DISCUSSION

1. In hemophilia A, it is generally accepted that a factor VIII level of 30 to 35% of normal activity is satisfactory for normal hemostasis.
2. Pooled normal plasma may be used in place of reference plasma. The advantage of reference plasma is that it has previously been assayed and therefore contains a known percent of factor VIII (IX).
3. Factor VIII- and factor IX-deficient substrates may be replaced by plasma known to be deficient in either factor VIII or factor IX. These plasmas may be stored at 0°C and thawed immediately before use. However, a plasma to be used as factor VIII- or factor IX-deficient must have a concentration of 0 to 1% of normal activity of the factor before it is acceptable. Also, even though the plasma is stored at 0°C, it may gradually become deficient in additional clotting factors, particularly factor V.

ASSAY FOR FACTOR V

The test results for factor V assay are expressed in percent, in relationship to the activity of factor V present in a normal plasma, or in a plasma containing a known concentration of the factor. The normal range for factor V is 50 to 200%.

REFERENCES

Biggs, R., and MacFarlane, R.G.: *Human Blood Coagulation and Its Disorders*, Blackwell Scientific Publications, Oxford, 1962.

Hardisty, R.M., and Ingram, C.I.C.: *Bleeding Disorders, Investigation and Management*, Blackwell Scientific Publications, Oxford, 1965.

REAGENTS AND EQUIPMENT

1. Water bath, 37°C.
2. Thromboplastin-calcium chloride mixture.
3. Factor V-deficient substrate. (Commercially available.)
4. Reference plasma with known factor V assay. (Commercially available.)
5. Ice bath.
6. Test tubes, 13 × 100 mm.
7. Stopwatch.
8. Two-cycle semilog graph paper.

SPECIMEN

Collect blood, using a syringe and 19- or 20-gauge needle. Citrated plasma is used: one part 0.11 M sodium citrate to nine parts whole blood. Place the tube of blood in a cup of ice as soon as it is collected.

PRINCIPLE

A prothrombin time is performed on factor V-deficient substrates containing varying dilutions of the patient's plasma. The patient's plasma is used to correct the prothrombin time. The amount of correction by the patient's plasma is compared with the results of the prothrombin time performed on a known reference plasma in place of the patient's plasma. The factor V content of the patient's plasma is expressed as the percent of normal.

PROCEDURE

1. Centrifuge blood at 2500 RPM for 20 minutes immediately after collection. Remove plasma and place in a cup containing crushed ice.
2. Pipet 0.2 ml of thromboplastin-calcium mixture into each of ten 13 × 100-mm test tubes and place in the 37°C water bath.
3. Reconstitute the factor V-deficient substrate, as directed, and place in the cup of crushed ice.
4. Prepare the following dilutions, as shown in Table 10.
5. Incubate each of the preceding plasma dilutions at 37°C for 3 minutes prior to testing. Care should be taken not to overincubate these plasmas.

TABLE 10. DILUTIONS FOR FACTOR V ASSAY

TUBE	PLASMA (OR REFERENCE PLASMA) (ml)	FACTOR V-DEFICIENT SUBSTRATE (ml)	DILUTION	PLASMA CONCENTRATION %
1	0.1	1.9	1:20	5
2	0.1	0.9	1:10	10
3	0.1	0.4	1:5	20
4	0.2	0.3	1:2.5	40
5	0.3	0.3	1:2	50

6. Forcibly blow 0.1 ml of the diluted patient's plasma into a tube containing 0.2 ml of thromboplastin-calcium mixture and simultaneously start the stopwatch.
7. Mix the contents of the tube and stop the watch at the first indication of clot formation. Perform duplicate prothrombin times on each plasma dilution, average results, and record.
8. Repeat steps 2 through 7 substituting the reference plasma for the patient's plasma.
9. Calculation of results:
   A. Using two-cycle semilog graph paper, plot each average clotting time in seconds against the plasma concentration in percent. Use the logarithmic scale for the plasma concentration. Plot 1 to 10% on the first cycle, and 20 to 100% on the second cycle. There will be ten points plotted.
   B. Draw the straight line that best connects the five points of the reference plasma. This represents the normal activity curve.
   C. By interpolation, determine from the graph the concentrations of reference plasma that give the same clotting times as the different dilutions of patient's plasma. Multiply the resulting reference plasma dilutions by the patient plasma dilution factors. (The calculations are similar to those for the factor VIII and IX assay. See Figure 130 for an example.)
   D. Average the five values received for the percent activity of patient's plasma. This result represents the percent of normal activity of factor V present in the patient's plasma.

DISCUSSION

1. Pooled normal plasma may be used in place of the reference plasma.
2. Factor V-deficient plasma may be used in place of the factor V-deficient substrate. If this is employed, a prothrombin time greater than 60 seconds should be obtained on the factor V-deficient plasma before it is used. (Factor V-deficient plasma may be prepared by incubating normal plasma at 37°C for 24 hours or by refrigerating the normal plasma at 4 to 10°C for 2 weeks.)
3. An assay of factor VII or factor X may be performed according to the previously described procedure. Factor VII- (or factor X-) deficient substrate and reference plasma with a known factor VII (or factor X) assay are used in place of factor V-deficient substrate and reference plasma.

TWO-STAGE PROTHROMBIN TIME

The two-stage prothrombin test determines the concentration of prothrombin in the plasma. Normally, there are 300 to 360