STUDY OF THE DEFIBRINATION SYNDROME ASSOCIATED WITH ACUTE HEPATIC FAILURE

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AFTER TOTAL HEPATECTOMY, a marked hemorrhagic diathesis develops. Meticulous mechanical hemostasis will reduce greatly the lymphorrhea which is so prevalent after extensive dissection below the diaphragm, particularly after dissection in the hepatic portal region and along the infrahepatic and infradiaphragmatic inferior vena cava.

The question of whether the bleeding tendency is mechanical or chemical in nature has not been fully settled. Undoubtedly, extensive surgical dissection occasionally is associated with bleeding. After hepatectomy in dogs, however, we (12) observed early, rapid defibrination of blood associated with an enhanced blood proteolytic activity, a feature that Drapanas and Rutherford and their associates were unable to confirm.

Recently, a marked alteration in the coagulation mechanism appeared already to be present early in the course of the hepatic dissection, long before the liver was resected (13a). Results obtained during the present studies suggest that these early changes, noted during operation, may persist after hepatectomy.

The present study of the changes in the coagulation mechanism after hepatectomy in dogs and rhesus monkeys was undertaken to establish the nature of the abnormalities, to determine the sequence in which they occur, to try to detect their cause, and to explore means for their alteration. The consideration that an answer to these questions would be of help in managing patients with acute liver failure, for example, during rejection of a liver homograft or after total hepatectomy as part of liver transplantation, served as a stimulus for this study.

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Studies supported by U. S. Public Health Grants No. HE 12324-02, No. HE 05341-08, and No. 7K4-HE 12,291.

MATERIALS

Preparation of radioactive fibrinogen. Dog plasma and Armour bovine fibrinogen served as sources of investigative material. The dog plasma was processed by a method that used Blombäck's fraction Io as the starting material, tannic acid being used to separate fibrinogen from antihemophilic factor by the method of Barnhart and Forman, and Saphedex® G 200 to separate plasminogen from fibrinogen, according to the method of Berg and Korsan-Bergsen. This fibrinogen preparation contained a 98 per cent clottable protein, free of demonstrable plasminogen, if clotted in the presence of urokinase. This preparation, however, clotted slowly.

In contrast, the material prepared from Armour bovine fibrinogen by the technique outlined by Atencio and his co-workers clotted normally. A 97 to 98 per cent clottable protein was, thus, obtained. Labeling with ^{125}I was carried out according to the principles outlined by McFarlane to the extent that the iodination did not exceed the mean of one-half atom of ^{125}I per protein molecule. The free ^{125}I was removed by dialysis of the protein solution in Versene® (edetic acid)-treated dialysis bags against frequent changes of saline solution at zero degree C. At the end of the dialysis, the supernatant fraction, after precipitation of the proteins with trichloroacetic acid, contained less than 1 per cent of the total radioactivity.

Reprint from SURGERY, *Gynecology & Obstetrics*, February, 1971, Vol. 132, 263–274

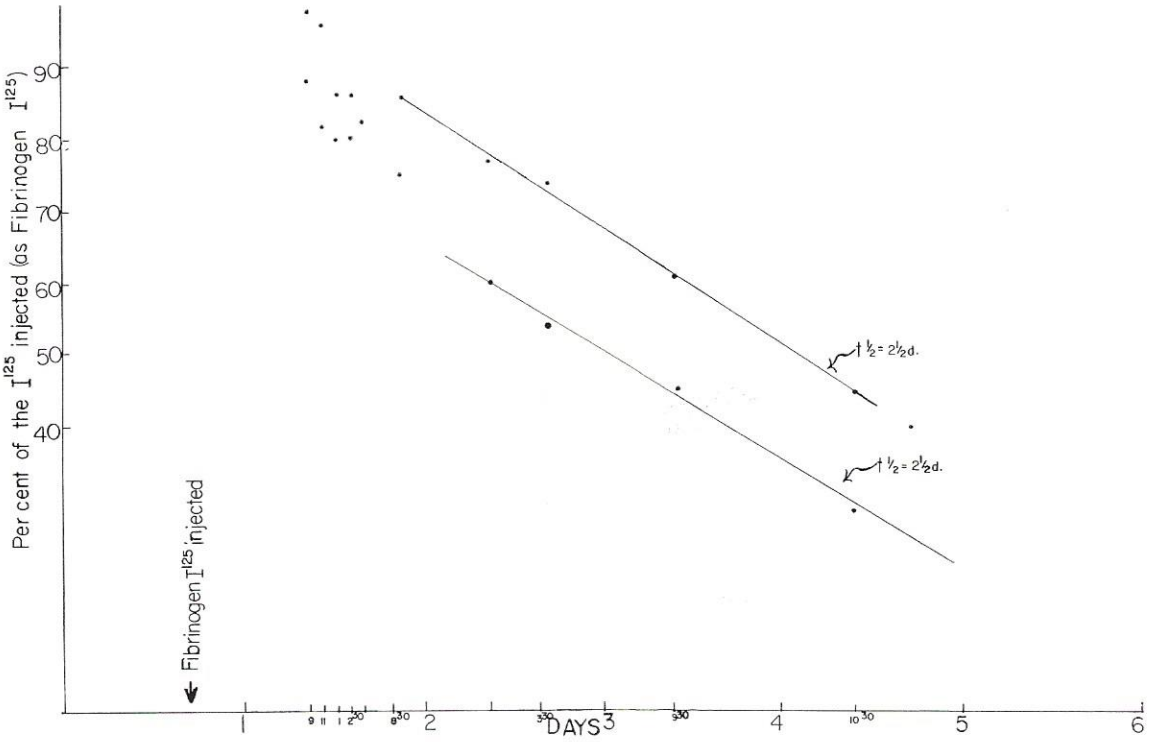

FIG. 1. Determination of the disappearance rate of labeled bovine fibrinogen in two dogs. Half of the labeled material disappears from the plasma in approximately two and one-half days.

Preparation of radioactive serum albumin. Sterile bovine serum albumin, 30 per cent, was labeled with ^{125}I according to the technique described by McFarlane. After labeling, the preparation was dialyzed against large quantities of distilled water.

