confirmed in the patients reported on in this paper. Exceptions of the rule will be discussed.

Various factors have been thought to influence the amount of bleeding. Several factors, as duration of perfusion, degree of acidosis, etc. have been evaluated with regard to their effect on the degree of plasminogen activator activity. So far, the finding that surgical correction for acquired heart disease is associated with release of significantly more plasminogen activator than that for congenital defects appears to be the most important factor uncovered by this study.

Methods and Materials

Blood (15 cm.^{3}) was collected before, during and after operation in non-siliconized tubes containing 0.3 cc. of 10 per cent sequestrene. The samples were obtained at various times.

Sample No. I was collected after the induction of the anesthesia at the time of introduction of a catheter in a vein in the

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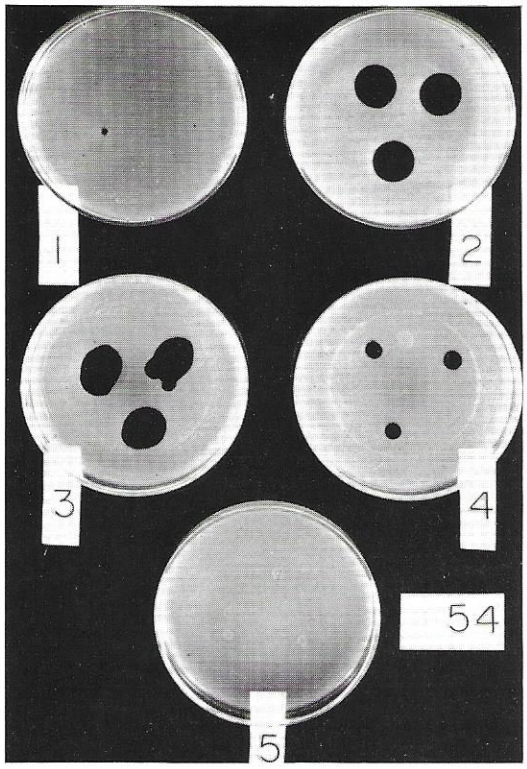


FIG. 1. Extent of lysis of Astrup plates after incubation of euglobulin samples of patient No. 54 (diagnosis: I. A. S. D. with Pulmonary Stenosis) for 24 hours at 37° C. Sample 1 obtained at the time of cutdown (10:30 a.m.). Sample 2 obtained 5 minutes after starting the cardiac bypass (12:15 p.m.). Sample 3 obtained at the end of the bypass (12:38 p.m.). Sample 4 obtained at the time of removal of femoral artery catheter (1:05 p.m.). Sample 5 obtained in the P.A.R. (2:45 p.m.).

groin. These samples were obtained prior to thoracotomy by the operating surgeon.

Sample No. II was collected five minutes after starting extracorporeal circulation. The samples were obtained directly from the pump oxygenator.

Sample No. III was collected before the release of venae cavae ligatures. Samples were obtained from the pump oxygenator.

Sample No. IV was collected after the release of the venae cavae ligatures, the patient being on partial bypass for approximately five minutes. The samples were obtained from the pump oxygenator.

Sample No. V was collected at the end of the cardiac bypass. Samples were obtained from the pump oxygenator. Average duration of cardiac bypass in 50 patients was 83 minutes.

Sample No. VI was collected before and Sample No. VII after the administration of polybrene. Both samples were taken by the operating surgeon via cardiac puncture. Average time span between samples V and VII was 30 minutes (32 cases).

Sample No. VIII was collected at the time of removal of the femoral artery catheter. Average time lapse between samples VII and VIII was 40 minutes (36 cases).

Sample No. IX was collected at the time of the patient's arrival in the Recovery Room. Average time lapse between samples VIII and IX was 150 minutes (41 cases).

All samples, after careful mixing with the anticoagulant, were immediately transported in ice from the operating room to the laboratory.

Plasminogen activator was obtained from plasma by removing inhibitors and by precipitating it in the euglobulin fraction.

Plasminogen activator activity was determined in two ways, namely by the Astrup plate technic, and by the euglobulin clot lysis method. In the Astrup plate technic, 30 lambda samples of the euglobulin solution are incubated on Astrup fibrin plates for 24 hours. The extent of lysis was determined as described by Astrup and Mullertz.[1] Each determination was performed in triplicate.

