Prevention of "Irreversible" Hemorrhagic Shock with Fibrinolysin *

COL. ROBERT M. HARDAWAY, MC, USA,** CAPT. DENNIS C. DRAKE, MC, USAF†

From the Division of Clinical Surgery, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington 12, D. C.

IN A PREVIOUS PAPER [5] evidence was presented that acute hemorrhage caused a shortening of the clotting time of blood in the dog. This hypercoagulability, together with a stagnant capillary circulation (as a consequence of low cardiac output, marked peripheral vasoconstriction, and opening of visceral arteriovenous shunts) apparently resulted in clotting in visceral capillaries of the bowel mucosa, liver, kidney, pancreas and lungs.‡ Probably as a result of this episode of intravascular clotting, endogenous heparin was activated producing incoagulability of the blood. This was considered to be a protective response made by the body to halt intravascular coagulation. It was postulated that if capillary thrombi were numerous and persistent enough they produced local tissue necrosis in liver, kidney, bowel mucosa and other organs. Even though the thrombi were subsequently dissolved and washed away, the tissue necrosis produced resulted in irreversible changes and death a few hours later.

It was shown that significant protection against this intravascular clotting could be provided if heparin were given before the hemorrhage.[5] Heparin given after the hemorrhage proved ineffective. In order for an agent to be of value clinically it must be effective if given after hemorrhage. Since fibrinolysin is known to dissolve fibrin it was postulated that proper doses of fibrinolysin given after hemorrhage should prevent irreversibility. This hypothesis was tested by the following experiment.

Materials and Methods

Experimental Model. Adult mongrel dogs in the weight range of 25 to 35 pounds and of either sex were randomized as to treatment groups and simultaneously subjected to our standardized bleeding procedure. This procedure now consists of a modification of the procedure previously described.[5] Significant among the changes in technic is the conversion from systolic to mean pressure in measuring control blood pressure levels. Also, the system of blood handling has been converted to a completely closed, completely plastic, and completely filtered system in order to insure sterility, minimize surface activation of clotting factors, and omit the possibility of small clot re-infusion. A step-by-step description of our hemorrhage technic in the control animal seems justified in view of our recent changes and particularly because this method had not been described

* Submitted for publication March 19, 1962.

** Director, Division of Clinical Surgery, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington 12, D. C.

† Resident in Anesthesiology, Walter Reed General Hospital, Walter Reed Army Medical Center, Washington 12, D. C.

‡ "Subsequent work, to be published, indicates that while hemorrhage and capillary stasis makes intravascular coagulation imminent, it does not actually occur unless blood is retransfused into the animal. Retransfusion of this blood into normotensive animals is harmless, but in hemorrhagic shocked animals, is toxic, producing results described in this and previous papers. It is possible that at least some of the "irreversibility" of irreversible hemorrhagic shock in dogs is due to retransfusion."

by other workers in the literature. As will be apparent, we believe this method is important because it enables us to obtain data on blood coagulation factors which cannot be obtained with preparations involving a reservoir and anticoagulation.

Adult mongrel dogs were fasted overnight but allowed to drink water up to the time of the experiment. Thirty minutes after an intramuscular injection of morphine sulfate (1.0 mg./lb.) the groins were shaved and washed with pHisoHex. Three animals were secured to an operating table, one for each of two treatment groups and a simultaneous control. With 1.0 per cent procaine skin infiltration for anesthesia, both common femoral arteries were exposed utilizing sterile technic. A 13-gauge polyethylene catheter was inserted through one femoral artery a measured distance into the abdominal aorta just above the bifurcation. This was used for the hemorrhage. A 15-gauge polyethylene catheter was inserted on the other side a measured distance to 3.0 cm. above the end of the larger catheter in the aorta. This was utilized for the continuous monitoring of central aortic blood pressure recorded on a Sanborn (Series 150) 4-channel recorder with Sanborn (267-B) strain gauges.

