occupies a little more than half of the original volume.

4. If a Lee and White clotting time was performed, use one of the three tubes of blood and check for clot retraction at 1, 2, 4, and 24 hours after the blood clotted.
5. Results are reported as the length of time it took for the clotted blood to retract. As an alternative method, the results may be reported as: normal, if clot retraction has occurred at 2 to 4 hours; poor, if retraction occurs after 4 hours and within 24 hours; nil, if no retraction occurs after 24 hours.

DISCUSSION

1. Clot retraction should be almost complete within 4 hours. In abnormal states, there may be variable degrees of retraction or no retraction at all.
2. Shaking or jarring of the tube of blood should be avoided. This may lead to a shortened clot retraction time.
3. Clot retraction varies inversely with the plasma fibrinogen concentration. That is, if the plasma fibrinogen level is elevated, clot retraction may be poor.
4. Clot retraction may be affected by the red cell mass. In blood containing a large mass of red cells, the degree of retraction is limited because of the large volume of red cells within the clot. In anemic states, the reverse occurs, and the degree of clot retraction is increased.
5. Generally there is a small amount of what is termed red cell fallout during clot retraction. This is seen as a few red cells at the bottom of the tube that have fallen from the clot. The significance of an increased amount of red cell fallout is not known. However, when the fibrinogen level is slightly decreased, there will be an increased number of free red cells at the bottom of the tube. Whenever red cell fallout is increased, a notation on the patient's report should be made.

CLOT LYSIS

The clot used in the clot retraction procedure should be kept at 37°C and examined at the end of 8, 24, 48, and 72 hours for clot lysis. Normally, there is no clot lysis before 72 hours. If the clot that was initially formed becomes fluid in less than 72 hours, abnormal clot lysis is present. The time at which lysis was observed is reported as the clot lysis time. If no lysis occurred, the results are reported as, "no clot lysis after 48 and 72 hours."

PROTHROMBIN TIME

The prothrombin time is a useful screening procedure for deficiencies in factors II, V, VII, and X. Deficiencies in factor I, although rare, may also be detected. This test may be used to follow the course of anticoagulant therapy in patients receiving coumarin drugs. Factors II, VII, IX, and X are inhibited by the coumarin drugs, with factor VII showing decreased activity first. Common causes of a prolonged prothrombin time are vitamin K deficiency, certain liver diseases, specific coagulation deficiencies, and coumarin drug therapy. The normal prothrombin time is generally 11 to 13 seconds. However, these values differ according to the method and reagents used in the performance of the test. Therefore, each laboratory should determine its own set of normal values.

Quick's One-Stage Prothrombin Time Method

REFERENCE

Quick, A.J.: *Bleeding Problems in Clinical Medicine*, W. B. Saunders Company, Philadelphia, 1970.

REAGENTS AND EQUIPMENT

1. Water bath, 37°C.
2. Thromboplastin-calcium chloride mixture.

3. Normal plasma control.
4. Test tubes, 13 × 100 mm.

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, within 1 hour of blood collection.

PRINCIPLE

The calcium in whole blood is bound by sodium citrate or sodium oxalate, thus preventing coagulation. Tissue thromboplastin, to which calcium has been added, is mixed with the plasma, and the clotting time noted.

PROCEDURE

1. Centrifuge anticoagulated blood at 2500 RPM for 10 minutes as soon as possible after blood collection.
2. Remove the plasma from the cells immediately and place on ice.
3. Pipet 0.2 ml of thromboplastin-calcium mixture into the appropriate number of 13 × 100-mm test tubes. Warm tubes in the incubator for at least 1 minute until they have reached 37°C. The incubation period for this mixture is not critical once it reaches 37°C.
4. Incubate the plasma for approximately 2 to 3 minutes, until it reaches 37°C. Plasma should be incubated for no longer than 5 minutes after reaching 37°C.
5. Forcibly blow 0.1 ml of patient's plasma into the tube containing 0.2 ml of thromboplastin-calcium mixture and simultaneously start the stopwatch.
6. Mix the contents of the tube, and if the tilt method is being used, remove the tube from the water bath and wipe dry. Gently tilt the tube back and forth until a clot forms, at which point the timing is stopped.
7. If the nichrome wire loop method is preferred, pass the wire loop through the mixture at the rate of two sweeps per second until a formed clot adheres to the loop.
8. Each test and control plasma should be performed in duplicate. The results should agree with each other within ±0.5 seconds, when the prothrombin time is below 30 seconds. Duplicate tests on a prothrombin time above 30 seconds will not agree as closely.
9. Average the two results and report both the patient and normal control values.