The euglobulin clot lysis time was determined on duplicate samples by clotting the euglobulin fraction with standard amounts of bovine thrombin (Parke-Davis) after which the euglobulin clots were incubated in a water bath at 37° C. Lysis time was measured from the moment of addition of thrombin till lysis of the clot had occurred.

The fibrin plates were prepared as described by Astrup.[1] The final concentration of the fibrinogen solution was measured by the method described by Fowell.[2]

Free plasmin activity was tested for by incubating 30 lambda samples of the euglobulin solution on the Lassen plates.[4]

The total number of patients studies was 61. The number of patients reported upon in this paper is 44.

Atrial Septal Defect	10
Ventricular Septal Defect	9
Tetralogy of Fallot	7
Mitral Disease	8
Aortic Disease	10
Totals	44

The majority of these patients received Epsilon Aminocarpoic Acid immediately at termination of cardiac bypass at the request of the operating surgeons. Dosage of the drug and mode of administration will be described elsewhere.

Results

Activator Activity in Individual Patient. The degree of activator activity before, during and after extracorporeal circulation was studied in individual patients by two technics (Fig. 1, 2). A 20-year old woman (U.M.H. 963733) who was operated upon for the correction of an I.V.S.D. may serve as an example (Table 1).

Over-all Changes in Euglobulin Clot Lysis Times. The euglobulin clot lysis times of all blood samples were placed in a scattergram (Fig. 3). The first sample had an average euglobulin clot lysis time of 105 minutes. Five minutes after the beginning of cardiac bypass, the average euglobulin clot lysis time was 56 minutes. At the termination of cardiac bypass, the mean value of the euglobulin clot lysis time was found to be 30 minutes. The average

Plasminogen Activator Activity in Individual Patient

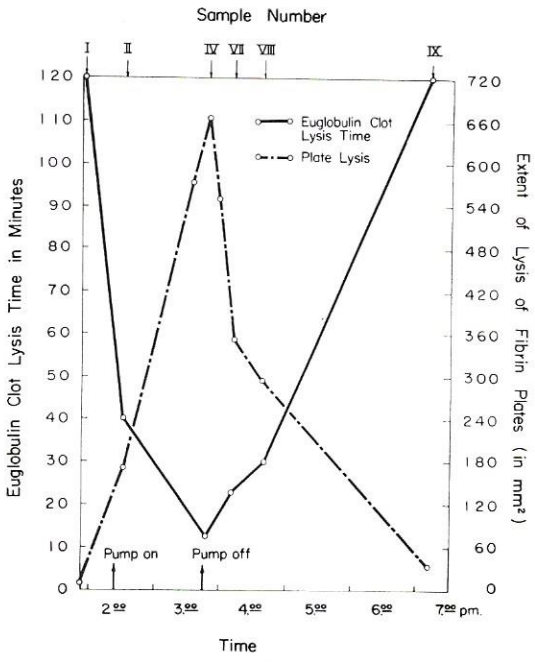


FIGURE 2.

TABLE 1

Time	Time at Which Specimen was Drawn	Euglob. Clot Lysis Time	Astrup Plate Lysis
1:21 p.m.	At time of introduction of caval catheters	>120′	9 mm.²
2:00 p.m.	Seven minutes after starting cardiac bypass	40′	156 mm.²
3:19 p.m.	Four minutes after termination of cardiac bypass	14′	630 mm.²
3:40 p.m.	Ten minutes after administration of polybrene	25′	352 mm.²
4:04 p.m.	At time of removal of femoral artery catheter	30′	282 mm.²
6:40 p.m.	Upon arrival in P.A.R.	>120′	35 mm.²

