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.

TABLE 12. CONCENTRATION OF PROTAMINE SULFATE FOR THE PROTAMINE TITRATION PROCEDURE

TUBE #	ml of STOCK PROTAMINE SULFATE	ml of 0.85% SODIUM CHLORIDE	CONCENTRATION OF PROTAMINE SULFATE IN μg/ml
1	1.00	0.00	1000
2	0.50	0.50	500
3	0.45	0.55	450
4	0.40	0.60	400
5	0.35	0.65	350
6	0.30	0.70	300
7	0.25	0.75	250
8	0.20	0.80	200
9	0.15	0.85	150
10	0.10	0.90	100
11	0.05	0.95	50

2. Prepare the following dilutions in 12 × 75-mm test tubes, according to Table 12.
3. Label 11, 12 × 75-mm test tubes, according to the final concentration of protamine, which will be 100, 50, 45, 40, 35, 30, 25, 20, 15, 10, and 5 μg per ml, when the blood is added (Table 13).
4. Transfer 0.1 ml of the protamine sulfate dilution into the preceding set of tubes.
5. As soon as the blood is collected from the patient, add 1.0 ml of whole blood to each of the tubes containing 0.1 ml of diluted protamine sulfate. Dispense the blood directly from the syringe into the test tube.
6. Invert each tube once, to mix, and set a clock for 15 minutes.
7. At the end of 15 minutes, tilt each tube to determine the smallest amount of protamine sulfate that causes the blood to clot. This may be interpreted as the amount of protamine sufficient to neutralize the heparin in 1 ml whole blood. Therefore, this amount of protamine sulfate, times the patient's total blood volume, gives the proper dosage of protamine to be given to the patient in order to overcome the effects of the heparin.

TABLE 13. FINAL CONCENTRATION OF PROTAMINE SULFATE FOR THE PROTAMINE TITRATION PROCEDURE

0.1 ml of PROTAMINE SULFATE FROM TUBE NO.	FINAL CONCENTRATION OF PROTAMINE SULFATE AFTER BLOOD HAS BEEN ADDED (μg/ml)
1	100
2	50
3	45
4	40
5	35
6	30
7	25
8	20
9	15
10	10
11	5

DISCUSSION

This test may be performed at room temperature or in a 37°C water bath. The same end point will be reached at both temperatures.

TOURNIQUET TEST (CAPILLARY FRAGILITY TEST)

The tourniquet test is a crude measure of capillary fragility. Since platelets function to maintain capillary integrity, the degree of thrombocytopenia will also correlate with the tourniquet test, as will the bleeding time. In normal patients, none to very few petechiae are formed during this test.