generation mixture, indicates a deficiency in factor VIII, IX, XI, or XII, if the prothrombin time is normal. It may also indicate the presence of an anticoagulant.

## THROMBOPLASTIN GENERATION TEST

The thromboplastin generation test measures the efficiency with which plasma thromboplastin is formed. It detects factor VIII and factor IX deficiencies and is able to distinguish between the two. Factor XI and XII deficiencies may be detected but cannot be differentiated from each other. If the patient's platelets are used in the test, a platelet abnormality may also be detected.

### *REFERENCES*

Biggs, R., and Douglas, A.S.: The thromboplastin generation test, J. Clin. Path., 6, 23, 1953.

General Diagnostics: *Manual for Teaching Blood Coagulation Techniques in the Routine Laboratory*, Warner-Chilcott Laboratories, Morris Plains, New Jersey, 1964.

### *REAGENTS AND EQUIPMENT*

1. Barium sulfate, powdered.
2. Water bath, 37°C.
3. Calcium chloride, 0.025 M.

| | |
|---|---|
| Anhydrous calcium chloride | 1.38 g |
| Distilled water | 500 ml |

4. Thromboplastin-calcium chloride mixture.
5. Sodium chloride, 0.85% (w/v).
6. Partial thromboplastin (platelet substitute). (Available commercially.)
7. Ice bath (crushed ice).
8. Test tubes, 13 × 100 mm.
9. Stopwatch.

### *SPECIMEN*

One tube of clotted blood and one tube of oxalated blood (one part 0.1 M sodium oxalate to nine parts whole blood), from both a normal patient (to be used as control), and from the patient to be tested.

### *PRINCIPLE*

The patient's diluted serum and adsorbed plasma are mixed with calcium chloride and a substitute platelet factor. This constitutes the patient's thromboplastin generation mixture. The amount of plasma thromboplastin formed by the patient's coagulation factors is measured by the ability of this mixture to clot a normal plasma (which supplies, mainly, prothrombin and fibrinogen). The patient's generation mixture is incubated for a total of 6 minutes. At 1-minute intervals during this time, samples of the generation mixture are added to a normal plasma, and the clotting time determined. Normally, there will be enough thromboplastin generated during this 6 minutes to clot normal plasma in 12 seconds or less. When an abnormal result is obtained, normal adsorbed plasma (source of factors I, V, VIII, XI, XII) and normal serum (source of factors VII, IX, X, XI, XII) are substituted, one at a time, in order to determine which corrects the patient's defect. If the prothrombin time is normal, factors I, II, V, VII, and X are assumed to be normal. Therefore, deficiencies in factors VIII, IX, XI, or XII may be detected.

### *PROCEDURE*

1. Preparation of patient and normal control plasma. (Source of factor VIII.)
   A. Centrifuge the normal control and patient's anticoagulated bloods at 2500 RPM for 10 minutes within 15 minutes of collection.
   B. Remove the plasma, and pipet 1.0 ml of each plasma into a separate 13 × 100 mm test tube containing 100 mg barium sulfate. Place the remaining patient's plasma in the refrigerator in case it is needed for future tests. Refrigerate the re-

maining control plasma for use as the plasma substrate.

C. Stir the plasma–barium sulfate mixtures with a glass rod for 10 minutes. Refrigerate for 10 minutes.

D. Centrifuge the two mixtures at 2500 RPM for 10 minutes. Remove the adsorbed plasmas and place in respective test tubes in crushed ice.

E. Perform a prothrombin time on each of the adsorbed plasmas. The prothrombin times should be greater than 3 minutes. If not, readsorb the plasma.

F. Refrigerate the adsorbed plasmas.

2. Preparation of the patient and normal control serums. (Source of factor IX.)

A. When the patient and normal control bloods have clotted, place the tubes in a 37°C water bath for 2 hours.

B. At the end of 2 hours, centrifuge the clotted bloods at 2500 RPM for 5 minutes. Remove the serums and place in respective test tubes in crushed ice.

3. Test.

A. Dilute the normal control and patient's adsorbed plasma 1:5 with 0.85% sodium chloride (0.1 ml adsorbed plasma and 0.4 ml 0.85% sodium chloride). Place in respective test tubes in crushed ice.

B. Dilute the normal control and patient's serum 1:10 with 0.85% sodium chloride (0.1 ml of serum and 0.9 ml of 0.85% sodium chloride). Place respective test tubes in crushed ice.

C. Pipet approximately 5 ml of 0.025 M calcium chloride into a 13 × 100-mm test tube and place in the 37°C water bath.

D. Place six 13 × 100-mm test tubes in the 37°C water bath and pipet exactly 0.1 ml of plasma substrate (unadsorbed and undiluted control plasma) into each tube.

E. Prepare the control generation mixture. Add the following solutions to a 13 × 100-mm test tube in the 37°C water bath:

1) 0.3 ml of diluted adsorbed control plasma.
2) 0.3 ml of partial thromboplastin.
3) 0.3 ml of diluted control serum.
4) 0.3 ml of 0.025 M calcium chloride.

Start a stopwatch at exactly the same time the last reagent (calcium chloride) is added to the mixture. If a clot forms in this generation mixture at any time, it should be removed so that it will not interfere with pipetting.

F. When 55 seconds have elapsed on the stopwatch, pipet 0.1 ml of the generation mixture. With the other hand, pipet 0.1 ml 0.025 M calcium chloride.

G. When 1 minute has elapsed on the stopwatch, blow the 0.1 ml of generation mixture into one of the tubes containing 0.1 ml of plasma substrate. Immediately, blow the 0.1 ml of calcium chloride into the same tube and simultaneously start a second stopwatch.