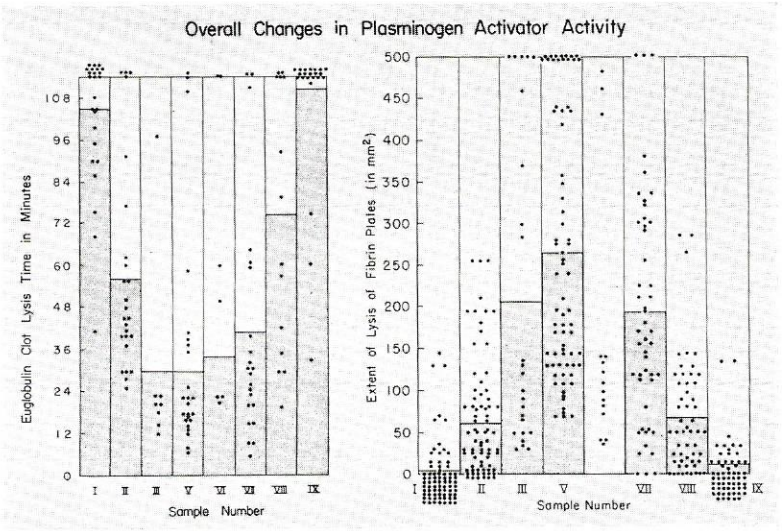


FIGURE 3.

lysis time returned to normal control values within several hours after termination of cardiac bypass. Extreme deviation in both direction and magnitude of the average pattern were observed. On the other hand in a number of instances, hardly any changes in euglobulin clot lysis time were found.

Over all Changes Observed in Astrup Plates. The extent of lysis of the Astrup plates of the typical control samples was found to be 0 mm². Five minutes after beginning of cardiac bypass the plate lysis had increased to 45 mm². Maximum plate lysis was observed at the end of the termination of cardiac bypass. The mean value of the plate lysis for these samples was found to be 265 mm². Subsequently, the plate lysis declined and returned to control value in the majority of instances when the patient arrived in the P.A.R. (Fig. 3).

The same deviations of the general pattern were observed here as were in the euglobulin clot lysis times. At the same time no free lytic activity of any significance was observed as measured on the Lassen plates (Fig. 4).

Time of Maximum Plasminogen Activator Activity. Maximum plasminogen activator activity was observed in most instances at the time of termination of cardiac bypass. Exceptions to the rule were

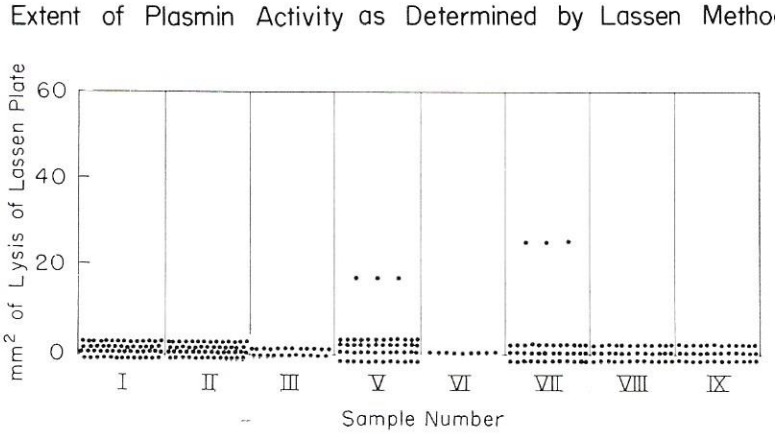


FIGURE 4.

found. The time of maximum plasminogen activator activity as observed in 61 case studies have been summarized (Table 2).

TABLE 2. *Time of Maximum Plasminogen Activator Activity*

Control sample	0
Sample obtained after cardiac bypass for five minutes	4
Sample obtained before the release of caval ligatures	3
Sample obtained at the end of cardiac bypass	37
Sample obtained after polybrene administration	8
Sample obtained at time of removal of femoral artery cutdown	5
Entirely negative studies	4
Total number of patients studied	61

Evaluation of the Influence of Various Factors on the Activation Mechanism

1. Effect of Operation and Anesthesia Upon the Degree of Plasminogen-Plasmin Activation. Patients undergoing major operation or thoracotomy were studied in a fashion similar to the one used for the patient undergoing open-heart surgery. No activator activity was observed in ten patients, six after thoracotomy, one after total colectomy, one after Billroth II gastrectomy, one after splenectomy, and one after abdominal perineal resection. The activator activity as measured by the euglobulin clot lysis time was never below 60 minutes while there was no lysis on the Astrup plate.