After initial pressure recording a prehemorrhage blood sample was drawn. The animal was then bled over a timed 15-minute interval to a mean blood pressure of 30 mm. Hg. The blood was collected in a sterile, sealed plastic bag after having been decalcified by passage through a resin-exchange column (Fenwal Laboratories-Ion Exchange Blood-Pack Unit, Code No. JB-2) to prevent clotting during temporary storage. A second sample was drawn at mean pressure 30 mm. Hg. The animal was then retransfused through a filter with the decalcified blood in an amount sufficient (30–60 ml.) to bring the pressure to 50 mm. Hg. mean. This pressure was maintained for 2½ hours by adding small increments of the decalcified blood or by removing small increments which were then heparinized for storage. The resin column could not be re-used for this purpose after the initial bleed. This somewhat tedious system of maintaining a constant mean pressure was necessary in order to avoid anticoagulation of the animal during the hypotensive period. None of the blood that was heparinized gained access to the circulation until the retransfusion period 2½ hours after a mean arterial pressure of 50 mm. Hg. was attained. All the resin decalcified blood was then retransfused, and only then was the remaining blood stored by heparinization also retransfused. Final pressures were recorded for at least 15 minutes and the wounds were closed. The animal was observed for a minimum of seven days if it survived that long.

Treatment Groups. With the above preparation as the control, two different dogs representing two different treatment groups were bled at the same time under identical conditions except for the treatment factor. In the heparin treatment group (Group A) the dogs received heparin sodium, 8.0 mg./Kg. intra-arterially, after the first blood sample and 10 minutes before the onset of hemorrhage. Previous experiments had shown that for heparin to be effective it must be given prior to the hemorrhage. In the fibrinolysin treatment group (Group B) the dogs received human fibrinolysin activated with streptokinase (Merck Sharp & Dohme "Thrombolysin")* 4,000 MSD u./Kg. in 25 25 ml. saline immediately following the initial hemorrhage and second blood sample. Decalcified blood was then transfused in amount sufficient (about 30 ml.) to stablize the blood pressure at 50 mm. Hg. mean and the remainder of the experiment was managed as in the control.

* Thrombolysin supplied through the courtesy of Merck Institute for Therapeutic Research, West Point, Penn.

Laboratory Methods. A 2-tube modified Lee and White clotting time was performed every 30 minutes in freshly siliconized glassware. Plasma fibrinogens were measured colorimetrically.[13] A one-stage method employing Simplastin (Warner Chilcott) was employed for prothrombin times. Factor V was determined according to the method of Stefanini.[24] Beef plasma filtered through 20 per cent asbestos in a Seitz filter was utilized for factor VII determinations.[20] Hematocrits were done by a micro-method. Blood volumes were determined with I^{131} tagged albumin.

Results

Survival. Animals were classified according to survival 48 hours following hemorrhage. This end point was selected on the basis of experience with over 700 dogs. Animals which survive a shock procedure for 48 hours, with few exceptions, survive indefinitely. The majority are observed over one month, and many have been reused by another investigator for different purposes. For this series a minimum of seven days observation was required, although most were observed longer. The animals which did not survive the hemorrhage died, in the majority of cases, within 24 hours. Two controls considered survivors did not fit this arbitrary definition, one dying at four days and another at 21 days. Pneumonia was a consideration in both. Within the limits imposed by our definition of survival, we believe we can say with statistical validity that both heparin and fibrinolysin offer significant protection against the lethal effects of acute hemorrhage in the dog. The figures are as follows: In Group A, the heparin treated series, we had a total of 18 paired experiments with 13 (72%) deaths and five (28%) survivors in the control animals and five (28%) deaths and 13 (72%) survivors in the heparin protected animals (Fig. 1). In Group B, the fibrinolysin

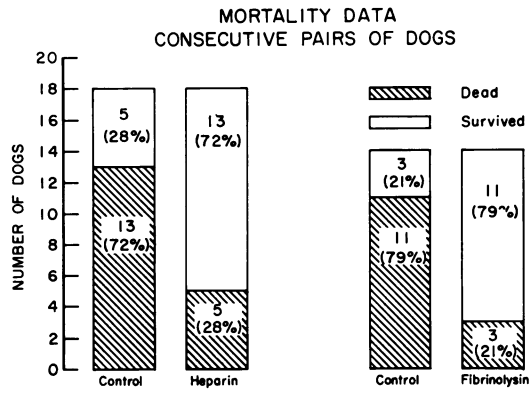

FIG. 1. Mortality data of the heparin series (left) and fibrinolysin series (right). Actual number of dogs and percentages are indicated. Each experimental animal and its control were done on the same table at the same time.

treated series, only 14 pairs were necessary to give statistical significance at the 5.0 per cent confidence level for at least a 50 per cent advantage. Eleven (79%) of the control animals died and three (21%) survived; three (21%) of the Thrombolysin treated animals died and 11 (79%) survived.

