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.

3. Allow to incubate for 30 seconds.
4. Pipet 0.1 ml of adenosine diphosphate solution into the tube and simultaneously start a stopwatch.
5. Agitate the mixture vigorously while keeping it in the 37°C water bath. Examine the mixture every 5 to 10 seconds for macroscopic agglutination. Normal clumping should occur within 30 seconds after the addition of adenosine diphosphate. Record the degree of platelet aggregation (1+, 2+, 3+, or 4+) and the time, after the addition of adenosine diphosphate, before clumping occurred.
6. Repeat steps 2 through 5, using the patient's platelet-rich plasma. If no aggregation with the patient's platelets has occurred after 2 minutes, place a small drop of the mixture on a slide. Examine the plasma mixture under the microscope for small clumps of platelets. (This may also serve as a check to determine if there are sufficient platelets in the plasma to give valid results.)

DISCUSSION

1. Failure of the normal control plasma to show normal platelet aggregation may be due to improperly prepared platelet-rich plasma. Therefore check the plasma microscopically for platelets. If the platelet-rich plasma shows a decrease in platelets, both control and patient bloods must be redrawn and the procedure repeated.
2. A more complete procedure for platelet aggregation is outlined in Chapter 7, Automation (Bio/Data Platelet Aggregation Profiler).

TEST FOR INACTIVATORS AND INHIBITORS (ANTICOAGULANTS)

Naturally occurring anticoagulants may be divided into two classes: (1) *inhibitors*, which act immediately to block the reaction between coagulation factors, and (2) *inactivators*, which progressively destroy the factors. Most cases of coagulation defects are due to deficiencies of one or more clotting factors. The presence of inactivators or inhibitors is rare but must be properly diagnosed when present. Inhibitors have been observed in certain chronic illnesses such as lupus erythematosus and chronic nephritis. Inactivators have been demonstrated in patients with hemophilia and Christmas disease, in patients with congenital deficiencies of other coagulation factors, and in some women soon after childbirth.

REFERENCE

Hardisty, R.M., and Ingram, C.I.C.: *Bleeding Disorders, Investigation and Management*, Blackwell Scientific Publications, Oxford, 1965.

REAGENTS AND EQUIPMENT

1. Water bath, 37°C.
2. Calcium chloride, 0.025 M.

Anhydrous calcium chloride	1.38 g
Distilled water	500 ml

3. Partial thromboplastin (platelet substitute).
4. Normal control plasma.
5. Test tubes, 13 × 100 mm.
6. Stopwatch.

SPECIMEN

Citrated plasma: one part 0.11 M sodium citrate to nine parts whole blood; or oxalated plasma: one part 0.1 M sodium oxalate to nine parts whole blood.

PRINCIPLE

Inactivators may be detected by performing an activated PTT on the patient's plasma, normal control plasma, and 1:1 mixtures of the patient and control plasmas at specifically timed intervals. These results are compared with each other and with a freshly made 1:1 mixture of patient and control plasma, to determine if the clotting time is progressively prolonged (inactivator present), or if the clotting time