2. Effect of Type of Pathologic Lesion and its Surgical Correction Upon the Degree of Plasminogen-Plasmin Activation. Since the maximum activity occurs at the termination of cardiac bypass it is interesting to observe the extent of plasminogen activator activity at this time in the different categories of patients. The average euglobulin clot lysis time values were shortest in patients with acquired aortic and mitral disease and longest in patients with congenital defects such as intra-atrial, intraventricular septal defects and tetralogy of Fallot (Fig. 5–8). Similar results were obtained using the Astrup plate method (Fig. 5–8). The over-all results for each group of patients are charted in Figure 9. It is interesting to note that several patients demonstrated short euglobulin clot lysis times for several hours after the cardiac

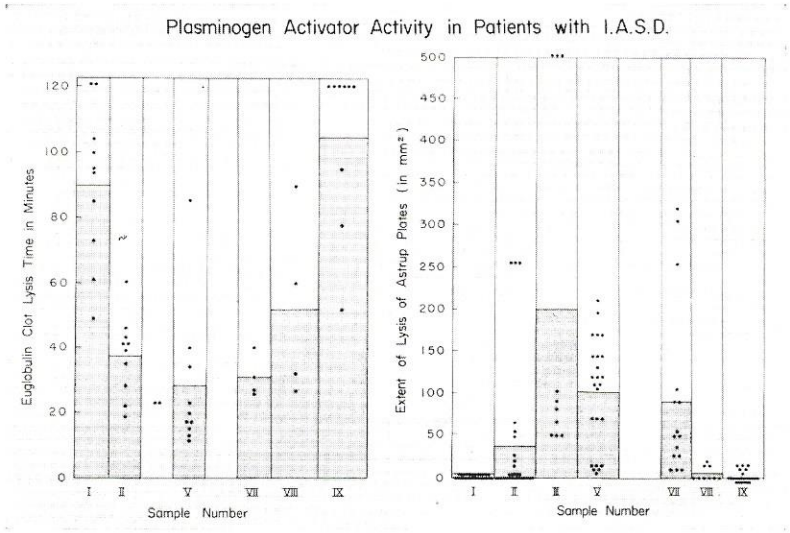


FIGURE 5.

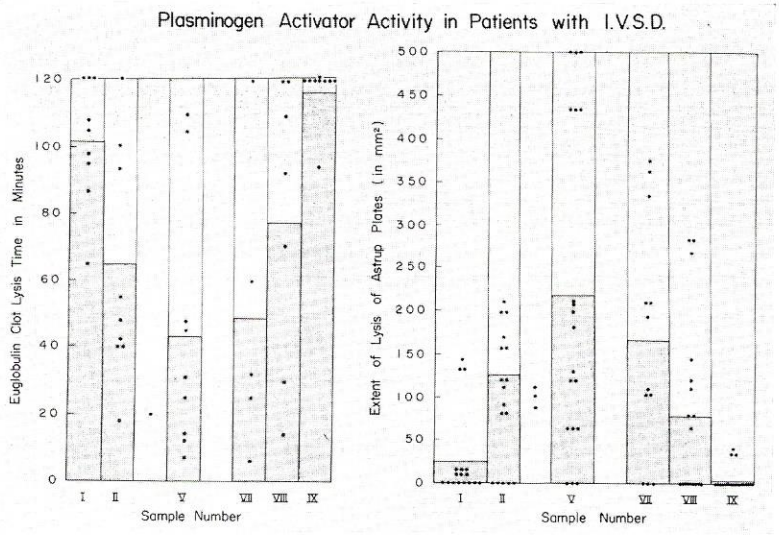


FIGURE 6.

bypass procedure, up to the time of arrival in the P.A.R.

3. **The Influence of Duration of Cardiac Bypass and the Degree of Plasminogen Activator Activity.** It appears from the data collected in Figure 10 that the longer cardiac bypass procedures are as a rule associated with more activator activity.

4. **The Effect of Acidosis on the Degree of Plasminogen Activator Activity.** The degree of acidosis does not seem to influence the activator activity to any great extent (Fig. 11).

5. **The Effect of Defibrillation on the Degree of Plasminogen Activator Activity.** The number of times a patient was defibrillated did not appear to affect the plasminogen activator activity (Fig. 12).