At the suggestion of statisticians,** we employed the method of sequential sampling [1] in order to have a running day-to-day chart of our approach toward statistical significance in the difference we were trying to demonstrate between our control and treater groups. The advantage of this method is considerable when using large and expensive laboratory animals, since it enables one to determine the moment the differences become significant or not significant within predefined limits. The experiment can therefore be terminated at the appropriate moment with a maximum economy of animals and effort. Such a chart is reproduced here in Figure 2 for the heparin-control comparison and in Figure 3 for the fibrinolysin-control comparison. The boundary lines as calculated on these charts are valid as long as the mor-

** Mr. E. L. Atwood and Major G. Stelter, Dept. of Epidemiology, WRAIR.

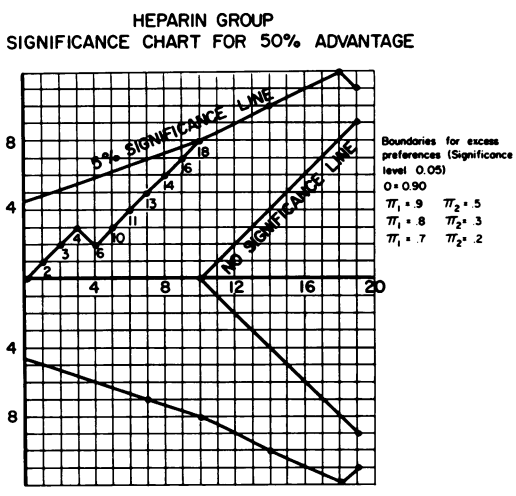

FIG. 2. Statistical treatment of the survival of the heparin group of dogs. A line is started on the left at the heavy horizontal line. If there is a difference in the outcome between the heparinized dog and his paired control, a line is drawn one square to the right and either one square up or down. If the line thus produced crosses the 5.0% significance line, the differences are statistically significant at the 5.0% level (see text). The numbers on the zigzag line refer to numbers of pairs of dogs necessary to achieve this point. Thus 18 pairs of dogs were used in this series.

tality in the control group is between 70 and 90 per cent. By arbitrary decision, the graph is set up to show significance or no significance for paired experiments showing at least a 50 per cent advantage in the treated group. Instructions for use of the graph are as follows: Statistics on survival are recorded in the paired experiment with an animal receiving treatment and one animal serving as control. There are four possible outcomes:

Outcome No.	Control	Treated
1	Death	Death
2	Survival	Survival
3	Death	Survival
4	Survival	Death

The results of Outcomes 1 and 2 in which the results in the two animals are the same, are not graphed. The outcomes in which the two members of the pair are different, are used on the graph. Survival is taken as the preference. If the treated member of the pair survives, then a diagonal line is made starting at zero and moving upward and to the right one-unit square. If the control member of the pair survives, a diagonal line is made moving downward and to the right starting at the point at which the last line terminated. If the zigzag line which results crosses either the upper or lower line, then the experiment is terminated with a significant result. (P = approx. 0.05). If the zigzag line crosses the V-shaped line at the right of the graph, the experiment is terminated with nonsignificant results. Although the chart shows a theoretical minimum of seven paired experiments required to demonstrate a 50 per cent advantage with 5.0 per cent confidence, we needed 18 pairs in Group A and 14 pairs in Group B. The numbers of pairs involved are indicated with the plots of results.

Clotting Time in Siliconized Glass. The changes in the clotting time following acute hemorrhage in the dog have been described previously.[5] The changes in coagulation as measured by this very nonspecific test have followed a similar pattern in our

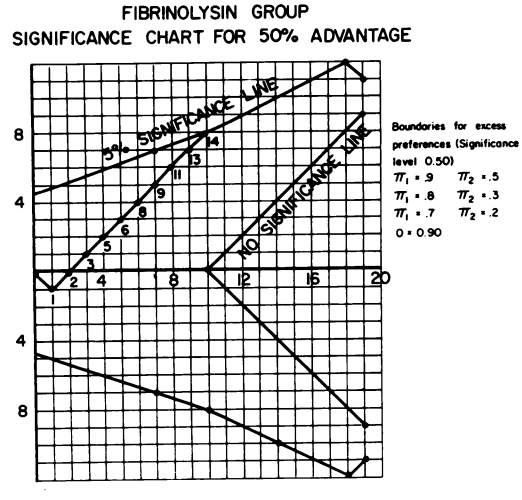

FIG. 3. Statistical treatment of the survival of the fibrinolysin group. See Figure 3 and text for discussion. In this case 14 pairs of dogs were required to achieve 5.0% significance.

