

CLOT OBSERVATION TEST FOR CLINICAL DIAGNOSIS OF CLOTTING DEFECTS

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ALTHOUGH the occurrence of excessive bleeding during surgery is not common, it is recognized that hypofibrinogenemia may be an important factor in the hemorrhagic diathesis which occurs in a number of pathological states. In many of these conditions an accompanying fibrinolysis has been reported.¹

One of the most valuable and reliable tests for fibrinogenopenia and fibrinolysis is the observation of the clot.² In this condition, the blood clot characteristically is either defective in bulk or quality, appears soft and likely to be ineffective physiologically and disappears from the blood sample on standing. This disappearance may require as long as four hours before it is noted, but in clinically significant cases the clot is likely to be dissolved within an hour.³

Fibrinolysis when first present in the blood stream may be corrected by adequate therapy. When the condition is allowed to progress for any length of time, the continued release of fibrinolysins may take place so rapidly that treatment is of no avail. It is therefore essential to establish this diagnosis as early as possible.³

A simple method of early diagnosis is desirable. We have undertaken to evaluate the usefulness of the clot observation test in clinical diagnosis of hypofibrinogenemia in patients undergoing operative procedures.

METHOD

Blood samples were drawn and clot observation tests carried out in 125 patients before induction of anesthesia, one or more times during operation and at the completion of the procedure and/or in the recovery room. Patients for sampling were selected to some extent, the majority having conditions in which

hemorrhagic tendencies have been reported, i.e., operations on uterus, pancreas, lung, prostate, patients with liver disease, carcinoma, chronic infections and those in whom multiple transfusions of banked blood were anticipated. With one exception, all patients were scheduled for elective surgical procedures. In an additional 5 patients, hemorrhagic disorders were suspected and sampling was done for diagnosis, during operation or postoperatively.

The sampling procedure was as follows: (1) Blood sample was drawn either from vein or from operative site, and placed in 5 or 10 ml centrifuge tube. (2) Time interval in which clotting takes place was noted, as well as the shape and form of clot, retraction, amount of "fall-out" of free cells. A graduated centrifuge tube was used to determine the relative percentage of clot, free cells and serum (fig. 1).

Figure 1, A: If there is an absence of fibrinogen and/or rapid fibrinolysis, the blood will not clot. The red cells in such a specimen will settle out to produce an even upper surface. *B and C:* If there is a decrease in the amount of fibrinogen and/or defects in the fibrin web due to fibrinolysis the clot may form but it will be of reduced volume. The red cells will escape from the fibrin web and will settle to the bottom of the tube as a high red cell column. *D and E:* If there is no fibrinogenopenia or fibrinolysis, the volume of the clot will be relatively great and there will be little red cell "fall-out."² The normal clot retracts and retains the majority of the red cells within the fibrin web.

Two criteria should be observed in an attempt to assess the relationship between fibrinolytic activity and excessive operative bleeding. Samples should be drawn during the period of active bleeding, since fibrinolytic activity often disappears when oozing ceases, and frequent samples should be obtained. Because of the rapid fluctuations in fibrinolytic activity a single sample may give misleading results.⁴

From a technical standpoint, we found that

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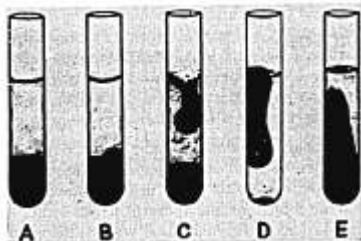


FIG. 1. Clot observation test. A. Afibrinogenemia; B. Fibrinogen deficiency; C. Defective clot from a patient with fibrinogenopenia with abnormal red cell "fall-out"; D. Normal clot. Note retraction and small amount of red cells escaping from clot; E. Normal clot with good retraction. From "Laboratory Procedures," Diggs, L. W., M.D. (Editor), Memphis, 1957, page 38.

unsatisfactory samples were obtained in the following circumstances: specimens taken from the extremity into which blood was being transfused, faulty venipuncture with admixture of tissue fluid in the blood sample, syringes or tubes which were wet or which contained foreign material.

RESULTS

Records were kept of the number and source of the specimen, time drawn, conditions under which sampling was done, time of beginning and completion of clot and a description of the clot. Samples were retained and read at intervals up to four hours or more.

In table 1 are shown the results of the clot observation tests. Of the 125 patients in whom clot observation tests were performed, approximately one in five exhibited abnormal

clots and an additional 25 per cent showed some abnormality as indicated by moderate fall-out of red cells. More than one-half (56 per cent) showed normal clots. No bleeding tendencies were observed in the normal or subnormal groups (81 per cent). Excessive bleeding was encountered in two patients of the group of 23 who exhibited abnormal clots. One of these was a patient undergoing right lobectomy. During the immediate postoperative period 400 ml. of blood was lost in the thoracotomy drainage bottle. Bleeding subsided without treatment. In the second patient, excessive bleeding occurred during transurethral resection of the prostate. No definite therapy was required other than replacement of the 900 ml. estimated blood loss.