6. **Effect of Pulmonary Hypoxia on the Degree of Plasminogen Activator Activity.** Samples collected immediately before and five minutes after the release of the

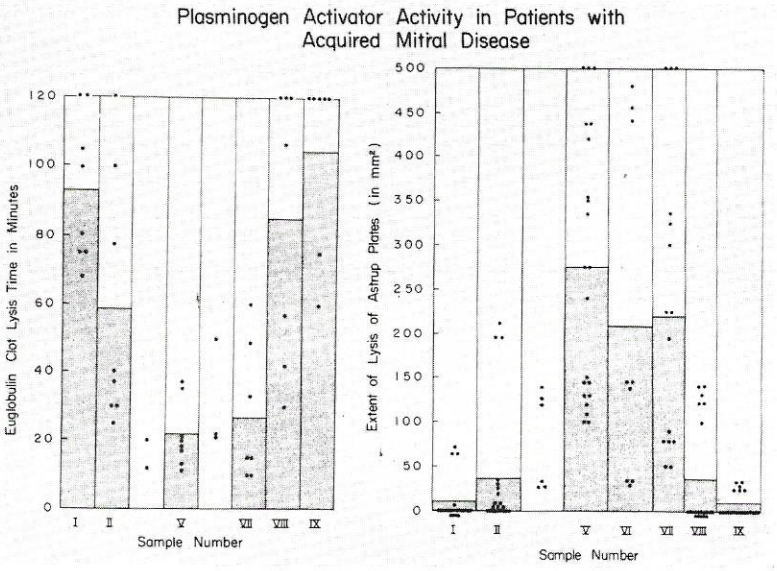


FIGURE 7.

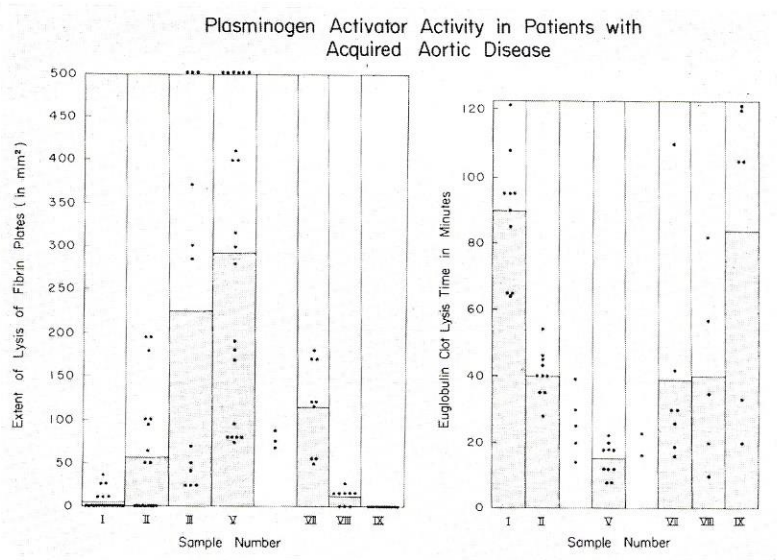


FIGURE 8.

caval vein ligatures did not exhibit marked changes in the degree of plasminogen activator (Fig. 13).

Discussion

The plasminogen activator activity can be best measured by means of the euglobulin clot lysis time method (v. Kaulla) and the fibrin plate technic (Nillson *et al.*). These methods have been shown to be reproducible and standardized. By and large there is close inverse relationship between the euglobulin clot lysis time and the Astrup plate technic. The results with the Astrup plate technic did have more scattering than those obtained with the euglobulin clot lysis method.*

* The reason for this is the result of difficulties in measuring accurately the fibrinogen concentration of the fibrinogen solution prepared as described by Astrup. But each individual patient had the appropriate samples measured on fibrin plates prepared from the same fibrinogen preparation.

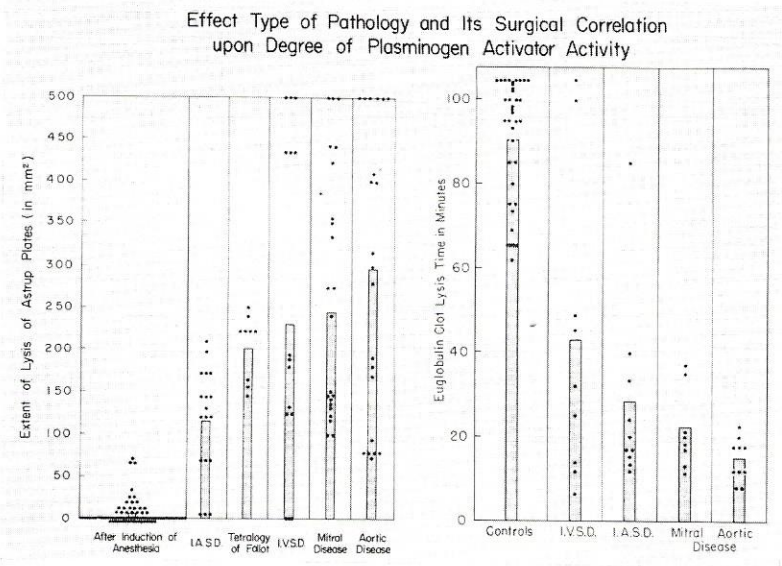


FIGURE 9.