present series. Immediately following a blood loss which approximates 50 ml. Kg., or around 50 per cent of the total blood volume in our procedure, the clotting time is greatly shortened. In almost every case the shortest clotting time is less than half the initial clotting time, and in many cases the changes are dramatic, with an initial clotting time of 20 minutes dropping to four minutes (Fig. 4). At the present time we have only speculation concerning the mechanisms responsible for this change, but it may be related to epinephrine or norepinephrine.[10] We hope to be able to present additional data in the future. Speaking teleologically, this increased coagulability of the blood might be considered a defense mechanism for controlling further hemorrhage. This state of relative hypercoagulability persists with moderate variation throughout the period of controlled hypotension. However, a second dramatic change in coagulability occurs in the dogs that go on to die within the next 48 hours. A marked prolongation of clotting time occurs, and dogs which may have had a clotting time of five minutes at 90 minutes of hypotension, develop clotting times in excess of 60 minutes at 120 minutes of hypotension. There is, of course, a range of variation, but if we draw an arbitrary line across our graph of clotting times at 35 minutes (1½ to 2 times the normal control level) and call all clotting times beyond 35 minutes abnormally prolonged, we find that there is a 100 per cent correlation between the occurrence of an abnormally prolonged clotting time in the latter half of the experiment and death of the animal (Fig. 4).

The animals which did not show this sudden hypocoagulability phenomenon survived the experiment in every case. The probability of such a high correlation due to chance is less than .001 in the case of those dogs which died and less than .05 in the case of the survivors. Again, with teleological thinking, one might consider this hypocoagulability a desperate but ineffective defense mechanism against intravascular clotting in vital organs. As pertains to this phenomenon we have better though not final information about the mechanism behind the hypocoagulability. With a protamine titration the clotting time can often be restored to normal levels. Also if plasma from a hypocoagulable dog is added to whole blood from a normal dog, the clotting time of this mixture will become greatly prolonged in most instances. We have interpreted these phenomena to represent the release of a heparin-like anticoagulant with its probable source in the liver. However, the circumstance also occurs that the blood from the normal dog will cause the blood from the hypocoagulable hemorrhaged dog to clot with a normal time.

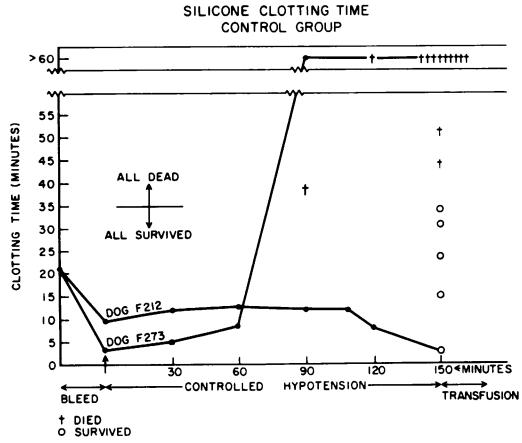

FIG. 4. Silicone clotting times in control group of dogs. Clotting times were taken before hemorrhage, at the end of hemorrhage and each 30 minutes during the 2½ hour shock period. The last clotting time was taken just before retransfusion of blood. A small retransfusion of decalcified blood (about 30 ml.) was given at the arrow at the beginning of the controlled hypotension period in order to bring a mean aortic blood pressure up to 55 mm Hg. Only terminal clotting times are plotted on all dogs. However, complete plots are given for two representative dogs. Dog F212 is representative of a minority group (5) of the controls which survived. All of these surviving dogs had terminal clotting times below 35 minutes. Dog F273 is representative of the majority group (13) which died. Note that all terminal clotting times on this group were above 35 minutes.

This may be due to a depletion of clotting factors in the shocked dogs' plasma due to their being used up in an intravascular clotting episode.

Though we have accumulated a large quantity of data on changes in prothrombin time, circulating levels of fibrinogen, Factors V and VII, we are not reporting these at the present time because we need further experimental information to take our interpretations out of the realm of speculation. An accurate evaluation of blood volume and fluid shifts is pertinent in this regard, and this type of information in our experimental model is being collected.

