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EPSILON Amino Caproic Acid (EACA), a synthetic antifibrinolytic agent, has produced dramatic cessation of bleeding (Nilsson *et al.*). The basis for the action of EACA is that of a competitive inhibitor of plasminogen activator activity (Alkjaersig *et al.*).

Because EACA was successful in the therapy of severe bleeding in a postcardiotomy patient (*vide infra*) the plasminogen plasmin system of patients undergoing open-heart surgery was investigated. The results obtained from this and other studies indicated the release of considerable amounts of plasminogen activator during open-heart surgery. Maximum activator activity was present at the time of termination of cardiac bypass. Quantity of plasminogen activator could be related to duration of cardiac bypass and the type of cardiac lesion.

Bleeding, as a result of increased plasminogen activator activity, does not respond to orthodox forms of therapy. The purpose of this study was to evaluate the inhibitor effects of EACA on the plasminogen activator activity and to determine the efficiency of the drug in the control of clinical hemorrhage.

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Introduction

In view of the complexity of the defects in the coagulation mechanism of patients undergoing open-heart surgery (i.e. decrease in platelet and fibrinogen concentrations, rise in antithrombin titer, etc.) it is hard to conceive that correction of one single defect will control severe postcardiac bypass hemorrhage. Yet in the patients studied it was demonstrated that EACA was of beneficial effect on several occasions and documentation was obtained in the laboratory of its efficacy.

The impetus for this study was engendered by the result of EACA administration in the following case.

Mr. G. W. (U. of M. Hosp. No. 953629), a 37-year-old man, was admitted with a preoperative diagnosis of severe aortic insufficiency, as a result of rheumatic heart disease. He was operated upon June 21, 1960, when a bicuspid, total, aortic prothesis was inserted below the coronary ostia under direct vision, utilizing a DeWall-Lillehei heart-lung machine. Selective cardiac hypothermia and total body hypothermia were used. Total perfusion time was 2 hours 9 minutes. The flow rate of 4,200 cc. per minute was increased subsequently to 4,500 cc. per minute.

Subsequent to bypass, bleeding became a problem. The patient continued to ooze from the aortotomy stitch holes. The patient received whole blood, low molecular dextran, polybrene, fibrinogen, Mephyton and calcium chloride. No evidence of clot formation in the chest was observed. Total

amount of blood administered till 9:30 p.m. was 56 units. The patient also received eight units of Dextran.

A blood sample was obtained at 9:45 p.m. after which 6.0 Gm. of EACA in 100 cm.^{3} of normal saline were administered over a 10 minute period. At 10 p.m. normal blood clots were observed in the chest cavity. These effected normal hemostasis. Chest closure was started. Another 4.0 Gm. of EACA were given slowly and a drip of 250 cm.^{3} of 5.0% dextrose. No recurrence of bleeding occurred.

Blood sample obtained from this patient prior to the administration of EACA contained marked plasminogen activator activity. The euglobulin clot lysis time of the sample was 7 minutes; lytic areas of 625 mm.^{2} were observed on the Astrup plate upon incubating euglobulin samples for 24 hours at 37° C. on these plates.

Methods and Materials

The patients reported upon in this study consist of 20 patients undergoing open-heart surgery. Ten patients were pretreated with EACA.* The rest of the patients were not preterated with this drug. Pretreated patients were systematically alternated with non pretreatment patients. Thus we were able to establish a control method which allowed us to compare the two groups.

Pretreatment consisted of the following: three grams of EACA per 1,000 cm.^{3} of circulating plasma ** were administered to the patient immediately after obtaining the control sample. After obtaining the second sample another 3.0 Gm. of EACA per 1,000 cm.^{3} circulating plasma were added to the pump oxygenator.

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

Sample I was collected after the induction of the anesthesia at the time of introduction of a catheter in a vein in the groin. These samples were obtained prior to thoracotomy by the operating surgeon.

Sample II was collected five minutes after starting extracorporeal circulation. The samples were obtained directly from the pump oxygenator.

Sample III was collected before the release of vena cavae ligatures. Samples were obtained from the pump oxygenator.