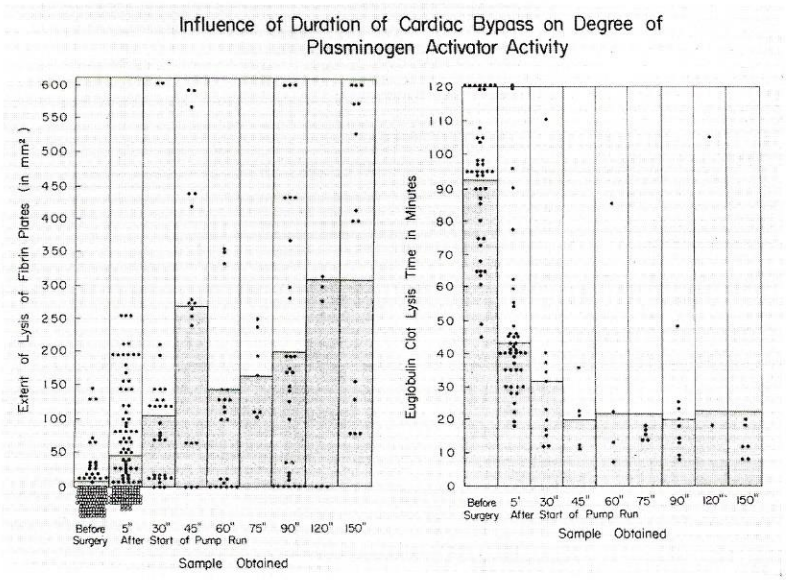
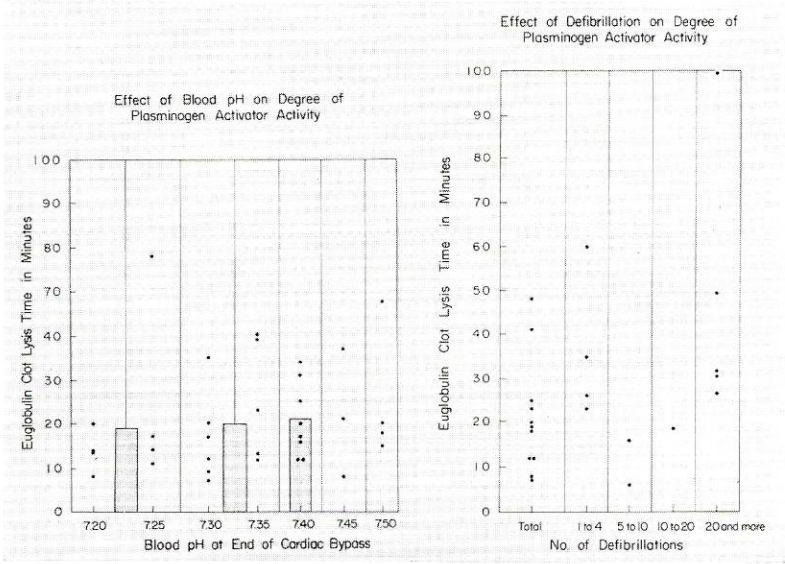


FIGURE 10.

From the data reported, it appears that in the majority of patients there is increase in the plasminogen-activator activity during extracorporeal circulation. Anesthesia, or operative surgery in general, or simple thoracotomy do not as a rule seem to be associated with an appreciable release of plasminogen activator. Extracorporeal circulation appears to be responsible for increase in plasminogen activator activity since it is demonstrated in considerable quantities for the first time after start of the cardiac bypass. Conversely, a few patients do not exhibit any change in plasminogen activator activity. No activity could be detected in four of 61 patients. Other patients have an extensive amount of plasminogen activator activity.

The amount of activator released varies. From results obtained in our study it appears that patients operated upon for correction of acquired heart disease (aortic



FIGURES 11, 12.

and mitral stenosis or insufficiency) released considerably more activator than those operated upon for correction of congenital lesions.