Labeled albumin and labeled fibrinogen were sterilized by Millipore® filtration, stored in sterile containers after quick freezing at minus 20 degrees until immediately prior to use.

The labeled material was injected intravenously two days before blood sampling to allow for equilibration and the clearance of proteins altered by the labeling procedure.

Antiproteolytic agents. The effects of Trasylol® (aprotinin) and Amicar® (aminocaproic acid) were studied to determine their ability to alter the proteolytic activity.

METHODS

Blood samples. These were obtained initially every two hours. In later experiments, they were obtained every four hours from a three way stopcock attached to the catheter in the femoral artery by a two syringe technique. The first 10 milliliters, containing saline solution and blood, were returned to the animal after obtaining the sample in a second syringe, and immediately the blood pH was determined. Samples were transferred to 100 by 13 millimeter test tubes with, and without, an anticoagulant. The anticoagulant was 0.06 milliliter of 15 per cent sodium ethylenediaminetetra-acetate solution in 0.2 milligram per milliliter potassium sorbate. Immediately, fibrinogen and euglobulin determinations were performed. The rest of the sample was quick frozen. Total protein, sodium, potassium, blood urea nitrogen, and albumin determinations were performed on these plasma samples.

Fibrinogen concentration. Fibrinogen concentrations were determined by the technique of Jacobsson with the use of com-

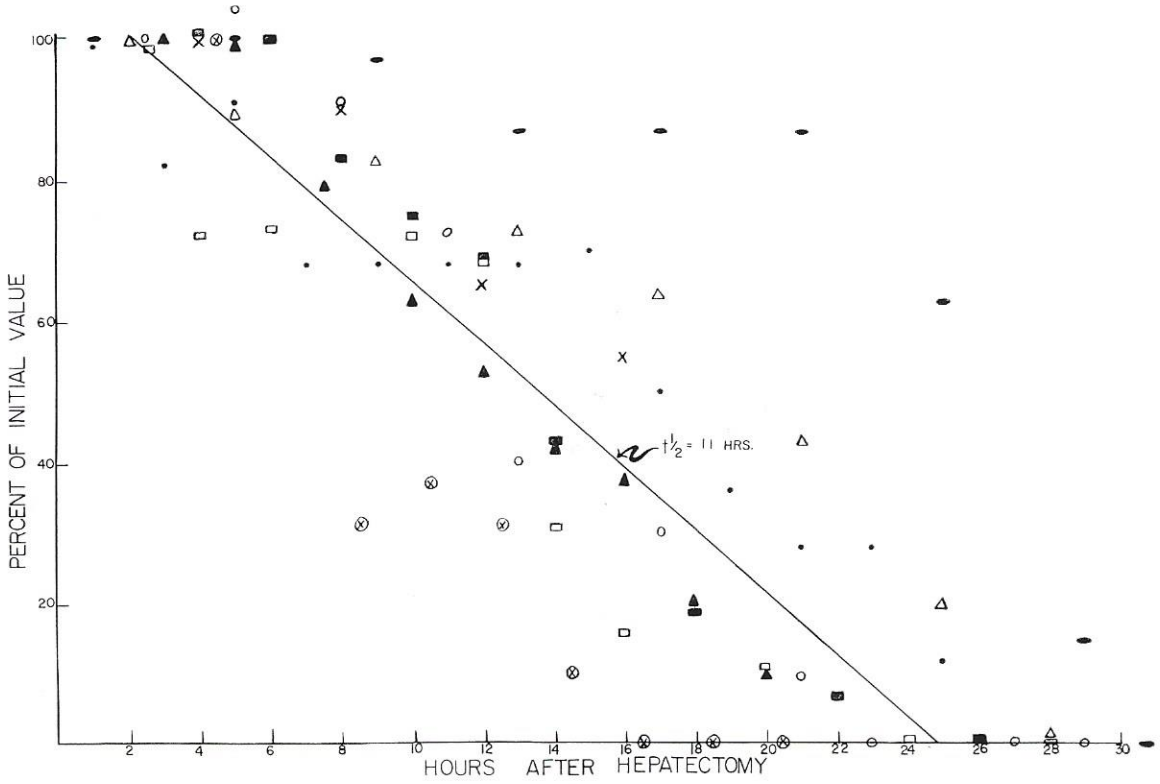

FIG. 2. Fibrinogen concentrations after hepatectomy, expressed in per cent of initial—immediate postoperative—value. After hepatectomy, fibrinogen levels rapidly decline, its in vivo half-life value is reduced to 11 hours.

mercially prepared bovine thrombin and a 40 per cent urea in 0.02 normal sodium hydroxide. In those instances in which no fibrinogen could be demonstrated by this technique, ammonium sulfate precipitation was used, as previously outlined by Fowell, to confirm the presence, or absence, of this plasma protein.

Determination of fibrinolytic activity. Since the results of the euglobulin clot lysis technique were found to parallel closely the changes observed with the fibrin plate technique, we (13) chose to use the former to test for the presence of plasminogen activator activity. Two milligrams of bovine fibrinogen were added to the euglobulin solution prior to clotting along with 20 units of thrombin.

Determination of plasminogen concentration. Plasminogen levels were determined in dog plasma on euglobulin samples by the technique described by Norman and on plasma samples by a procedure described by Alkjaersig and her colleagues.