Sample IV was collected after the release of the vena cavae ligatures, the patient being on partial bypass for approximately 5 minutes. The samples were obtained from the pump oxygenator.

Sample 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 VI was collected before and Sample VII after the administration of polybrene. Both samples were taken by the operating surgeon via cardiac puncture. Average time span between Sample V and VII was 30 minutes (32 cases).

Sample 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 IX was collected at the time of the patient's arrival in the Recovery Room. Average time lapse between Sample 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

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* Epsilon Amino Caproic Acid obtained through the courtesy of Dr. N. Schimmel, Merck, Sharp and Dohme Research Laboratory, West Point, Pa.

** Each patient's blood and plasma volume was determined preoperatively. Radioactive iodinated serum albumen was used for this purpose.

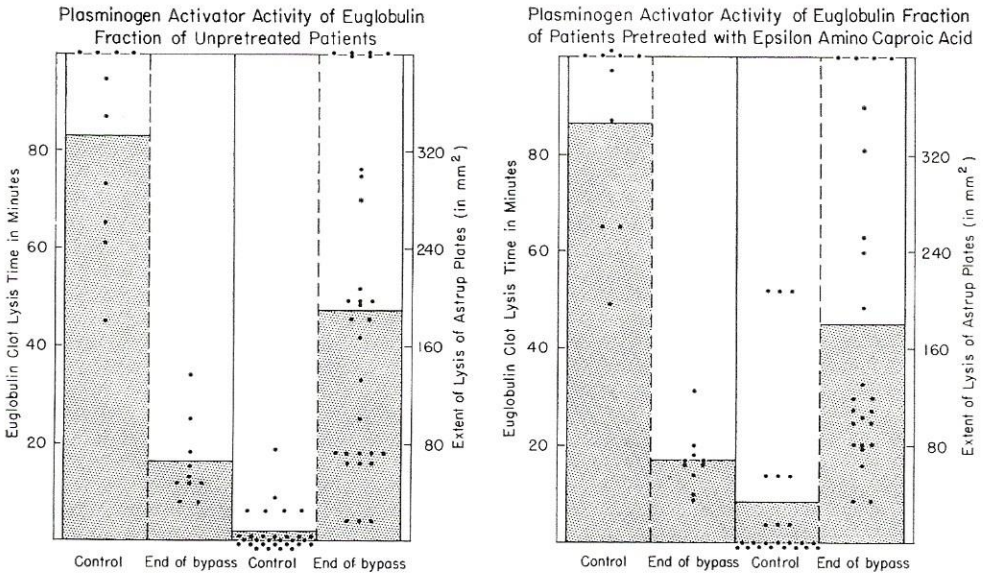

FIGURE 1.

clot lysis method. In the Astrup plate technic thirty lambda samples of the euglobulin solution are incubated on Astrup fibrin plates for 24 hours. 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. The final concentration of the fibrinogen solution was measured

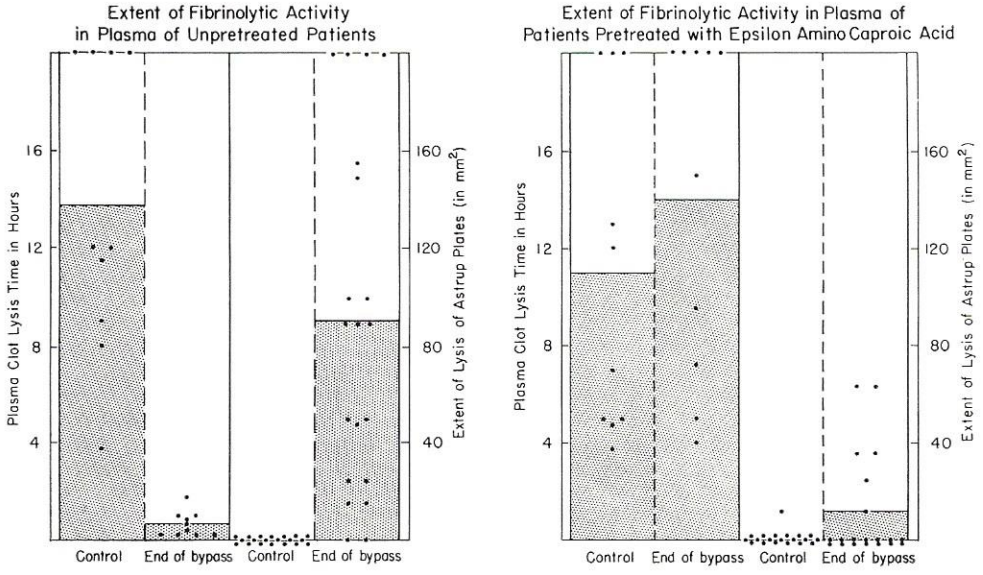

FIGURE 2.

