9. Use of an abnormal prothrombin control is recommended. This should be run with the morning group of prothrombin times and each time a new bottle of thromboplastin-calcium mixture is opened.
10. If the patient is receiving heparin, the prothrombin must be drawn at least 4 hours after the last injection, or the results obtained for the prothrombin time will be invalidated.

ACTIVATED PARTIAL THROMBOPLASTIN TIME

The activated partial thromboplastin time (APTT) is the single most useful procedure available for routine screening of coagulation disorders. The PTT, or activated PTT, measures those coagulation factors present in the intrinsic system, except for platelets and factor XIII. (Factor VII, of the extrinsic system, is also not measured.) The normal values for the activated PTT (by manual methods) are generally 35 to 45 seconds with results of 45 to 50 seconds being considered borderline and results over 50 seconds considered abnormal. As previously explained for the prothrombin time, each laboratory should determine its own set of normal values based on the method of clot detection and the reagents used.

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 (commercially available).
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. Oxalated plasma is not recommended unless the test is going to be performed promptly after blood collection. Immediately after blood collection, place the tube of blood in a cup of crushed ice and deliver to laboratory.

PRINCIPLE

The calcium in whole blood is bound by the anticoagulant to prevent coagulation. The plasma, after centrifugation, contains all intrinsic coagulation factors except calcium and platelets. Calcium, a phospholipid substitute for platelets (partial thromboplastin), and an activator (to ensure maximal activation), are added to the plasma. The time required for the plasma to clot is the activated partial thromboplastin time.

PROCEDURE

1. Centrifuge the anticoagulated blood at 2500 RPM for 10 minutes as soon as possible after the blood has been collected.
2. Remove the plasma from the cells immediately and place on ice.
3. Incubate a sufficient amount of 0.025 M calcium chloride at 37°C.
4. Pipet 0.2 ml of normal control plasma (or patient's plasma) into a 13 × 100-mm test tube.
5. Pipet 0.2 ml of the partial thromboplastin (containing activator) into the test tube containing the control (or patient's) plasma.
6. Mix the contents of the tube quickly and place in a 37°C water bath for 3 minutes.
7. After exactly 3 minutes, blow in 0.2 ml of the prewarmed calcium chloride and simultaneously start the stopwatch.
8. Mix the tube once, immediately after

adding the calcium chloride. Allow the tube to remain in the water bath, while gently tilting the tube every 5 seconds. At the end of 30 seconds, remove the tube from the water bath. Quickly wipe off the outside of the tube with a clean gauze, so that the contents of the tube can be clearly seen.

9. Gently tilt the tube back and forth until a clot forms, at which point the timing is stopped.
10. Control and patient plasmas must always be run in duplicate, and the two results averaged, to obtain the final value. The two results should check within ±1.5 seconds of each other. If they do not, another test should be performed. When the clotting time is prolonged, however, duplication of results is more difficult, and the allowable range of variation is wider. In certain abnormal states, clot formation is markedly prolonged. If formation of the clot has not started by the end of 2 minutes, the test may be stopped, and the results reported as greater than 2 minutes.
11. Report both the patient results and the normal control results. (Normal control results must always fall within the normal range; otherwise something is wrong with the reagents, equipment, or technique being used, and the entire test must be repeated.)

DISCUSSION

1. When the activated PTT is abnormally prolonged, there may be a deficiency in one of the coagulation factors, or there may be an inhibitor(s) present in the patient's plasma. To differentiate between these two abnormal states, perform, in duplicate, as described previously, an activated PTT, mixing 0.1 ml of normal control plasma with 0.1 ml of patient's plasma (in place of the usual 0.2 ml of patient's plasma). If the results are closer to the value received for the normal plasma control, the problem is probably due to a deficiency of one of the coagulation factors. (The activated PTT gives normal results when there is a 50% concentration of the coagulation factors present.) If, however, the results received are closer to the original results (using 0.2 ml of the patient's plasma), the defect is thought to be due to an inhibitor(s) present in the patient's plasma.
2. The partial thromboplastin time (without an activator) is performed in exactly the same way as the activated PTT, using partial thromboplastin not containing an activator. The normal results for the PTT performed in this way are generally 40 to 100 seconds, with a result of 120 seconds or longer being considered abnormal. The activator in the activated PTT allows for maximum activation of the contact factors and gives more consistent and reproducible results.
3. The activated PTT does not test for factor VII or platelets. It detects deficiencies in factors I, II, V, VIII, IX, X, and is also sensitive to circulating anticoagulants or inhibitors. The test is somewhat insensitive to deficiencies in factors XI and XII, the contact factors.
4. The PTT and activated PTT are much more sensitive to coagulation factor deficiencies than is the whole blood clotting time.
5. If the partial thromboplastin becomes frozen before use (for example, in shipment during the winter), the results of the PTT may be prolonged by as much as 15 or more seconds.
6. If there are sufficient stopwatches available, it is possible to do more than one test at a time by starting each of the 3-minute incubations at 2-minute intervals.

7. When sodium oxalate is used as an anticoagulant, the test must be performed within 1 hour of blood collection. Citrated blood should be spun down within 30 minutes of blood collection and may be stored on ice up to 1½ hours. Plasma allowed to sit longer than the recommended times gives prolonged and abnormal results.

PLASMA RECALCIFICATION TIME

(Plasma Clotting Time)

The plasma recalcification time is a measure of the overall intrinsic coagulation process. In the procedure outlined here, a deficiency in platelets, or platelet activity, is not detected. The normal plasma recalcification time on platelet-poor plasma is 90 to 250 seconds. A decrease in any of the clotting factors present in the intrinsic system will cause a prolonged clotting time.

REFERENCES

Biggs, R., and MacFarlane, R.G.: *Human Blood Coagulation and Its Disorders*, Blackwell Scientific Publications, Oxford, 1962.

Eli Lilly and Company: ART coagulation test advocated in pre-surgery cases, Clinical Laboratory Forum, 5, 4, 1970.

Miale, J.B.: *Laboratory Medicine: Hematology*, 5th Edition, C. V. Mosby Company, St. Louis, 1977.

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. Sodium chloride, 0.85% (w/v).
4. Normal platelet-poor 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

Platelet-poor plasma is mixed with sufficient calcium chloride to neutralize the effects of the anticoagulant, and the clotting time is then recorded.

PROCEDURE

1. Immediately after collection, centrifuge blood at 2500 RPM for at least 20 minutes in order to obtain a platelet-poor plasma.
2. Incubate, at 37°C for 2 to 3 minutes prior to each test, each of the following, in separate test tubes:
   A. Patient's platelet-poor plasma
   B. Normal platelet-poor control plasma
   C. Calcium chloride, 0.025 M
   D. Sodium chloride, 0.85%
3. Into a 13 × 100 mm test tube, in the 37°C water bath, pipet 0.1 ml 0.85% sodium chloride and 0.1 ml of patient's plasma. Mix.
4. Blow in 0.1 ml 0.025 M calcium chloride and simultaneously start a stopwatch.
5. Allow the tube to remain in the 37°C water bath for 90 seconds, tilting the tube gently every 30 seconds.
6. After 90 seconds, remove the tube from the water bath and gently tilt. Stop the watch as soon as a clot forms, and record the results.

DISCUSSION

1. The plasma recalcification time varies according to the number of platelets present in the plasma. As the number of platelets increases, the plasma recalcification time shortens. Therefore, it is important to centrifuge the blood in the prescribed manner.
2. The plasma recalcification time may be performed on platelet-rich plasma, in which case the normal range is 90