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.

(Petechiae are minute hemorrhages under the skin and appear as small bruises.) A positive tourniquet test (presence of numerous petechiae) will be found in thrombocytopenia, purpura, and von Willebrand's disease.

REFERENCE

Cartwright, G.E.: *Diagnostic Laboratory Hematology*, Grune & Stratton, Inc., New York, 1963.

REAGENTS AND EQUIPMENT

1. Stethoscope.
2. Blood pressure cuff.

PRINCIPLE

An inflated blood pressure cuff on the upper arm is used to apply pressure to the capillaries. At the end of 5 minutes, the arm is examined for petechiae. If a patient has thrombocytopenia, there will not be enough platelets present to maintain capillary integrity, and small bruises will form on the arm.

PROCEDURE

1. Apply a blood pressure cuff on the upper arm, above the elbow, and take a blood pressure reading.
2. Inflate the blood pressure cuff to a point halfway between the systolic and diastolic pressures. (However, never exceed a pressure of 100 mm of mercury.) Maintain this pressure for 5 minutes.
3. Remove the blood pressure cuff.
4. Examine the forearm, hands, and fingers for petechiae. Disregard any petechiae within one-half inch of the blood pressure cuff, since this may be due to pinching of the skin by the cuff.
5. The test results are graded roughly as follows:
   1+ = A few petechiae on the anterior part of the forearm.
   2+ = Many petechiae on the anterior part of the forearm.
   3+ = Multiple petechiae over the whole arm and back of the hand.
   4+ = Confluent petechiae on the arm and back of the hand.

DISCUSSION

1. An alternative procedure uses the inflated blood pressure cuff at a pressure of 80 mm of mercury, regardless of the patient's blood pressure.
2. The test should not be repeated on the same arm within 7 days.
3. At times, the petechiae may not appear until several minutes after the blood pressure cuff has been removed. If this occurs, these petechiae should be included in the grading of the test results.
4. For a more quantitative test, a circle, 5 cm in diameter, may be drawn on the anterior surface of the forearm, about 4 cm below the anterior bend of the elbow. If this is done, the test is performed as just described, and the number of petechiae present in the circle is counted. In normal patients, there should be no more than 10 to 20 petechiae present. The one drawback to this method occurs when many petechiae appear on the arm, but very few are present within the circle.

TEST FOR PLATELET FACTOR-3 AVAILABILITY

Platelets activated during the coagulation process liberate platelet factor-3, a phospholipid, which is essential for normal blood coagulation. Normal values for this method are determined by the correlation of patient and control results. The patient's platelet-rich plasma should give a clotting time similar in length to that of the normal platelet-rich control plasma. Increased clotting times occur in thrombocytopenia and in defects in platelet factor-3 availability, as found in thrombasthenia and some uremic patients.