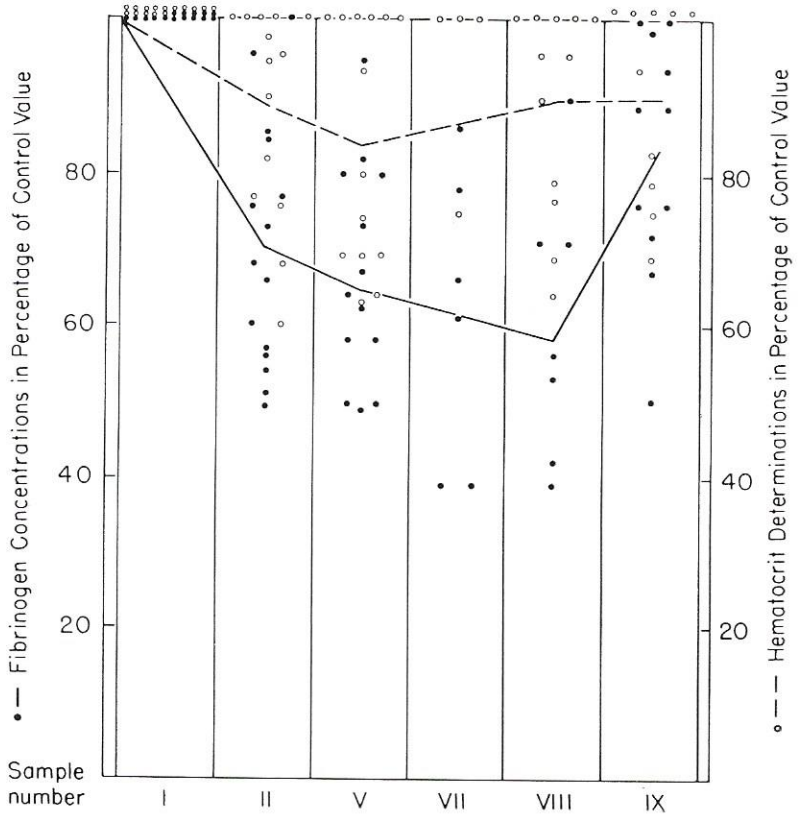

FIG. 3. Fibrinogen concentrations and hematocrit determinations in 2 groups of patients. The patients of the first group (11) were pre-treated with EACA, those of the second (4) showed no evidence of fibrinolytic activity during or after cardiac bypass.

by the method described by Fowell. The extent of lysis was determined as described by Astrup and Mullertz.

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

Hematocrit values were determined by micro hematocrit method using an International Hematocrit Centrifuge.

Fibrinogen concentration was measured by method of K. Jacobsson. *EACA levels in the blood* were determined by the method described by Nilsson *et al.*

The extent of fibrinolytic activity was determined by two methods. The first one consisted of the plasma clot lysis time. In the second one the extent of lysis observed after 24 hour incubation of 30λ samples of plasma on Astrup plates was determined. The latter methods were resorted to when it became apparent that the euglobulin solution prepared from plasma, as previously described, were devoid of all the administered EACA. *Thrombin time determinations* were done as described by Biggs and McFarlane using 5 units of bovine thrombin (Parke-Davis topical thrombin, 5,000 units per vial). Normal values for this test were found to range from 20 to 30 seconds.

Postoperatively both pretreated and unpretreated patients were studied with weekly complete blood counts, including platelet counts, BSP, urinalysis, BUN, and one stage prothrombin time determinations. The purpose of these tests was to study the effect of EACA on hepatic, renal and bone marrow function.

