TABLE 15. PLASMA DILUTIONS (TEST FOR INHIBITORS)

TUBE NO.	PATIENT'S PLASMA (ml)	NORMAL CONTROL PLASMA (ml)	PATIENT PLASMA DILUTION	ACTIVATED PTT (seconds)
1	0.0	1.0	0	
2	0.2	0.8	1:5	
3	0.4	0.6	2:5	
4	0.5	0.5	1:2	
5	0.6	0.4	3:5	
6	0.8	0.2	4:5	
7	1.0	0.0	—	

longer than the original, should not be as prolonged as that of tubes No. 1 and No. 3. It generally shows a clotting time closer to that of the control plasma. In the presence of an inhibitor, or factor deficiency, tubes Nos. 1, 2, 3, and 4 will not show more than a normal increase in the clotting time as the time interval increases. The clotting time of tube No. 4 will more closely parallel that of tube No. 3.

16. If an inactivator has been found to be present, the procedure may be stopped here. However, to distinguish between an inhibitor and factor deficiency, continue with the procedure as follows.
17. Label seven 13 × 100-mm test tubes, No. 1 through No. 7, and carefully prepare the indicated dilutions of patient's plasma with normal control plasma, as shown in Table 15.
18. Perform an activated PTT, in duplicate, on each of the preceding seven tubes. Average the results and record.
19. Interpretation of results: In the presence of an inhibitor, all tubes containing the patient's plasma (tubes No. 2 through No. 7) will show a prolonged clotting time. If a coagulation factor deficiency exists, normal clotting time results will occur on the contents of tubes Nos. 2, 3, and 4. (The clotting time from tube No. 1 must always be normal.) The clotting time on the plasmas from tubes No. 5 and No. 6 may or may not be prolonged, depending on the severity of the factor deficiency.

EUGLOBULIN CLOT LYSIS TIME

The euglobulin clot lysis time is a screening procedure for the measurement of fibrinolytic activity and is more sensitive than the clot lysis time. The euglobulin fraction of plasma contains plasminogen, fibrinogen, and activators capable of transforming plasminogen to its active state, plasmin. Normally occurring inhibitors of this reaction (plasminogen to plasmin) are not present in this fraction of the plasma. Once a clot is formed, clot lysis occurs more quickly than in whole blood. Increased fibrinolytic activity has been associated with circulatory collapse, adrenalin injections, sudden death, pulmonary surgery, pyrogen reactions, and obstetric complications. Normally clot lysis does not occur in less than 2 hours but is usually complete within 4 hours. Clot lysis in less than 2 hours is indicative of abnormal fibrinolytic activity.

REFERENCE

Bucknell, M.: The effect of citrate on euglobulin methods of estimating fibrinolytic activity, J. Clin. Path., *11*, 403, 1958.

REAGENTS AND EQUIPMENT

1. Calcium chloride, 0.025 M.
   Anhydrous calcium chloride 1.38 g
   Distilled water 500 ml
2. Acetic acid, 1% (v/v).

3. Borate solution, pH 9.0.
   Sodium chloride 9.0 g
   Sodium borate 1.0 g
   Dilute to 100 ml with distilled water.
4. Water bath, 37°C.
5. Test tube, 15 × 125 mm.

SPECIMEN

Oxalated plasma: one part 0.1 M sodium oxalate to nine parts whole blood. Citrated plasma should not be used, since the presence of citrate tends to increase fibrinolytic activity.

PRINCIPLE

The plasma euglobulins are precipitated with 1% acetic acid and resuspended in a borate solution. The euglobulins are then clotted by the addition of calcium chloride. The clot is incubated, and time of lysis reported.

PROCEDURE

1. Collect blood with a plastic or siliconized syringe. Mix with the appropriate anticoagulant and immediately place on ice.
2. Centrifuge the blood specimen at 2500 RPM for 10 minutes, and immediately proceed with the test. The procedure must be carried out within 20 minutes of blood collection.
3. Into a 15 × 125-mm test tube, pipet 9.0 ml distilled water, 0.5 ml patient's plasma, and 0.1 ml 1% acetic acid.
4. Refrigerate the preceding mixture for 30 minutes at 4°C to allow for euglobulin precipitation.
5. Centrifuge at 2500 RPM for 5 minutes.
6. Pour off the supernatant and invert the tube on filter paper to drain.
7. Add 0.5 ml of the borate solution and place in a 37°C water bath.
8. Stir the mixture gently with a glass rod.
9. Add 0.5 ml 0.025 M calcium chloride to the mixture.
10. Record the time of clot formation.
11. Incubate the tube in the 37°C water bath, and periodically check for clot lysis. When clot lysis begins, check the tube every 5 minutes until the lysis is complete.
12. Report results as the length of time from clot formation to complete clot lysis.

PROTAMINE SULFATE

The protamine sulfate procedure tests primarily for the presence of fibrin monomers. During stage 3 of the coagulation process when thrombin acts on fibrinogen, fibrin monomers are formed that then polymerize to form a fibrin clot. Also detected in this procedure are early fibrin-(fibrinogen) split products (fragments X and Y). During the process of fibrinolysis, plasmin breaks down fibrin and fibrinogen to fragments X, Y, D, and E. Under certain pathologic conditions, intravascular coagulation may be stimulated. In these instances there is widespread appearance of fibrin clots in the blood vessels of the microcirculation. Fibrin monomers are therefore present in the plasma. Due to the presence of coagulation, there is stimulation of the fibrinolytic system and the formation of fibrin- (fibrinogen) split products. Rapid detection of the presence of fibrin monomers is an important aid in the diagnosis of disseminated intravascular coagulation. Normally, there should be no fibrin monomers present in the plasma.

REFERENCES

Dade Division, American Hospital Supply Corporation: *Protamine Sulfate* Set, Dade Division, American Hospital Supply Corporation, Miami, Florida, 1975.

Williams, W.J., Beutler, E., Erslev, A.J., and Rundles, R.W.: *Hematology*, 2nd Edition, McGraw-Hill Book Company, New York, 1977.