Discussion

Fibrinolysis has long been known to be associated with shock. It has been documented in burn, hemorrhagic and traumatic shock,[8, 26] toxemia of pregnancy,[23] endotoxin shock [7, 25] and death.[29] Blood transfusion reaction,[8] cardiopulmonary bypass, malignant disease,[2] multiple transfusion pyrogens [27] and other conditions have been productive of a bleeding tendency due all or in part to fibrinolysis. A substance so often found in association with such bad company must be bad, guilty by association if by no other means. *Cum hoc ergo propter hoc.* It has been correctly blamed for episodes of hemorrhage in various clinical situations. Carcinoma of the prostate has been accompanied by fibrinolysin activation which resulted in near fatal hemorrhage.[3] Life was only saved by the use of Epsilon-aminocaproic acid.[3] This chemical has also been hailed as the protector of dogs from endotoxin shock [25] due to its remarkable and unique ability to prevent the activation of endogenous fibrinolysin. The mechanism of this protection is explained through the activation of histamine by fibrinolysin which was in turn activated by endotoxin. The prevention of fibrinolysin activation prevented activation of histamine. The activation of histamine by endotoxin has only recently been suggested as the mechanism of action of endotoxin [22] reviving an old theory of shock. The validity of this idea remains to be determined and a good deal of work is now going on studying the possible therapeutic effect of Epsilon-aminocaproic acid in shock.

Fibrinolysin has also been assigned the role of destroyer of fibrinogen and responsible for various hypofibrinogenemic and afibrinogenemic states. This mechanism of development of afibrinogenemia was postulated by Moloney [19] and has many backers. However, McKay [16] denies that fibrinolysin will attack fibrinogen. It will attack its breakdown components which result from the action of thrombin. Certainly dramatic drops in fibrinogen take place rapidly without the presence of fibrinolysin,[21] and the blood level of fibrinogen can rise in the face of the activation of marked amounts of fibrinolysin.[6] It seems likely that rapid drops in fibrinogen levels must at least be initiated by an episode of intravascular coagulation rather than by fibrinolysin. Even assuming this to be true, fibrinolysin is still on the defense in its role in shock.

However, fibrinolysin is not without its defenders. It may be that fibrinolysin is present as a fireman attempting to put out the fire rather than as an arsonist who started it. Lasch *et al.*[15] working with a limited number of cats were able to protect four out of five from otherwise irreversible hemorrhagic shock with fibrinolysin whereas five controls died. Epsilon-aminocaproic acid given with the fibrinolysin seemed to be determental rather than beneficial.

Recent experiments by us gave evidence that both endotoxin and hemorrhage resulted in an episode of intravascular coagulation, most prominant in visceral capillaries.[5, 7] It was postulated that these clots interferred with the capillary perfusion of the liver, kidney, gastro-intestinal mucosa and other organs enough to produce focal

and local tissue necrosis. These thrombi are apparently largely washed out after a period of time [4, 6, 9, 11, 17, 18] synchronous with the appearance of endogenous heparin and fibrinolysin in the blood stream. However, this washing out and disappearance of the thrombi frequently came too late to avoid focal necrosis of tissue and the animal later succumbed because of liver, kidney or mucosal damage. If this theory is true, substances which 1) prevent coagulation should prevent irreversibility of shock; and possibly 2) substances which destroy thrombi might be effective even after thrombosis has occurred. The first possibility was tested using exogenous heparin in both past [5] and again in present experiments and found to protect significantly against irreversible hemorrhagic shock.

The second possibility, that of the therapeutic action of fibrinolysin, was tested in the present experiments. A significant protection was shown even when the fibrinolysin was given after the hemorrhage. Previous experiments had shown that heparin was not effective at this time but must be given before the hemorrhage.

The dosage of fibrinolysin given resulted in increasing the fall in fibrinogen over the fall seen without fibrinolysin. The exact mechanism of action of fibrinolysin is unknown and will be the subject of a future paper.[12] It is thought likely that the fibrinolysin is not acting on the fibrinogen directly but on its breakdown products (including fibrin) which were produced as the result of the initiation of intravascular coagulation. Anyway, because of one or the other of these actions, the fibrinogen level did fall. This fact indicates two possible mechanisms of action of fibrinolysin in protecting against irreversible hemorrhagic shock:

1. Thrombi when formed are quickly dissolved.

2. Fibrinogen was so reduced that not enough was left in the circulating blood to form good clots.

The former possibility would seem to be much more important therapeutically. The mode of action of fibrinolysin and its possible time of administration will be the subject of future work in this laboratory.