Results

Plasminogen Activator Activity. As can be seen from Figure 1 there is no significant difference in the plasminogen activator activity as determined by euglobulin

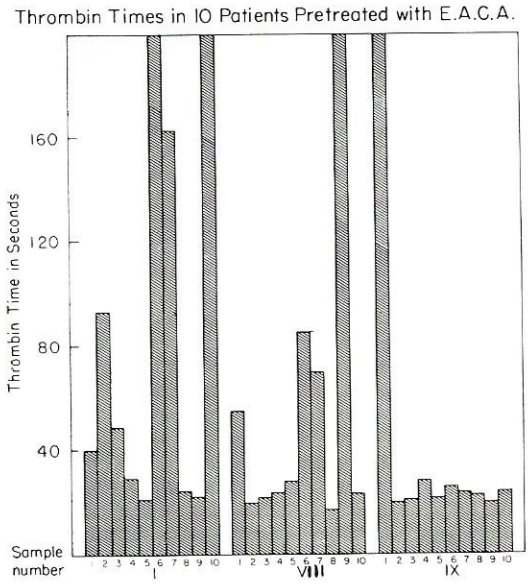

FIGURE 4.

clot lysis and euglobulin plate lysis technics in the two groups of patients undergoing open-heart surgery.

Effect of EACA on the Fibrinolytic Activity of Whole Plasma. Fibrinolytic activity of plasma became greatly enhanced during cardiac bypass in patients who did not receive EACA. This fact is illustrated by the reduction of the plasma clot lysis time from 14 hours for the control sample to an average of 46 minutes in the samples obtained at the end of cardiac bypass. At the same time the extent of plate lysis increased from an average of none to an average of 95 mm.[2] These changes were not present in the group of patients pretreated with EACA. The plasma of those patients did not reveal any evidence of increased fibrinolytic activity by either method used (Fig. 2).

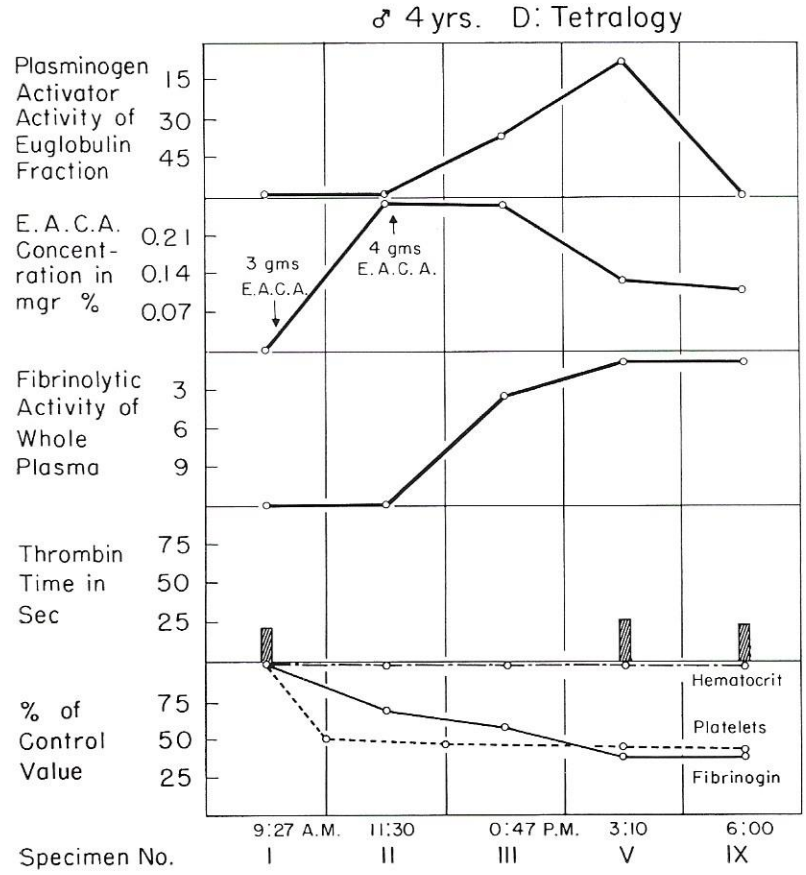

FIG. 5. Plasminogen Activator Activity (as euglobulin clot lysis time in minutes), EACA concentration (in mg.%), fibrinolytic activity of whole plasma (plasma clot lysis time in hours), thrombin time (in seconds) and hematocrit, platelet counts and fibrinogen concentration (in % of initial value) as observed in four patients undergoing open-heart surgery. (See Figures 6, 7, and 8.)