Observation of the clot was used as a test for fibrinogen deficiency in an additional five patients who exhibited excessive bleeding during or after operation. Two of these patients had emergency Cesarean sections for obstetrical complications. It is now the usual procedure to perform serial clot observation tests on all patients with suspected abruptio placentae. Bleeding was reported in two patients in the recovery room following transurethral resection of the prostate. Blood samples were drawn immediately for clot observation tests. The first of these two occurred prior to this study. In the second case, serial clot observation tests indicated that the defect continued through the third postoperative day. The fifth patient had a bone graft of the femur performed for nonunion of a fracture. This individual exhibited bleeding in the early postoperative period. Clot observation tests were abnormal and signs of hemoglobinuria became evident.

TABLE 1
RESULTS OF CLOT OBSERVATION TESTS

	Otolaryngology	Thoracic Surgery	General Surgery	Urology	Gynecology	Orthopedics	Total
Abnormal	1	3	5	5	7	2	23
Subnormal	1	1	13	6	10	0	31
Normal	3	4	20	30	14	0	7
Total	5	8	38	41	31	2	125

Abnormal: soft clot, marked fall-out of red cells.

Subnormal: formed clot, poor retraction, moderate fall-out.

Normal: good retraction, minimal fall-out.

Recheck of the blood administered during the operation revealed incompatibility.

DISCUSSION

The recognition that multiple clotting defects may be a dangerous complication of anesthetic and operative procedures has increased study of this phenomenon. In general, the liver damage produced by disease, hypoxia, and anesthesia increases the danger of hemorrhage and this is further increased by the effects of stress of preoperative preparation and the operative procedure. Transfusion reactions and allergic responses to drugs increase the possibility of hemorrhage. One must be alert to the importance of traces of bleeding, unexplained rise in body temperature, and other signs.² The change most frequently associated with excessive oozing during operative procedures was hypofibrinogenemia.^{1,4}

The clot observation test has proved to be a rapid and practical means for the early recognition of the hypofibrinogenic state.^{2,3,6} This simple test involves the withdrawal of 5 ml. of the patient's blood into a clean dry tube and observing it at frequent intervals for clot formation and stability. Normal blood will clot within 8-12 minutes and the clot will remain intact for at least 24 hours. In cases of fibrinogen deficiency, the blood may not clot, or if it does, may undergo partial or complete dissolution within 30-60 minutes. In the event of the latter finding, hypofibrinogenemia and the likely presence of fibrinolytic activity are indicated.

Mechanism of Fibrinogen Depletion. The possible mechanism for the initiation of fibrinogen depletion includes:^{7,8} (1) defective formation of fibrinogen by the liver, (2) increase in fibrinogen consumption due to activation of intra-vascular clotting, and (3) destruction or digestion of fibrinogen by fibrinolytic or proteolytic enzymes circulating in the blood stream.

Reviews of the present concepts of the fibrinolytic enzyme systems can be found in a number of publications.^{1,9-11} The terminology of these lytic systems is not standardized. To briefly summarize current thinking, the proteolytic enzyme system is a complicated system consisting of proenzyme(s), activators, active enzyme(s), inhibitors and substrates.

In plasma there exists a balance with delicate equilibrium between the active enzyme, *plasmin*, and its inhibitor, *antiplasmin*. The precursor of plasmin is termed *plasminogen*. An imbalance of this system may occur in pathological conditions resulting in severe bleeding manifestations. The mechanisms which activate the fibrinolytic enzyme are not clearly understood. This enzyme system may be activated by a number of agents, including body tissues. Tissues, particularly the lung, uterus, pancreas, and prostate are said to contain activators which may be released into the circulation during operative trauma. Stress, shock and hypoxia can activate fibrinolysis, and may play a role in operative patients. While fibrinolysis is usually thought of as acting only on fibrinogen and fibrin, it must be remembered that it is a general proteolytic enzyme, and may destroy other circulating blood clotting factors. Therefore, multiple clotting deficiencies may result from the activation of the fibrinolytic enzyme.^{1,4,12}

Clinical Conditions. The clinical conditions leading to the presence of fibrinogen defect include the following:^{2,8} (1) obstetrical complications: premature separation of the placenta; prolonged retention of a dead fetus; amniotic fluid embolism; toxemia of pregnancy and related syndromes; and induced abortion; (2) major operative procedures on the prostate, lungs or pancreas; (3) major pathological conditions: hepatic malignancy, stress or hypoxia; (4) hemorrhagic or burn shock, and (5) transfusion reaction: massive transfusions or transfusion of incompatible blood.

