7. Using the tilt-tube method, determine the clotting time. It should be approximately 30 seconds.
8. Continue to add 0.2 ml 0.025 M calcium chloride to each succeeding tube at 1-minute intervals. That is, when the clock has 6 minutes remaining, add calcium chloride to tube No. 2, and so forth, so that calcium chloride is added to each tube exactly 20 minutes after the kaolin was added.
9. Average the duplicate results and record.
10. Perform a platelet count on the patient's platelet-rich plasma, and the normal platelet-rich control plasma.
11. Interpretation of results: the plasma mixtures in tubes No. 2 and No. 7, and in No. 3 and No. 6, differ only in their source of platelets. If the average clotting times of these two groups agree within 2 to 3 seconds of each other, and the platelet counts on the patient's and normal control platelet-rich plasmas are within 100,000 to 300,000 per cu mm, the patient has no significant defect in platelet factor-3 availability. If, however, the clotting time in tubes No. 3 and No. 6 are more prolonged and differ more widely from tubes No. 2 and No. 7, this may be due to decreased platelets (thrombocytopenia) in the patient or to defective platelet factor-3 availability. If the platelet count performed on the patient's platelet-rich plasma is within the range of 100,000 to 300,000 per cu mm, the prolonged clotting time is probably due to defective platelet factor-3 availability. A patient with abnormal platelet function generally has a clotting time about 15 seconds longer than the control.

PLATELET ADHESIVENESS TEST

One of the functions of platelets is their participation in hemostasis, where they adhere to each other and to the walls of damaged blood vessels to form a hemostatic plug. The adhesiveness of blood platelets is measured, in vitro, by their ability to adhere to glass surfaces. The normal values for this test are 26 to 60% platelet adhesiveness. Decreased values, using the procedure to be described, are found in thrombasthenia, where there is a qualitative disorder in platelets, von Willebrand's disease, and in some cases of myeloid metaplasia and thrombocythemia. Increased platelet adhesiveness has been reported in venous thrombosis, pulmonary embolism, coronary disease, following splenectomy, and in diabetes mellitus.

Salzman Method

REFERENCE

Salzman, E.W.: Measurement of platelet adhesiveness, a simple in vitro technique demonstrating an abnormality in von Willebrand's disease, J. Lab. Clin. Med., 62, 724, 1963.

REAGENTS AND EQUIPMENT

1. A double-ended, 20-gauge, Vacutainer needle.
2. Hypodermic needle, 20 gauge.
3. Vacutainer holder.
4. Vacutainer tubes (two) containing EDTA anticoagulant.
5. Siliconized ML-ML adapter, obtainable from Becton-Dickinson Company, Rutherford, N.J.
6. Siliconized 3200 A adapter, obtainable from Becton-Dickinson Company, Rutherford, N.J.
7. Polyvinyl tubing (inner diameter of 0.113 inch).
8. Grease-free, soda-lime-silica glass beads, with an average diameter of 0.0185 inch. (Obtainable from Minnesota Mining Company as "Superbrite," type 070.)
9. Siliconized nylon mesh, with openings of 0.002 inch.

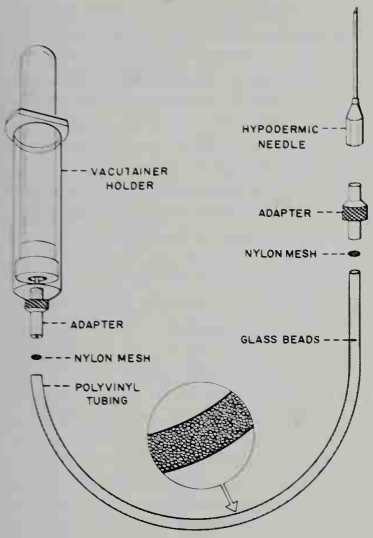

FIG. 131. Salzman glass bead collecting system.

10. Duco cement.
11. Materials necessary for two platelet counts.
12. Glass bead filter.
    A. Cut two pieces of siliconized nylon mesh to fit exactly over the ends of the two siliconized adapters.
    B. Using Duco cement, glue a piece of the nylon mesh to one end of each of the adapters.
    C. Attach one end of the polyvinyl tubing to that end of the adapter to which the nylon mesh is glued (Fig. 131).
    D. Fill the polyvinyl tubing with 1.3 g of glass beads.
    E. After packing the glass beads into the tube, cut the tubing. Allow a little extra unfilled tubing to remain in order to fit over that end of the second adapter that contains the nylon mesh. (The degree of packing of the glass beads, and therefore, the length of the polyvinyl tubing, should be such that it takes 40 to 50 seconds for the blood to be collected through this system.)