Spec. 1: obtained after induction of anesthesia, II: obtained 5 min. after starting the pump oxygenator, III: obtained at the end of cardiac bypass, V: obtained at the time of removal of the femoral artery catheter, IX: obtained in the PAR.

Other Changes Noted in Pretreated Patients. Decline of fibrinogen concentration was observed. This decline was found to be considerable, reaching values as low as 58 per cent of normal. Decline in hematocrit was also observed. Magnitude of changes was much less than those observed in the fibrinogen concentration (Fig. 3).

Prolongation of thrombin time was at times observed. These prolonged thrombin times occurred in control samples as well as in postoperative blood samples, as appears from Figure 4.

The various changes observed in the clotting mechanism in individual patients have been summarized in Figures 5, 6, 7 and 8. These are essentially as described above.

Evaluation of Toxicity of EACA. The values obtained with various function tests were evaluated postoperatively. Renal, hepatic and bone marrow function changes in the postoperative period in pretreated patients were found to be not different in nature or in magnitude from those observed in patients not receiving the drug. Up until the present time no obvious toxic manifestations have been observed with the clinical use of this drug in the dosages mentioned.

Discussion

The effect of EACA, a specific inhibitor of the plasminogen activator activity was studied in patients undergoing open heart surgery. Two similar groups of patients, one pretreated, the other not pretreated

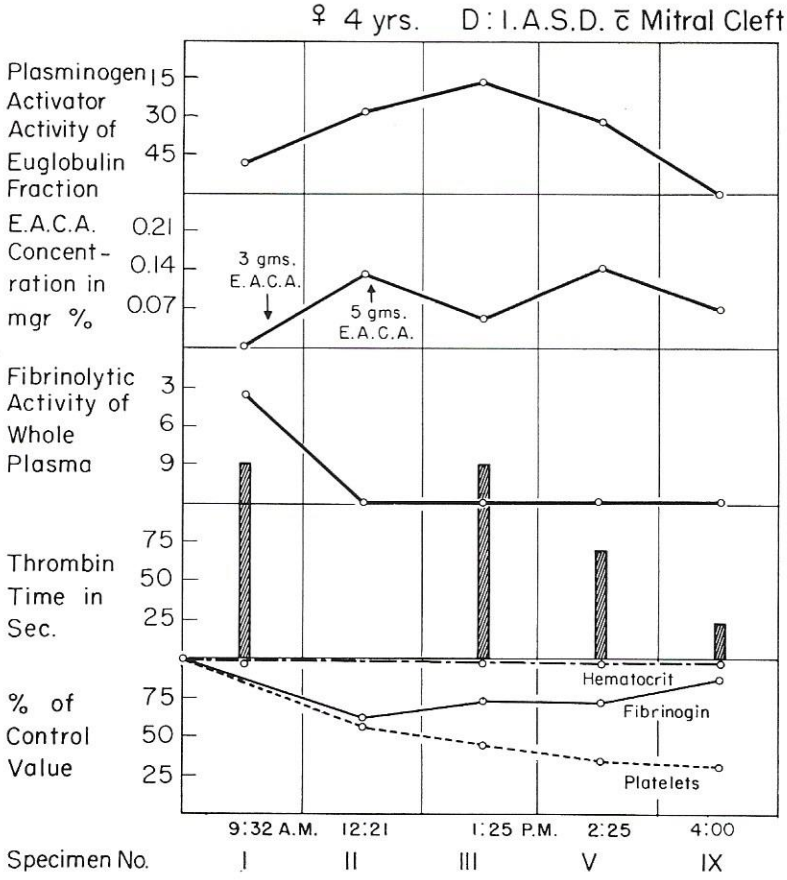

FIGURE 6.