Acquired hypofibrinogenemia is frequently an obstetrical complication. The most probable cause is intravascular clotting following the entrance of thromboplastic material into the circulation with the resultant increase in fibrinogen consumption. Since decidual and placental tissues contain thromboplastin, it is conceivable that the clotting mechanism may be activated intravascularly and fibrinogen may be deposited as fibrin emboli. Such a deposition of fibrin must be followed by an activation of fibrinolytic enzyme system. Furthermore, the enzyme can contribute to the fibrinogen depletion by hydrolysis of the fibrinogen itself. In addition, it has been demonstrated that the myometrium of the pregnant uterus contains

potent activators of this enzyme system which can be demonstrated even during normal delivery.⁷

Abnormal bleeding during and following prostatic operations has been attributed to fibrinolysis. Fibrinolysis occurs primarily in these cases in which the venous sinuses are opened by the operative procedure.¹³ Prostatic tissue is an activator of the fibrinolytic system and in addition contains a fibrinolytic system of its own. The process may be due entirely to the intravascular release of prostatic proteolytic enzyme or may be due to intravascular release of enzyme activators from the prostate triggering the plasmin system.

Hypofibrinogenemia is occasionally observed after pulmonary surgery.^{8, 12} An investigation of fibrinolysis following thoracic surgery in comparison with general surgery demonstrated a significant increase in lysis within two hours in thoracic patients compared with a minimum increase in general surgery patients.¹⁴ The higher percentage of thoracic patients showing lysis can be correlated with the high concentration of tissue activator of plasminogen which has been demonstrated in the lung. Surgical manipulation of the lung could cause a release of the activator into the blood stream causing an increase conversion of plasminogen to plasmin with a resulting increase in fibrinolysis. As a factor accompanying malignant disease,^{7, 8, 12, 15} the lack of fibrinogen has been noted with leukemia, carcinoma of the stomach and lung and in cases of carcinoma of the prostate with metastases. In carcinoma of the prostate, an actual fibrinolysin is elaborated by the carcinoma cells themselves.

The coagulation defect that is observed after hemolytic transfusion reactions and after giving massive amounts of blood may be essentially the same as the bleeding state encountered in other hypofibrinogenic states.^{9, 11, 12, 16, 17, 18} As in the latter, activity of circulating fibrinolysins is an important factor. These enzymes, normally present in the blood in an inactive form, are activated by a series of events of which shock, hypoxia, trauma and even bleeding itself may be listed. The bleeding tendency in hemolytic reactions is due probably to insidious intravascular clotting brought about by the liberation of a powerful clotting agent, erythrocytin, from the hemolyzed

erythrocytes. As a result, hemostatic efficiency is impaired by utilization of all available fibrinogen and activation of profibrinolysin or fibrinolysin. A bleeding condition may develop from the transfusion of a large amount of blood even when it is compatible. Bank blood is likely to be low in certain of the coagulation factors. With the transfusion of large amounts of such blood it has been postulated that the recipient's coagulation factors may be diluted to the hemorrhagic range.¹⁶

Treatment. The first step in treatment of hypofibrinogenic states consists of the administration of adequate amounts of fibrinogen.^{1, 2, 19, 20} There is no present knowledge of the correct dosage. The usual recommendation is to administer 2-3 units of fibrinogen and to repeat in thirty minutes if oozing does not disappear. The clot observation test also is recommended for estimating the amount of fibrinogen therapy necessary to restore normal blood coagulation. By withdrawing venous blood before therapy is started and at intervals during the course of treatment and saving each clot for observation, a continuous record of progress and response of the patient to therapy is provided. Since the active enzyme is rapidly destroyed and if sufficient fibrinogen is given to control the hemorrhage for a short period of time, the process may then be reversed as the patient's own protective mechanisms start to resynthesize the various factors involved, i.e., fibrinogen, inhibitors and profibrinolysin.

If fibrinogen is not available, blood transfusions are indicated. The freshest blood available should be used. In the absence of blood or fibrinogen, blood plasma is indicated.

Proteolytic inhibitors such as toluidine blue and klot are said to be effective in the prophylactic prevention of fibrinolysis.¹² More recently, the intravenous administration of fat emulsion has been reported to inhibit circulating fibrinolysins.²¹ Cortisone also is said to prevent the activation of fibrinolytic enzymes.

SUMMARY

Clot observation tests were performed routinely in 125 patients to assess the relationship between fibrinolytic activity and excessive surgical bleeding. Twenty-three of the 125 patients sampled exhibited abnormal clots and in two of these patients bleeding was trouble-

some. In 5 other patients in whom bleeding tendencies were evident, clot observations tests were performed for diagnosis. The blood either did not clot or formed soft unstable clots with abnormal retraction. Failure of freshly drawn venous blood to clot or to form a normal sized stable clot is believed sufficient evidence to conclude that a clinically significant defect of clotting mechanism is present.

The pathogenetic mechanism of clotting defects in various clinical conditions is discussed.

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