Plasmin activity was determined by a modification of the caseinolytic technique described by Remmert and Cohen. To 1 milliliter of enzyme solution was added 1 milliliter of 4.4 per cent lysine phosphate buffer—pH 7.4 and 2 milliliters of 4 per cent casein. After 30 minutes of incubation at 37 degrees C., reaction was terminated with 2 milliliters of 20 per cent trichloroacetic acid. The blank consisted of the same constituents; however, trichloroacetic acid was added before the casein. After precipitation, the substance was separated by filtration. The crystal-clear filtrate was read in a D.U. spectrophotometer at 280 millimicrons. After correction for the optical density of the blank, the activity of the sample was expressed in micrograms of tyrosine released, a value obtained from a reference

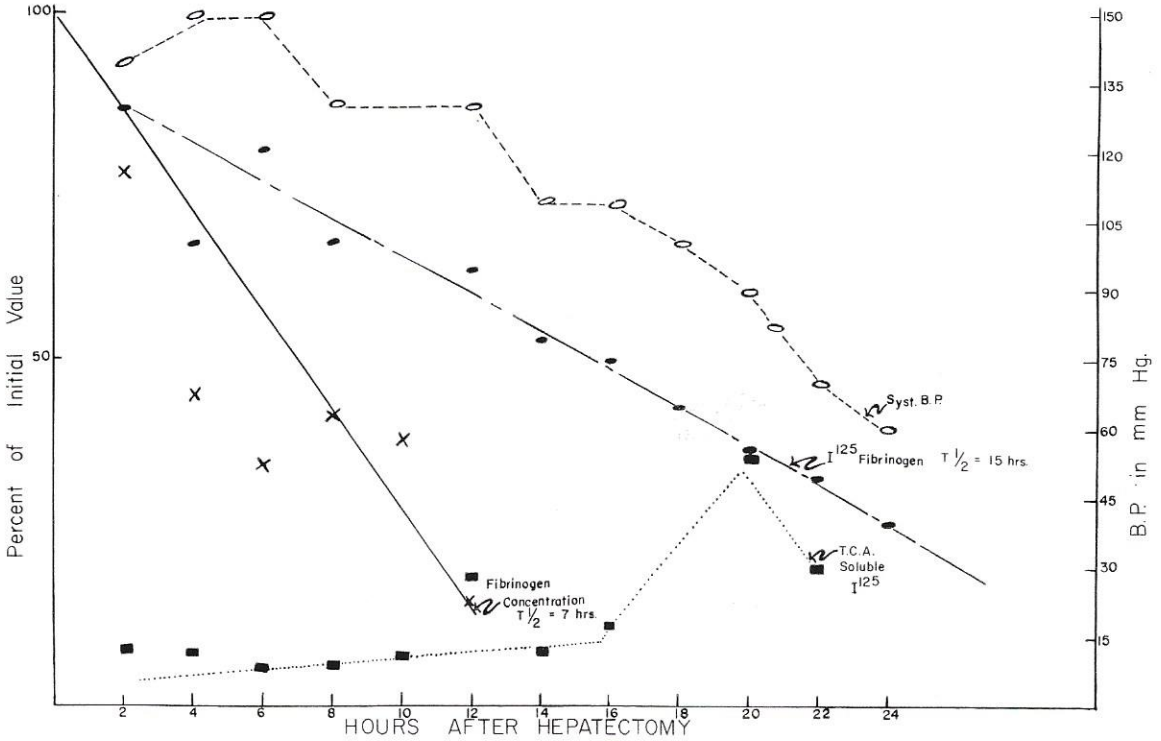

FIG. 3. Relationship of the decline in fibrinogen concentration to the changes in blood pressure. The onset of decline in fibrinogen precedes the drop in blood pressure by many hours. Comparison of half-life values between clottable and radioactive proteins: The half-life value of the clottable protein, $t\frac{1}{2}=7$ hours, is considerably shorter than of labeled fibrinogen, $t\frac{1}{2}=15$ hours. Prior to death, the major portion of the label is soluble in trichloroacetic acid, hence it is no longer protein bound.

curve expressing the relationship between the optical density at 280 millimicrons versus the concentration of L-tyrosine in micrograms per milliliter.

Determination of antiplasmin and antithrombin. Antiplasmin and antithrombin activity were determined as previously described (14).

Immunoelectrophoresis. Immunoelectrophoresis on 1 per cent agarose gel was carried out for one hour at 7 volts per centimeter at zero degree in Michaelis diethyl barbiturate buffer—pH 8.2. Rabbit antidog fibrinogen was allowed to diffuse at room temperature into the agarose, which was placed in a moist-closed chamber for 24 hours prior to washing, fixing, and staining of the precipitin arcs with ponceau red.

Determination of radioactivity. Radioactivity of 1 milliliter of blood, 1 milliliter serum, and 1 milliliter trichloroacetic acid soluble supernatant of plasma was determined in a sodium-iodide well crystal with a single channel spectrometer. Each sample was counted for a sufficient length of time to give a statistical counting error of 2 per cent, or less, and was corrected for background and decay, if necessary.

RESULTS

Control studies. Turnover of fibrinogen ^{125}I and of albumin ^{125}I in normal dogs was started two days after the intravenous injection of the labeled material. Blood samples were collected twice daily and processed as previously described.

Fibrinogen ^{125}I of dog and bovine origin has an in vivo half-life of two and a half days (Fig. 1). Bovine fibrinogen turnover rates become markedly accelerated four to