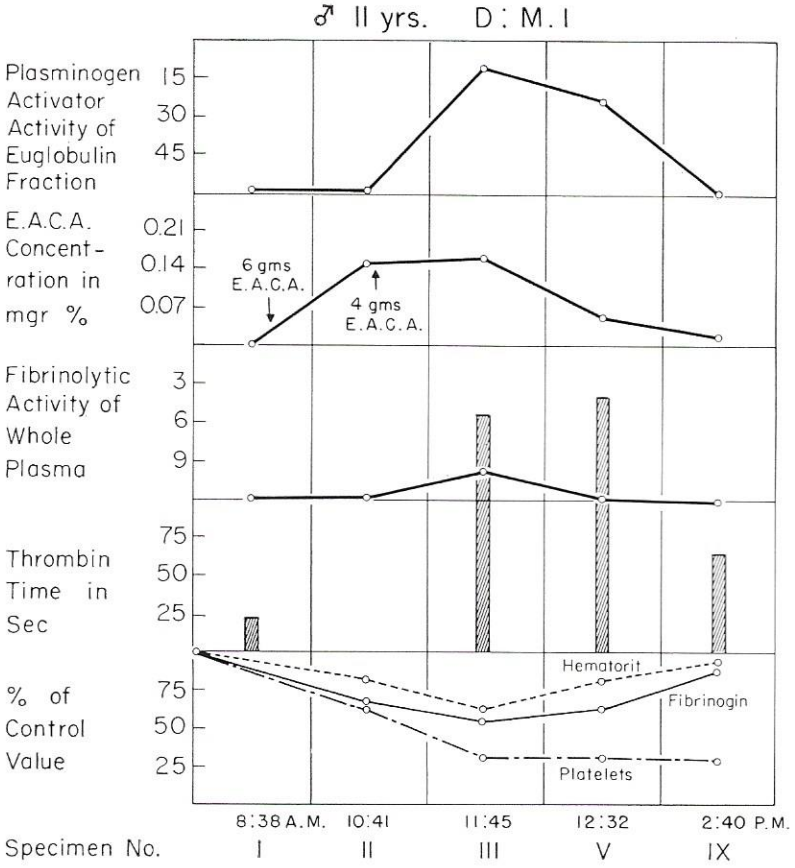

FIGURE 7.

with EACA, were compared. As a result of cardiopulmonary bypass equal amounts of plasminogen activator were released in the two groups of patients.

Fibrinolytic activity was found to be absent in plasma of patients pretreated with EACA. Considerable fibrinolytic activity was present in plasma of patients not receiving the drug. From this data it appears that EACA, in the dose administered, is effective in inhibiting plasminogen activator activity in patients undergoing open-heart surgery.

Though EACA will correct defects which result from increased fibrinolytic activity of the blood, it was shown that several other coagulation defects remain unaltered. Changes in antithrombin titer, decline in number of circulating platelets and fall in fibrinogen concentration were not different from those changes observed in patients not receiving EACA. Consequently these changes appear to be unaffected by the use of the drug.

From these findings one may conclude that other therapeutic measures are needed for the correction of the remaining defects. It is obvious that the use of EACA does not make the use of polybrene, blood, vitamin K, etc. unnecessary. EACA only serves as an adjunct, adding to existing forms of therapy.

No obvious toxic manifestations have been observed with the clinical use of the drug. This fact, as well as the above noted observations, support observations made by others. McNichol *et al.* (1960) noted the beneficial effect of EACA where they