Autogenous heparin appears late in the shock phase (possibly as a protective mechanism) and causes a hypocoagulability of blood up to several hours.[5] Evidence that this anticoagulant is heparin includes its neutralization by protamine and its lipid clearing action.[5] It is postulated that the stimulation for activation of this endogenous heparin is the appearance of fibrin in the blood. In both the present and past experiments [5] the appearance of endogenous heparin and hypocoagulability was correlated with subsequent death with remarkable consistency. The appearance of hypocoagulable blood thus seemed to mark the onset of irreversibility which is the onset of an episode of intravascular coagulation. This hypocagulability is probably the only clinical or laboratory test which will predict irreversible shock. The finding of autogenous heparin and hypocoagulability in shock has also been reported in anaphylactic shock in dogs [14] by Jaques and Waters. These workers utilized an elaborate extraction procedure which they applied both to blood and to the liver. The crystalline anticoagulant substance obtained had properties similar to commercial heparin and their evidence pointed to the liver as the source. The shocked liver was low in heparin content, and liver masts cells were depleted. Hepatectomized dogs showed no heparin in anaphylaxis.

The finding of autogenous heparin and incoagulability has recently been correlated with a fatal outcome in humans.[28] We are not prepared to make a definite statement at this time about the effect of quantitative depletion of clotting factors in contributing to the prolongation of the clotting time but the few examples we have observed in which normal dog blood causes the hypocoagulable plasma to clot in a shorter time

suggests that a deficiency of clotting factors (due to their being used up in the intravascular clotting episode) may contribute to some of the gross increases in coagulation time.

A word might be said about the difference in the present experimental method and that used in former experiments.[5] Formerly the systolic pressure was used to monitor the aortic pressure as it was a clinical determination. The mean pressure in those experiments was about 60 mm. Hg. It was decided that the mean pressure more truly reflected the effective pressure so it was used in the present experiments. In addition, in order to insure against contamination, the technic was revised to use a closed system for the blood consisting entirely of plastic tubes, plastic syringes and plastic bags instead of glass syringes and open beakers. When this was done it found that the mean pressure must be reduced to 50 mm. Hg and the shock period extended from two hours to 2½ hours in order to cause a high mortality in the control animals. The reason for this is unknown but may be related to surface activation of clotting factors (such as the Hageman factor) by the use of glass.

Summary and Conclusions

1. It is possible to protect significantly against irreversible hemorrhagic shock in dogs by the administration of fibrinolysin after the hemorrhage.

2. Previous work showing protection against irreversible hemorrhagic shock by heparin administered before hemorrhage was confirmed.

3. This is additional evidence for the theory that intravascular coagulation occurs in hemorrhagic shock which is treated by return of blood.

4. It is possible to predict a fatal outcome for hemorrhagic shock in the dog by the appearance of a prolonged clotting time in the latter part of the shock period. Evidence that this is due to autogenous heparin is pointed out as well as collaborative evidence in humans. It is postulated that the stimulus for the activation of autogenous heparin is the appearance of intravascular coagulation (fibrin) in the blood stream.

5. The mechanism of action of fibrinolysin in protection against irreversible hemorrhagic shock is probably by one or both of the following:

a. Dissolution of established clots in visceral capillaries.

b. Destruction of fibrinogen making clotting impossible.

Evidence in support of the former mechanism will be presented in a subsequent paper.