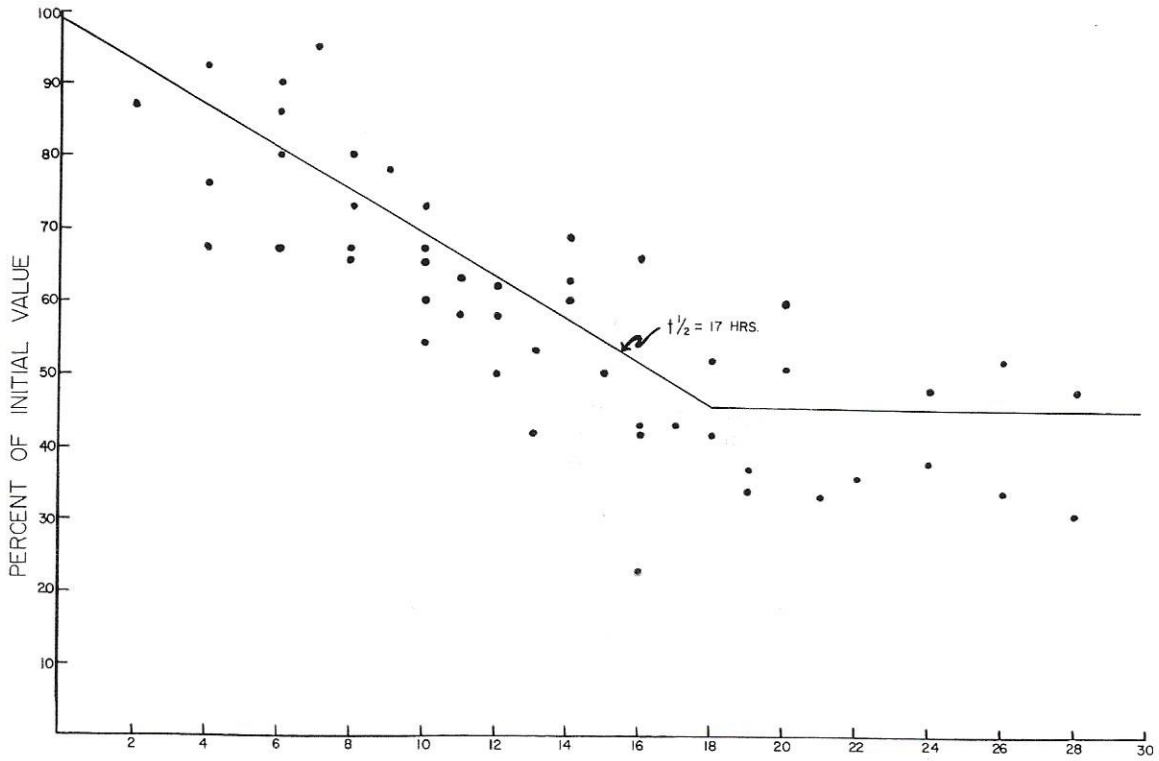

FIG. 4. Disappearance of radioactive fibrinogen after hepatectomy. Half-life equals 17 hours. Turnover suddenly ceases 18 hours after resection of the liver, at which time values level off at approximately 45 per cent of the initial radioactivity.

six days after injection, presumably due to the development of antibovine fibrinogen antibodies.

Posthepatectomy studies. Plasma fibrinogen levels decline after hepatectomy (Fig. 2). The onset of the decline precedes the first clinical evidence of shock by six to ten hours (Fig. 3). As a rule, this change coincides with the decline in stroke volume.

Plasma fibrinogen turnover rates after hepatectomy are greatly accelerated. Half-life values for the clottable protein are slightly shorter—11 hours—than for the radioactive fibrinogen—17 hours—(Fig. 4). Radio-fibrinogen turnover rather suddenly ceases approximately 18 hours after hepatectomy, when values level off at approximately 45 per cent of the initial fibrinogen value (Fig. 4). Most of this activity, however, no longer represents protein-bound radioactivity (Fig. 3).

One dose of heparin—0.5 to 1 milligram per kilogram—can prevent the decline in fibrinogen in these dogs for as long as eight hours (Fig. 5).

Blood proteolytic activity. Euglobulin clot lysis time values decline during operation. A few hours postoperatively, euglobulin clot lysis times are longer than those immediately after resection of the liver. The plasminogen activator activity observed initially after hepatectomy is quite labile. In the presence and the absence of its natural inhibitors, this plasminogen activator activity decays relatively rapidly (Fig. 6). In contrast, the plasminogen activator activity in the plasma obtained prior to the death of the animal is stable and does not change appreciably upon incubation at 37 degrees C. for 24 hours, or longer. Whether this finding indicates the release of different plasminogen activators presently is not known. Heparin therapy does not alter the plasminogen activator activity.

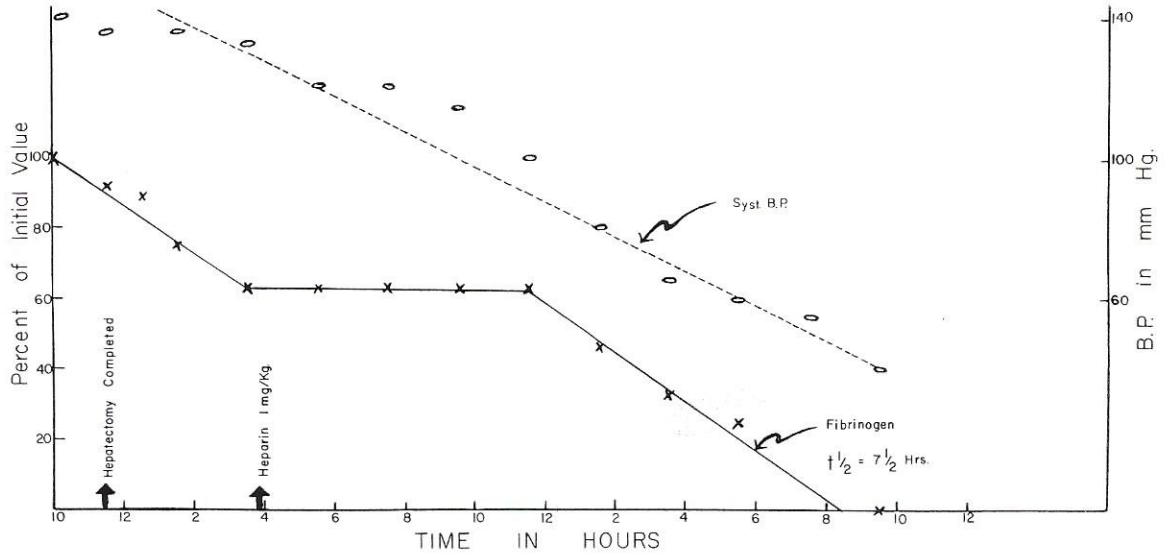

FIG. 5. Effect of heparin on fibrinogen turnover. A single dose of heparin, 1 milligram per kilogram, prevented the decline in fibrinogen for eight hours.