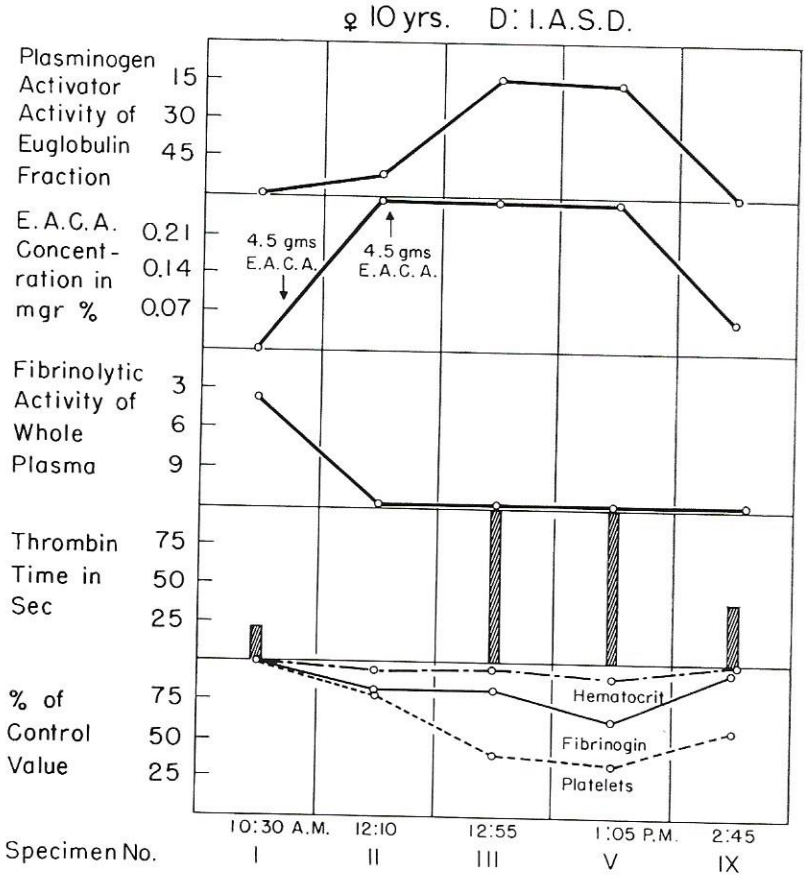

FIGURE 8.

described a decrease in blood loss in a group of patients receiving this drug after transuretheral resections. Nilsson *et al.* (1960) were able to control hemorrhage in patients who suffered from purpura fibrinolytica with the use of EACA. Recently, Grossi *et al.* (1961) were able to inhibit the spontaneous proteolytic activity of plasma of patients with cirrhosis with the use of EACA.

The observations of Blix (1961) indicates that intravenously infused EACA exerts a marked inhibitory effect on fibrinolysis which only lasts for approximately 2 hours. Data summarized in Figure 5 tend to confirm the fact that the *in vivo* effect of the drug is only shortlived. The drug was administered in this patient at a time when there was no signicant activator activity. Several hours later the plasminogen activator activity was found to have increased considerably. Now very little EACA remained in the plasma. It is therefore not surprising to observe that considerable fibrinolytic activity could be demonstrated at the time of increased plasminogen activator activity in the plasma of this patient.

EACA acts as a competitive inhibitor of plasminogen activator (Alkjaersig *et al.*). Several experiments have indicated that various EACA plasma concentrations are adequate to procure sustained inhibition of plasminogen activator activity. A danger to be noted is that *in vitro* experiments illustrate that undertreatment may induce activation rather than inhibition of the plasminogen-plasmin system. For this reason it is imperative to give an adequate dose of the drug.

Summary

The purpose of this study was to evaluate the inhibitor effects of EACA on the plasminogen activator activity released during cardiac bypass procedures and to determine the efficiency of the drug in the control of postcardiac bypass hemorrhage.

Two similar groups of patients, one pretreated, the other unpretreated with EACA showed release of equal amounts of plasminogen activator. Patients not receiving EACA had greatly enhanced fibrinolytic activity in their plasma. These changes were not present in the group of patients pretreated with EACA.

Though EACA will correct defects which result from increased fibrinolytic activity of the blood, it was shown that several other coagulation defects remain unaltered. Changes in anti-thrombin titer, decline in number of circulating platelets and fall in fibrinogen concentration were not different from the changes previously observed in patients not pretreated with EACA. Consequently, these changes appear to be unaffected by the use of the drug.

On several occasions it was noted that the clinical use of the drug in patients with post cardiac bypass hemorrhage resulted in normal clot formation where this feature had been absent before the administration of the drug.

Since maximum plasminogen activator activity is observed at the end of cardiac bypass, as was demonstrated previously, EACA is presently no longer given preoperatively. Instead it is administered at the end of cardiac bypass.

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