H. Determine the clotting time of this mixture by the tilt tube method. If clotting has not occurred within 40 to 45 seconds, have a second person continue tilting the tube until clotting occurs. Record the results. If the clotting time is greater than 60 seconds, record as over 60 seconds.

I. Repeat steps F, G, and H at 1-minute intervals for all six tubes containing the 0.1 ml of plasma substrate. (Start the second clotting time when 1 minute 55 sec-

onds have elapsed on the first stopwatch.) Do not stop the first stopwatch at any time during this procedure.

J. If any of the six tubes has a clotting time of 12 seconds or less within the 6-minute incubation of the generation mixture, the test is considered normal. If the control test performed on the plasma substrate is abnormal, the entire test must be repeated.

K. Place six 13 × 100-mm test tubes in the 37°C water bath and pipet exactly 0.1 ml of plasma substrate (unadsorbed and undiluted control plasma) into each tube.

L. Prepare the patient's generation mixture. Add the following solutions to a 13 × 100-mm test tube in the 37°C water bath:
   1) 0.3 ml of diluted adsorbed patient's plasma.
   2) 0.3 ml of partial thromboplastin.
   3) 0.3 ml of diluted patient's serum.
   4) 0.3 ml of 0.025 M calcium chloride.

   Start a stopwatch at exactly the same time the last reagent (calcium chloride) is added to the mixture. If a clot forms in this generation mixture at any time, it should be removed so that it will not interfere with pipetting.

M. Repeat steps F through J.

N. If none of the tubes has clotted in less than 12 seconds using the patient's generation mixture, proceed to section 4, Substitutions. If any of the preceding six tubes has a clotting time of 12 seconds or less, the results are normal, and section 4 of this procedure may be omitted.

4. Substitutions.

A. Pipet 0.1 ml of plasma substrate into each of six 13 × 100-mm test tubes and place in a 37°C water bath.

B. Prepare a generation mixture. Add the following solutions to a 13 × 100-mm test tube in the 37°C water bath:
   1) 0.3 ml of diluted adsorbed control plasma.
   2) 0.3 ml of partial thromboplastin.
   3) 0.3 ml of diluted patient's serum.
   4) 0.3 ml of 0.025 M calcium chloride.

   Start a stopwatch at exactly the same time the last reagent (calcium chloride) is added to the mixture. Remove clot, if formed.

C. Repeat steps F through J, as described previously in section 3, Test.

D. Repeat step A and prepare another generation mixture as follows:
   1) 0.3 ml of diluted adsorbed patient's plasma.
   2) 0.3 ml of partial thromboplastin.
   3) 0.3 ml of diluted control serum.
   4) 0.3 ml of 0.025 M calcium chloride.

   Start stopwatch. Remove clot, if formed.

E. Repeat steps F through J, as described previously in section 3, Test.

5. Interpretation of results. See Table 6.

*DISCUSSION*

1. Abnormal platelet activity may be detected using the patient's platelets, in place of partial thromboplastin, in the patient's generation mixture. If this procedure is employed, a prolonged clotting time, using the patient's serum, platelets, and adsorbed plasma, must be followed up by a substitution test, using partial

**TABLE 6. INTERPRETATION OF THE THROMBOPLASTIN GENERATION TEST**

| FACTOR DEFICIENCY | CLOTTING TIME CORRECTED BY ADSORBED PLASMA | NORMAL SERUM | PROTHROMBIN TIME |
|---|---|---|---|
| VIII | yes | no | normal |
| IX | no | yes | normal |
| XI or XII | yes | yes | normal |
| anti-coagulant | no | no | may be normal |

thromboplastin in the patient's generation mixture. Correction of the generation clotting time by partial thromboplastin indicates defective platelet activity. The patient's platelets for this procedure may be prepared as follows:

A. Centrifuge oxalated blood for 10 minutes at 1000 RPM immediately after collection.
B. Transfer the platelet-rich plasma to a siliconized or plastic test tube and note the volume of the plasma.
C. Centrifuge platelet-rich plasma at 3000 RPM for 15 minutes. A platelet button will be formed.
D. Pour off supernatant plasma and refrigerate until 20 minutes before use.
E. Resuspend platelets in 0.85% sodium chloride and centrifuge at 3000 RPM for 15 minutes.
F. Pour off the supernatant sodium chloride and resuspend the platelets in a volume of 0.85% sodium chloride, equal to one-third of the original plasma volume.

2. The presence of circulating anticoagulants may give abnormal results in this test.
3. Oxalated plasma must be used if the plasma is to be adsorbed by barium sulfate. If citrated plasma is employed, aluminum hydroxide must be used as the adsorbing agent.
4. The thromboplastin generation test may also be performed on the Fibrometer.

## PARTIAL THROMBOPLASTIN SUBSTITUTION TEST

The partial thromboplastin substitution test may be performed if the PTT, or activated PTT, is abnormal in order to identify factor deficiencies in stage 1 or 2 of the coagulation process.

### *REFERENCE*

Proctor, R.R., and Rapaport, S.I.: The partial thromboplastin time with kaolin, Am. J. Clin. Path., 36, 212, 1961.

### *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 containing an activator (platelet substitute with an activator). Obtainable commercially.
4. Citrated normal control plasma.
5. Sodium chloride, 0.85% (w/v).
6. Sodium citrate, 0.1 M
   Sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) 2.94 g
   Distilled water 100 ml
7. Test tubes, 13 × 100 mm.
8. Stopwatch.
9. Adsorbed plasma (rich in factors V, VIII, XI, and XII). Prepare as follows:
   A. Add 100 mg of barium sulfate to