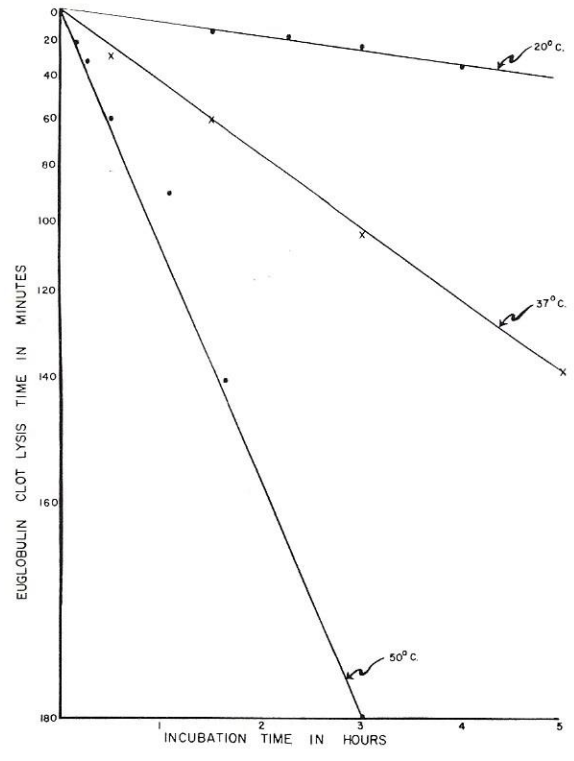

FIG. 6. Rate of in vitro decay of plasminogen activator activity after its incubation at different temperatures. Its slow decay at room temperature was the noteworthy feature of this study.

Plasminogen levels also decline. A regression curve prepared of the plasminogen values provides a posthepatectomy in vivo half-life value of 20 hours (Fig. 7).

After the disappearance of the clottable fibrinogen from the blood, fibrinogen occasionally still could be detected in plasma samples collected in epsilon aminocaproic acid. Those samples from which all coagulable proteins had disappeared by both techniques revealed the presence of at least two precipitin bands on immunoelectrophoresis against rabbit antidog fibrinogen, one of which coincided with dog fibrinogen (Fig. 8).

The effect of Trasylol® and Amicar® on the proteolytic activity of the euglobulin fraction was assessed in vitro. Trasylol® in concentrations of 1 unit per milliliter and higher completely inhibited the proteolytic activity. Similarly, epsilon aminocaproic acid inhibited this activity in vitro in concentrations that ranged from 4×10^{-4} to $2\text{-}\ 4\times10^{-3}$M (Fig. 9).

Hemodilution. Since a postoperative hemorrhagic diathesis develops which results in bleeding that is accentuated by frequent blood sampling without blood replacement, hemodilution occurs which is aggravated by

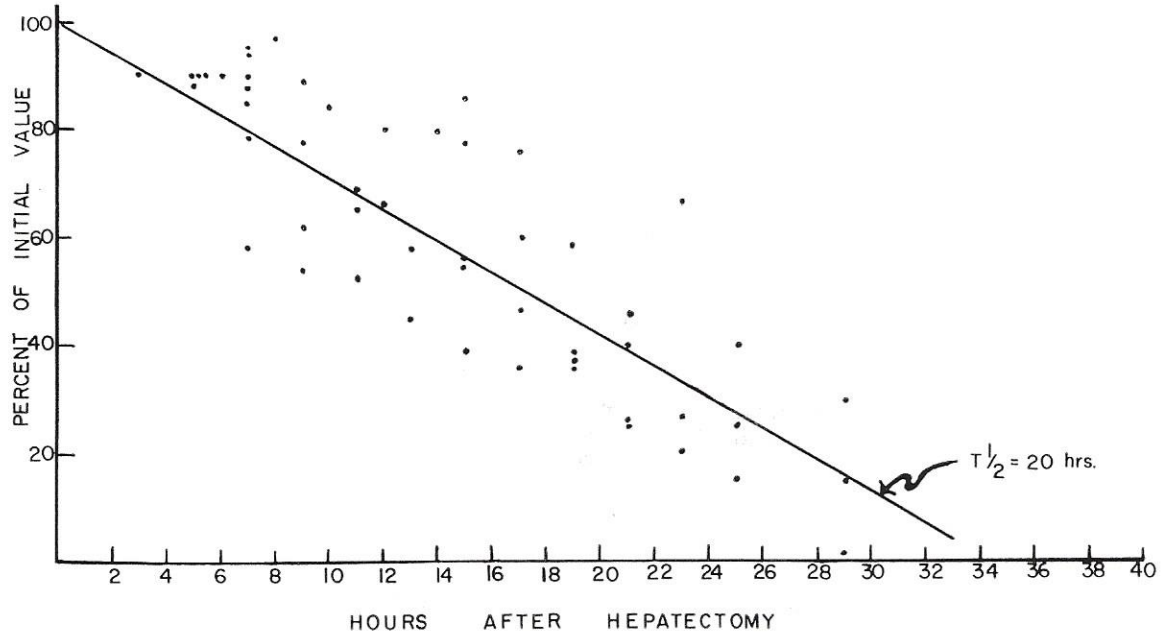

FIG. 7. Plasminogen levels after hepatectomy. Plasminogen concentration declines; its half-life value is 20 hours. This is only slightly longer than that of fibrinogen.

the continuous intravenous infusions of glucose, especially after the development of oliguria and anuria. The extent of the hemodilution and its effect on the turnover of proteins remained to be assessed. Hence, bovine serum albumin ^{125}I was administered to three dogs, two days prior to hepatectomy and its fate determined after hepatectomy. The regression curve for the data thus obtained reveals that the in vivo half-life of albumin which in the intact dog is six to eight days after hepatectomy is reduced to 50 hours (Fig. 10). With the albumin curve as a base line, half-life values for the in vivo turnover of fibrinogen, plasminogen, and fibrinogen ^{125}I were corrected to account for postoperative hemodilution. Corrected in vivo half-life values are fibrinogen, 14 hours; plasminogen, 32 hours; and labeled fibrinogen, 26 hours (Fig. 11).

Proteolysis and the kinin system. In view of the considerable proteolytic activity, it was found necessary to study the effect of bradykinin on blood pressure and pulse rate in the anhepatic dog. Synthetic bradykinin was injected in doses ranging from 0.005 to 0.02 milligram per kilogram intravenously. After hepatectomy, the observed changes, both in duration and magnitude, were not different from those observed in nonoperated control animals.