SPECIMEN

One tube of whole blood collected according to routine procedure, using the Vacutainer assembly with a 20-gauge needle, and drawing the blood directly into an EDTA vacuum tube. A second specimen of blood is collected through the glass bead collecting system directly into an EDTA vacuum tube.

PRINCIPLE

A platelet count is performed on both specimens of blood. The number of platelets in the blood, collected through the glass bead collecting system, will be lower than the number obtained by routine venipuncture. This is due to the adhesiveness of the platelets, which have adhered to the glass beads in the filter system. The results of this procedure are expressed as the percent of platelets retained in the filter.

PROCEDURE

1. Perform two clean venipunctures, at separate sites, with and without the use of the glass bead collecting system. (The blood collection rate through the glass bead collecting system should be 6 to 10 ml per minute.)
2. Perform a platelet count on both blood samples.
3. Calculate the percent of platelet adhesiveness as shown below.

$$\text{Percent of platelet adhesiveness} = \frac{\text{Platelet count without glass beads} - \text{Platelet count with glass beads}}{\text{Platelet count without glass beads}} \times 100$$

DISCUSSION

1. If the glass beads are siliconized, there will be no platelet adhesion.
2. There is no correlation between the patient's platelet count and platelet adhesion.
3. Heparin therapy does not interfere with platelet adhesiveness.
4. It is recommended that each laboratory determine its own set of normal values. Slight differences in technique and the length of the polyvinyl tubes are important factors.
5. It is felt by E. W. Salzman that calcium ions are necessary for platelet adhesion under the conditions of this test. For this reason, the blood passes through the filter system prior to being anticoagulated.
6. Modifications of this procedure may be utilized to produce more standardized and reproducible results. Becton, Dickinson and Company (Rutherford, New Jersey) market the B-D Platelet Retention Column (similar to the previously described glass bead column, but more standardized). Also, use of a constant-flow syringe pump provides a constant rate of blood flow through the column. (Blood is collected in a syringe. The glass bead column is then attached to the syringe and the syringe placed in the pump. The pump is turned on, forcing the blood through the glass bead column, at a standardized rate of speed, into tubes containing EDTA.)

PLATELET AGGREGATION TEST

There is evidence that adenosine diphosphate (ADP), in the red cells or platelets, is responsible for the clumping of platelets during the coagulation process and for the formation of a platelet plug in injured blood vessels. Normally, platelets undergo rapid aggregation (clumping) when adenosine diphosphate is added to platelet-rich plasma. In thrombasthenia, the platelets fail to aggregate and this test is, at present, used to diagnose this disorder. Decreased platelet aggregation may also be found in uremia and macroglobulinemia.

REFERENCE

Dacie, J.V., and Lewis, S.M.: Practical Hematology, 5th Edition, Churchill Livingstone, New York, 1975.

REAGENTS AND EQUIPMENT

1. Barbitone buffer, pH 7.35 to 7.4.
   Sodium diethyl barbiturate, 0.1 M — 570 ml
   Hydrochloric acid, 0.1 N — 430 ml
   Sodium chloride — 5.67 g
   Dilute with an equal volume of 0.9% sodium chloride before use.
2. Adenosine diphosphate.
   Adenosine diphosphate — 10 μg
   Barbitone buffer — 1 ml
   This solution must be made up fresh each day the test is performed.
3. Test tubes, 12 × 75 mm.
4. Water bath, 37°C.
5. Stopwatch.

SPECIMEN

Citrated platelet-rich plasma: one volume of 0.11 M sodium citrate to nine volumes whole blood. Collect a specimen of blood from a normal control at the same time the patient's blood is obtained.

PRINCIPLE

Adenosine diphosphate is added to platelet-rich plasma, and the specimen is observed for platelet aggregation.

PROCEDURE

1. Centrifuge patient and control bloods at 1000 RPM for 10 minutes immediately after collection.
2. Pipet 0.2 ml of normal control platelet-rich plasma into a 12 × 75-mm test tube in the 37°C water bath.