TABLE 11. THROMBIN UNITS (TWO-STAGE PROTHROMBIN TEST)

CLOTTING TIME (SECONDS)	THROMBIN UNITS/ml OF PLASMA
13.0	1.20
13.5	1.15
14.0	1.10
14.5	1.04
15.0	1.00
15.5	0.96
16.0	0.92
16.5	0.88
17.0	0.85

C. If a plasma, diluted 1:25, gave a clotting time of 15.0 seconds, the units of prothrombin would be calculated as shown:

$$10 \times 25 \times 1.00 = 250 \text{ units of prothrombin/ml of plasma}$$

D. The results may also be reported as a percent of normal, by comparing the patient results with the control results as shown below:

$$\frac{\text{Prothrombin units/ml in patient's plasma}}{\text{Prothrombin units/ml in normal control plasma}} \times 100 = \text{Percent of normal}$$

DISCUSSION

1. For accurate results, a further correction should be made for the dilution of the blood with anticoagulant. Determine the hematocrit and then calculate the dilution of the plasma. If the hematocrit was 35%, 65% of the blood collected is plasma (65% of 4 ml, if 4 ml of blood was collected). Therefore, 2.6 ml of plasma was diluted with 0.5 ml of citrate to give a final plasma dilution of 2.6 : 3.1, or a factor of 1.19.
2. The reactivity of different preparations of fibrinogen varies. This causes a variation in the units of prothrombin. For this reason, it is desirable to report the results in percent of normal.
3. When the clotting times are done at 1-minute intervals, the time reaches a minimum and then slowly increases due to the action of antithrombin. The shortest clotting time achieved should, therefore, be used as the final result.
4. It is advisable to perform the routine one-stage prothrombin time before starting the two-stage procedure. In this way, it is possible to determine if there may be a decreased concentration of prothrombin. (However, remember that a prolonged prothrombin time may be caused by deficiencies other than prothrombin.)

STYPVEN TIME

The Stypven time is capable of detecting deficiencies in prothrombin and factor V and X. It therefore differs from the prothrombin time in that deficiencies in factor VII are not detected. The normal Stypven time is 20 to 25 seconds.

REFERENCE

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

REAGENTS AND EQUIPMENT

1. Water bath, 37°C.
2. Stypven brand Russell's viper venom. (Obtainable from Burroughs Wellcome Co., Research Triangle Park, N.C.) Prepare according to directions on package.
3. Calcium chloride, 0.025 M.
   Anhydrous calcium chloride 1.38 g
   Distilled water 500 ml
4. Normal plasma control.
5. Test tubes, 13 × 100 mm.
6. Stopwatch.

SPECIMEN

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

PRINCIPLE

Russell's viper venom (Stypven) is a thromboplastic substance that contains a factor VII-like substance. A prothrombin time using Stypven as a source of tissue thromboplastin and factor VII is performed. Deficiencies in factors V, X, and prothrombin may be detected.

PROCEDURE

1. Centrifuge blood at 2500 RPM for 10 minutes as soon as possible after blood has been collected.
2. Remove the plasma from the cells immediately and place on ice.
3. Incubate each of the following in separate test tubes, at 37°C for 3 minutes:
   A. Patient's plasma.
   B. Normal control plasma.
   C. Calcium chloride, 0.025 M.
   D. Russell's viper venom (Stypven).
4. Into a 13 × 100-mm test tube, in the 37°C water bath, pipet 0.1 ml of Stypven and 0.1 ml 0.025 M calcium chloride. Mix.
5. Blow in 0.1 ml of patient or control plasma and simultaneously start the stopwatch.
6. Record the clotting time, as is done in the one-stage prothrombin time.
7. Each patient and control plasma should be performed in duplicate.

DISCUSSION

1. If the one-stage prothrombin time is normal, the Stypven time need not be performed.
2. In a factor VII deficiency, the prothrombin time would be prolonged and the Stypven time normal.
3. To make the test more specific for factor X and also for factor V, the Stypven time may be performed by the addition of 0.1 ml of bovine charcoal-filtered plasma as a source of factor V. This bovine plasma is obtainable from Colorado Serum Company, Denver, Co.

PROTAMINE TITRATION

When patients undergo open-heart surgery, heparin is used to prevent activation of the coagulation process. At the completion of surgery, protamine is administered in order to neutralize the effects of the heparin. However, protamine in excess is capable of interfering with factor IX activity and with thromboplastin generation. The protamine titration, therefore, is used to estimate the minimum required dose of protamine. All preparations for the protamine titration must be made prior to receiving the patient's blood.

REFERENCE

Perkins, H.A., Osborn, J.J., Hurt, R., and Gerbode, F.: Neutralization of heparin in-vivo with protamine; a simple method of estimating the required dose, J. Lab. Clin. Med., 48, 223, 1956.

REAGENTS AND EQUIPMENT

1. Sodium chloride, 0.85% (w/v).
2. Protamine sulfate, 1%. Obtain from the hospital pharmacy. (This is the same protamine that is used by the patient.) Store at 4°C.
3. Test tubes, 12 × 75 mm.

SPECIMEN

One 20-ml syringe filled with 15 ml whole blood.

PRINCIPLE

A specific amount of whole blood is added to varying dilutions of protamine sulfate. At the end of 15 minutes, the tubes are tilted to determine the lowest concentration of protamine sulfate that causes the blood to clot.

PROCEDURE

1. Prepare a stock protamine solution (1000 μg per ml): 1 ml 1% protamine sulfate in 9 ml 0.85% sodium chloride.