Is disseminated intravascular coagulation the cause of shock? Since the defibrination preceded the onset of shock by several hours, the question arose whether or not disseminated intravascular clotting causes shock in the anhepatic animal. In an effort to discern a possible cause or effect relationship, dogs were subjected to a thrombin-induced defibrination episode. Immediately after the removal of the liver, 2,500 units of bovine thrombin were added to 500 milliliters of 5 per cent dextrose in water which was allowed to enter the femoral vein at a rate of 4 milliliters per minute. After two hours, the animal was completely defibrinated. Shortly after beginning the thrombin infusion, the systolic pressure dropped to 90 to 95 millimeters of mercury, without an appreciable change in pulse rate. Fifteen minutes later, it had returned to the preinfusion level, where it remained for the next four to eight

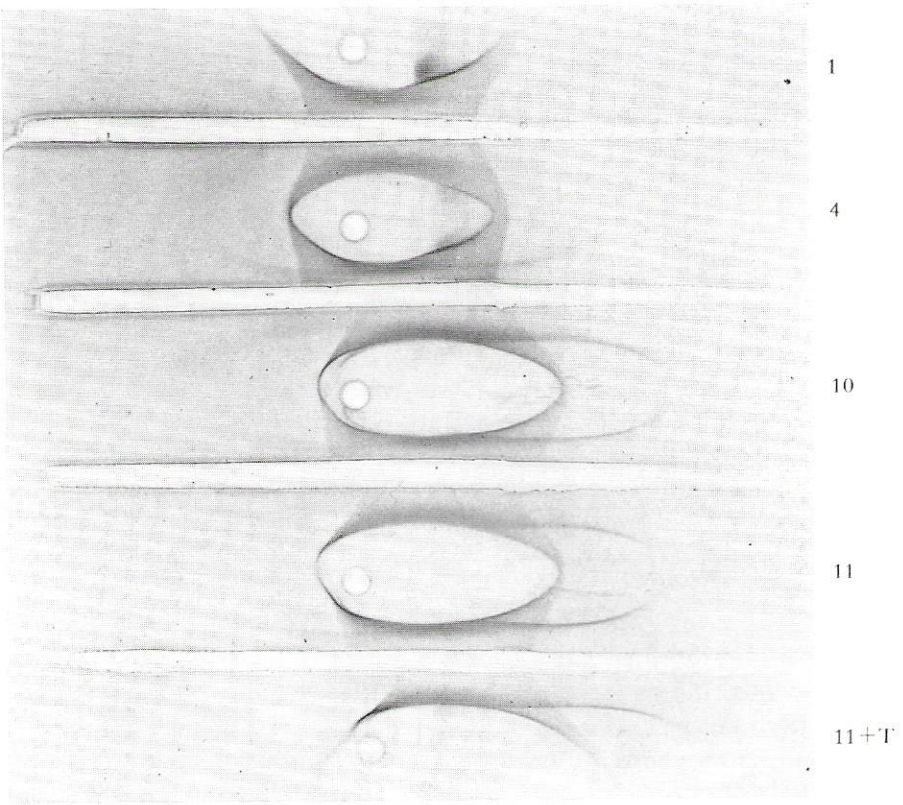

FIG. 8. Immunoelectrophoresis of dog plasma against rabbit antidog fibrinogen. After hepatectomy: Samples 1, 4, 10, and 11 were obtained zero, eight, 26, and 30 hours after hepatectomy. Sample 11+T was obtained 30 hours after hepatectomy. Thrombin was added and the serum was filtrated. Note the development of a new kationic component which shares an antigenic determinant with fibrinogen, while the original fibrinogen band shifts slightly and becomes longer.

hours. Then, the blood pressure declined in a manner similar to that observed and described previously.

DISCUSSION

Defibrination and an enhanced fibrinolytic activity were noted previously (13a) during hepatectomy. The relationship of defibrination to disseminated intravascular coagulation could be demonstrated and attributed to a failure of the liver to eliminate coagulative and proteolytic enzymes from the blood. Changes in hepatic blood flow accompanying the manipulations of the organ during its resection presumably are sufficiently severe to account for these changes in the clearance function. The defibrination and enhanced proteolytic activity continues in the postoperative period. It, again, results from intravascular coagulation, since heparin treatment prevents its progression. It becomes more pronounced in time. Since the hepatic clearance function at this time is absent, it is not altogether an unexpected finding. Disseminated intravascular clotting is neither the cause of bradycardia or shock, as results of the thrombin infusion studies would suggest, nor the effect of shock, since defibrination precedes the development of shock by several hours. Instead, the likelihood that one and the same agent is responsible for the observed abnormalities, which in the absence of the liver is either no longer eliminated or no longer produced, should be considered. Irrespective of the cause, however, the first changes that are

noticeable are a decline in stroke volume and an incipient bradycardia (21). Simultaneously, fibrinogen values decline. The latter occurs much faster than expected, as a result of the normal decay of this protein. In the present experiments in the dog, in vivo half-life values of six days were found for fibrinogen. After hepatectomy, the in vivo half-life of fibrinogen is reduced to 11 hours. When corrected for hemodilution, the value is 17 hours. Therefore, fibrinogen turnover is greatly accelerated. The enhanced turnover rate suggests that after resection of the liver a defibrination syndrome develops, thus confirming the previous observations of Gans, Rutherford, and Bergentz and their associates. This event is delayed greatly by heparin. Hence, disseminated intravascular coagulation appears to be the cause of the accelerated fibrinogen turnover rate.

The effect of heparin is prolonged excessively; one dose of heparin—1 milligram per kilogram—delays the further decline of fibrinogen for more than eight hours. In intact animals, the in vivo half-life value of heparin has been estimated at slightly longer than an hour. Heparin requires a protein-heparin cofactor for its activity. Presumably, heparin cofactor is identical with antithrombin. After hepatectomy, the antithrombin activity of plasma rapidly declines. Whether the decline in antithrombin is responsible for prolonged action or whether the prolonged half-life of heparin in the dog which has undergone hepatectomy suggests that the liver possibly plays a significant role in heparin degradation remains to be established. It should be noted that Jacques (16) previously showed that heparin is inactivated by rabbit liver extracts and suggested that it might be the result of an enzyme termed heparinase. Subsequently, he and his associate (17) demonstrated its presence in livers in other animals as well and also in kidney extracts. Whether heparinase really exists or merely represents the binding of heparin to proteins presently is not really clear.