Factors which may contribute toward release of plasminogen activator were evaluated. It should be noted that plasminogen activator activity has been demonstrated in the past in patients under a variety of conditions. Of these, acute anoxia or acidosis (Mullertz) electro shock (Sherry *et al.*), adrenalin injection (McFarlane), and local ischemia (Mullertz) could be directly applied to our problem.

The result of our studies so far indicate that defibrillation (electro shock) had no effect whatsoever on the amount of activator released. Moreover, the duration of the procedure did not seem to affect greatly the quantity of activator detectable in circulation. Latter findings suggest that the maximum release of plasminogen activator occurs shortly after the beginning of cardiac bypass to reach a maximum after approximately 60 minutes. On occasion the release of plasminogen activator continues for considerable periods of time and maximum activity has been observed up until one hour or more after termination of cardiac bypass (Table 2).

The effect of pulmonary hypoxia was negligible since no increase in activator activity was observed immediately after release of the caval vein ligatures. This latter finding, however, should not be interpreted as meaning that plasminogen activator activity is not derived from lung tissue.

Two mechanisms have been recognized through which activation of the plasminogen plasmin system can exert its effect. First and probably most important is the effect on fibrin. During the conversion of fibrinogen, both plasminogen and activator are absorbed to the fibrin mesh, which constitutes the framework of a clot. In the presence of considerable amounts of activator rapid clot lysis can and does occur.[3]

The second mechanism of action is observed during conditions of hyperplasminemia. Since plasmin can break down fibrinogen, factor V, VII and AC globulin (Soulier *et al.*[11]) as well as fibrin, multiple

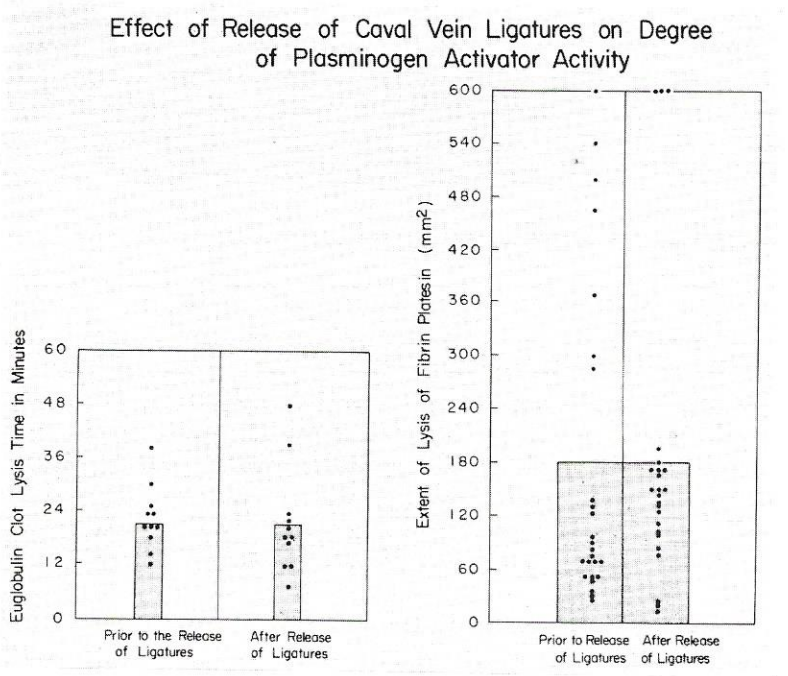


FIGURE 13.

coagulation defects result. In the patients reported here, no free plasmin could be demonstrated by means of the Lassen plate technic.

Summary

Cardiac bypass is associated with release of *plasminogen activity* in over 90 per cent of the patients. In a few patients no activator activity of any significance could be demonstrated. The degree of activator activity was greater in patients operated upon for the correction of acquired heart defects, and persisted in some patients for considerable periods of time after termination of cardiac bypass. Interesting was the finding that in 10 per cent of the cases studied the maximum activity occurred at an average of 70 minutes after cardiac bypass.

The free plasmin activity during and after cardiac surgery was found to be negligible. Consequently we have to assume that the bleeding tendency observed in some of these patients is, at least in part, the result of breakdown of fibrin rather than that of fibrinogen and other clotting factors.

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