References

1. Armitage, P.: Evaluation by Preferences. *In* Sequential Medical Trials. Charles C Thomas, 1960, p. 25.
2. Brown, R. C., *et al.*: Increased Fibrinolysin with Malignant Disease. A. M. A. Arch. Int. Med., **109**:129, 1962.
3. Gollub, S. and A. W. Ulin: Unpublished data.
4. Hardaway, R. M.: Disseminated Intravascular Coagulation Syndromes. A. M. A. Arch. Surg., **83**:842, 1961.
5. ——, W. H. Brune, E. F. Geever, J. W. Burns and H. P. Mock: Studies on the Role of Intravascular Coagulation in Irreversible Hemorrhagic Shock. Ann. Surg., **155**:241, 1962.
6. ——, *et al.*: Alterations in Blood Coagulation Mechanism after Intra-aortic Injection of Thrombin. A. M. A. Arch. Surg., **81**:983, 1960.
7. ——, *et al.*: Endotoxin Shock. A Manifestation of Intravascular Coagulation. Ann. Surg., **154**:791, 1961.
8. ——, *et al.*: Lower Nephron Nephrosis. Am. J. Surg., **87**:41, 1954.
9. ——, *et al.*: Pathologic Study of Intravascular Coagulation Following Incompatible Blood Transfusion in Dogs. I. Intravenous Injection of Incompatible Blood. Am. J. Surg., **91**:24, 1956.
10. ——, *et al.*: Role of Norepinephrine in Irreversible Hemorrhagic Shock. Ann. Surg. **156**:57, 1962.

11. ——, and F. H. Weiss: Intracapillary Clotting As the Etiology of Shock. A. M. A. Arch. Surg., **83**:851, 1961.
12. ——, and D. Johnson: Mechanism of Action of Fibrinolysin in the Prevention of Irreversible Hemorrhagic Shock. Ann. Surg., in press.
13. Holburn, R. R.: Estimation of Fibrinogen in Small Samples of Plasma. *In* The Coagulation of Blood Methods. L. M. Tocantins, ed. Grune & Stratton, N. Y., pub. 1955, p. 155.
14. Jaques, L. B. and E. T. Waters: The Identity and Origin of the Anticoagulant of Anaphylactic Shock in the Dog. J. Physiol., **99**:454, 1941.
15. Lasch, H. G., *et al.*: The Effect of Fibrinolysin on the Course of Hemorrhagic Shock. Klin. Wchnschr., **39**:1137, 1961.
16. McKay, D. G., A. Kliman and B. Alexander: Experimental Production of Afibrinogenemia and Hemorrhagic Phenomena by Combined Fibrinolysis and Disseminated Intravascular Coagulation. New England J. Med., **261**: 1150, 1959.
17. ——, *et al.*: Pathologic Study of Intravascular Coagulation Following Incompatible Blood Transfusion in Dogs. II. Intra-aortic Injection of Incompatible Blood. Am. J. Surg., **91**:32, 1956.
18. ——, *et al.*: Alteration in Blood Coagulation Mechanism after Incompatible Blood Transfusion. Am. J. Surg., **89**:583, 1955.
19. Moloney, W. C., W. J. Egan and A. J. Gorman: Acquired Afibrinogenemia in Pregnancy. New England J. Med. **240**:596, 1949.
20. Owren, P. A. and K. Aas: Control of Dicumarol Therapy and the Quantitative Determination of Prothrombin and Proconvertin. Scand. J. Clin. & Lab. Invest., **3**:201, 1951.
21. Reid, D. E., *et al.*: Intravascular Clotting and Afibrinogenemia, the Presumptive Lethal Factors in the Syndrome of Amniotic Fluid Embolism. Am. J. Obst. & Gynec., **66**:465, 1953.
22. Shayer, R. W.: Recent Progress and Present Problems in the Field of Shock. Federation Proceedings, July 1961, Supplement No. 9, p. 154.
23. Smith, V. S.: Seminar Conference. Dept. of Physical Chemistry, Harvard Medical School 1945.
24. Stefanini, M. and W. Dameshek: The Hemorrhagic Disorders. Grune and Stratton, New York, pub. 1955.
25. Spink, W. W. and J. A. Vick: Endotoxin Shock and the Coagulation Mechanism: Modification of Shock with Epsilon-Aminocaproic Acid. Proc. Soc. Exper. Biol. and Med., **106**:242, 1961.
26. Tagnon, H. J., *et al.*: The Occurrence of Fibrinolysis in Shock, with Observations on the Prothrombin Time and Plasma Fibrinogen During Hemorrhagic Shock. Am. J. Med., Sc., **211**:88, 1946.
27. von Kaulla, K. N. and R. L. Schultz: Methods for the Evaluation of Human Fibrinolysis. Am. J. Clin. Path., **29**:104, 1958.
28. von Kaulla, K. N.: Spontaneous Anticoagulants in Surgery. Proc. 7th Congress of the European Society of Haematology. p. 135. S. Karger, New York, 1960.
29. Yudin, S. S.: Transfusion of Stored Cadaver Blood. Lancet, **2**:360, 1937.