Heparin therapy failed to affect the fibrinolytic activity observed in these animals. Therefore, the enhanced blood proteolytic activity occurs independently from the disseminated intravascular coagulation. Previously, Drapanas and Rutherford and their co-workers were unable to detect an increased proteolytic activity of the blood after total hepatectomy. As in a previous study (12), we found consistent changes of the euglobulin clot lysis time and plasminogen concentration, compatible with an activation of the fibrinolytic enzyme system. Euglobulin clot lysis times progressively shorten; antiplasmin levels, both slow and fast, rapidly decline.

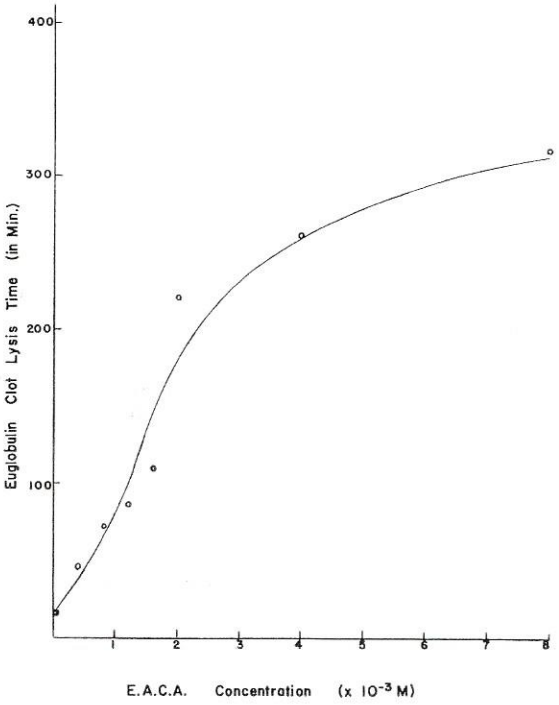

FIG. 9. Effect of aminocaproic acid on euglobulin clot lysis time, *E.A.C.A.*, in concentrations ranging from 3×10^{-4} to 3×10^{-3}M progressively inhibit plasminogen activator activity, at 37 degrees C.

The possibility that the gradual decline in plasminogen and antiplasmin levels represents their normal turnover for the anhepatic animal was considered, assuming then that these proteins are produced by the liver. If this should be a valid assumption for antiplasmin, extrahepatic sources of plasminogen production have been described by

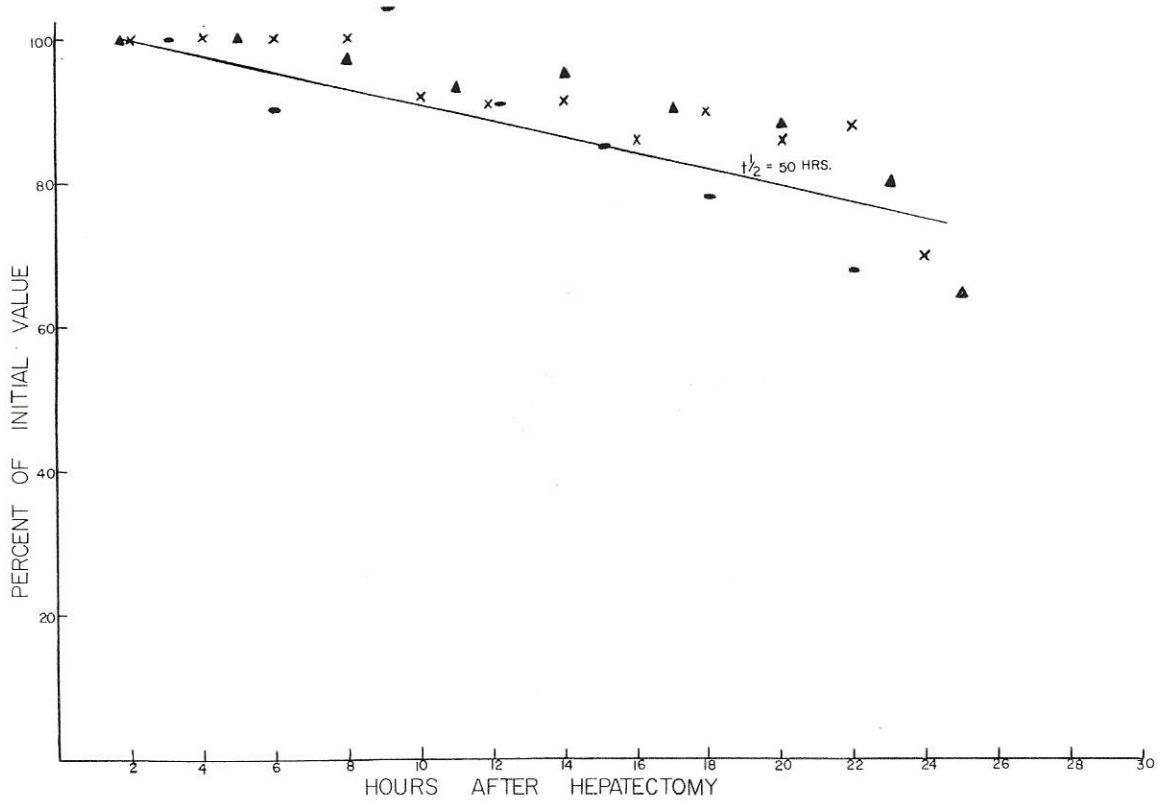

FIG. 10. Fate of bovine serum albumin ^{125}I after hepatectomy. The turnover of bovine serum albumin ^{125}I in intact dogs is slow—half-life, six to nine days—in dogs after hepatectomy half-life equals 50 hours. The accelerated turnover rate observed after hepatectomy is the result of hemodilution, which occurs secondary to hemorrhage and blood sampling, to the continuous intravenous infusion of glucose, to the development of oliguria, and possibly to other factors.