DISCUSSION

1. The prothrombin time may also be performed by a semiautomated method using the fibrometer, or by a more completely automated method employing optical density readings.
2. Prothrombin time results may be reported in percent of activity. In this method, multiple dilutions of normal plasma control are made with 0.85% sodium chloride solution. A prothrombin time is run on each dilution. The results are charted on a graph, plotting the control plasma prothrombin time, in seconds, on the ordinate (vertical) axis, and the percent concentration of the control plasma on the abscissa (horizontal) axis. A curve similar in shape to that shown in Figure 128 will be obtained. The undiluted control plasma is graphed at 100% concentration. A separate curve must be available for each normal plasma control value. For example, a curve must be made when the undiluted normal control plasma has a prothrombin time of 12.5 seconds, a separate curve when it is 13.0 seconds, and another curve when the value is 13.5 seconds.

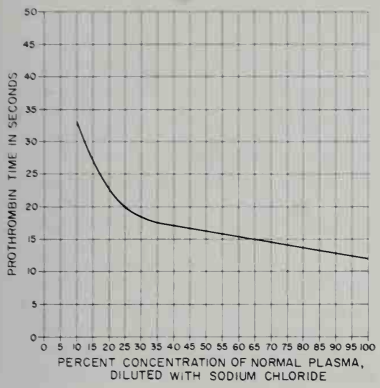

FIG. 128. Prothrombin activity curve.

When reporting the patient's prothrombin time in percent activity, use the appropriate curve, based on the normal plasma control value (control value of 100% activity). Using the patient's prothrombin time in seconds, refer to the curve in order to determine the percent activity of the patient's prothrombin time. The results reported must then include the normal control value in seconds, the patient's prothrombin time in seconds, and the percent activity for the patient's prothrombin time. The latter must never be reported by itself. Reporting prothrombin times in percent activity is of questionable value and is not used in all laboratories. Among the problems in this system are:

A. A new dilution curve must be drawn with each new lot of thromboplastin-calcium mixture used.
B. The percent activity and dilution curve vary with the material used as a diluent and with different thromboplastins. This variation causes discrepancies between different laboratories.
C. The curve is shaped such that, in the range of 25 to 100% activity, there is a relatively small change in the prothrombin time. In the range of 0 to 25% activity, there is a large range in the prothrombin times. Thus the interpretation of these results can be extremely confusing.

3. The prothrombin time should be performed within 1 to 2 hours of blood collection when sodium oxalate is used as the anticoagulant. If sodium citrate is employed, the test should be completed within 4 hours of collection. Factor V is labile and decreases in activity as the blood or plasma sits. The plasma may be frozen and stored up to 1 week without an appreciable effect on the prothrombin time.
4. A normal plasma control must be run with each group of tests performed, and each time a new bottle of thromboplastin-calcium mixture is opened.
5. When reconstituting the thromboplastin-calcium solution, mix well. Excessive shaking does not affect this solution.
6. Generally, the thromboplastin-calcium mixture is a suspension and not a homogeneous solution. It is, therefore, imperative that the suspension be well mixed whenever it is used.
7. Patients receiving coumarin drugs for thromboembolic disorders generally have prothrombin times of 20 to 30 seconds or 1.5 to 2.5 times their normal prothrombin time.
8. Normal plasma control values must fall within the laboratory's normal range. If the control results fall outside of this range, there is something wrong with the equipment, reagents, or techniques used, and the test must be repeated.

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