Barnhart and Riddle. Other evidence suggests, however, that after hepatectomy the turnover of these proteins is greatly enhanced. First, the euglobulin clot lysis time is markedly shortened, an indication of the presence of a large quantity of plasminogen activator, capable of activating plasminogen. Second, the absence of clottable fibrinogen in plasma collected in sodium ethylenediaminetetra-acetate and its presence in plasma collected in aminocaproic acid would indicate that proteolysis continues in the test tube to account for the observed discrepancy. Aminocaproic acid, which inhibits fibrinolysis and, thus, the destruction of fibrinogen in the plasma or of fibrin during fibrinogen determination, prevents these changes. This finding suggests that at this time the plasminogen activator activity persists in vitro for longer periods of time and, hence, that this plasminogen activator is more hardy than the materials previously described by Fletcher and his colleagues. We were able indeed to show the rapid decay of activator activity observed shortly after completion of the operation. Its persistence in an unaltered form before, and at the time of, death tends to confirm this impression. Finally, the evolution of increasing amounts of fibrinogen breakdown products could be demonstrated. These materials interfere with hemostasis and with fibrin formation.

The development of these breakdown products and their delayed excretion by the kidneys, especially in the preterminal stages, probably accounts for the difference in turnover rates between the clottable and the

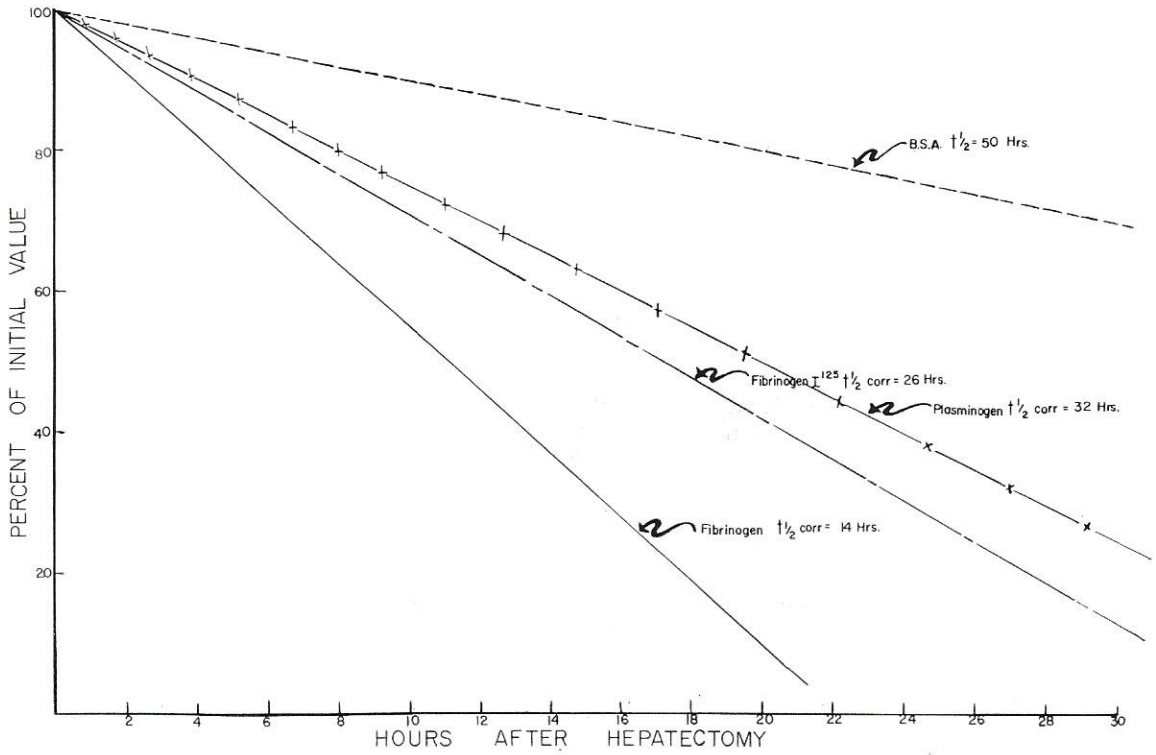

FIG. 11. Half-life values for fibrinogen—clottable and radioactive—and plasminogen, corrected for postoperative hemodilution. The corrected half-life for clottable fibrinogen is 14 hours, for radio-active fibrinogen 26 hours, and for plasminogen 32 hours.

labeled fibrinogens. Part of the label remains in the plasma attached to the fibrinogen split products. These accumulate as renal function deteriorates. On immunoelectrophoresis of plasma samples that no longer contain clottable proteins, the evolution of substances that share antigenic determinants with dog fibrinogen which normally are absent in dog serum, could be demonstrated. The noted changes were similar to those previously described by Nussenzweig and Seligmann as a result of the effect of plasmin on human fibrinogen. Fibrinogen breakdown products, reflecting the partial and total proteolysis of fibrinogen, thus were visualized.

These findings suggest that the proteolytic activity of the blood is sufficiently severe to cause breakdown of fibrin and possibly also of fibrinogen. In some instances, the proteolytic activity was found to proceed in vivo to completion with formation of proteolysis products of the D and E type. The defibrination and the development of proteolysis products which exert anticoagulant and antiplatelet-aggregating activities inevitably precipitate an enhanced bleeding tendency. The longer the anhepatic state is allowed to persist, the more severe the observed changes. One way to prevent their development during liver transplantation is to reduce the time during which the recipient is without a liver.

Chronic liver failure in man was found to be associated with slow defibrination, secondary to a disseminated intravascular coagulation, according to Beaumont, Thomas, Letterquist and their associates as well as Johansson. Results of the present study suggest that such a process also takes place during acute liver failure, even though markedly accelerated, with manifestations that are so magnified as to become life-threatening.

SUMMARY

During the anhepatic state, defibrination of blood is observed. Its onset coincides with the decrease in stroke volume and pulse rate. It results from disseminated intravascular coagulation because it is prevented by heparin. It could be established that the defibrination is neither the cause nor the effect of the ensuing shock.

This syndrome is accompanied by enhanced proteolytic activity of the blood. This is already present during operation, but it declines slightly after hepatectomy, despite the absence of the liver. Then, the activity expands progressively, while some of its characteristics, particularly its in vitro stability at 37 degrees C. undergo changes. The effect of the proteolysis on the coagulation mechanism appears to be quite significant; the enzyme digests fibrinogen and fibrin, both in vitro as well as in vivo. This is associated with the elaboration of fibrinogen breakdown products. Heparin does not affect the proteolytic activity. The synthetic antiplasmins, however, completely reverse its effect.

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