6. For an interpretation of results, refer to Table 8.

DISCUSSION

1. In order for the patient's prothrombin time to be considered as corrected, the corrected result should fall within a lower range, close to the results received for the normal plasma control.

ASSAY FOR FACTORS VIII AND IX

Test results for the assays for factors VIII and IX are expressed in percent, in relationship to the amount of activity of the factor present in a normal plasma or in a plasma containing known concentrations of the factor. The assays for factors VIII and IX are performed separately, but according to the same general procedure. The normal range for both factor VIII and factor IX is 50 to 200%. A factor VIII deficiency is found in classic hemophilia and von Willebrand's disease. Decreased amounts of factor IX are present in Christmas disease (also known as hemophilia B), liver disease, vitamin K deficiency, and in the newborn.

REFERENCE

Hardisty, R.M., and MacPherson, J.C.: A one-stage factor VIII assay and its use on venous and capillary plasma, Thromb. Diath. Haemorrh., *7*, 215, 1962.

REAGENTS AND EQUIPMENT

1. Partial thromboplastin containing an activator (platelet substitute with an activator). Obtainable commercially.
2. Water bath, 37°C.
3. Calcium chloride, 0.025 M.

Anhydrous calcium chloride	1.38 g
Distilled water	500 ml

4. Factor VIII deficient substrate. Factor IX deficient substrate. Obtainable commercially.
5. Reference plasmas with known factor VIII and IX assay. Obtainable commercially.
6. Sodium chloride, 0.85% (w/v).
7. Ice bath.
8. Stopwatch.
9. Test tubes, 13 × 100 mm.
10. 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 immediately after collection.

PRINCIPLE

An activated PTT is performed on factor VIII- (or factor IX-) deficient substrates containing varying dilutions of the patient's plasma (Table 9). The patient's plasma is used to correct the activated PTT. The amount of correction by the patient's plasma is then compared with results of the activated PTT, using a known normal, or reference, plasma in place of the patient's plasma. The factor VIII or factor IX 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. Proceed with the test immediately.
2. Maintain the partial thromboplastin (with activator) at room temperature.
3. Reconstitute the factor VIII- (or factor IX-) deficient substrate as directed, and place in the cup of crushed ice.
4. Incubate sufficient 0.025 M calcium chloride at 37°C.
5. Add 0.85% sodium chloride to four 13 × 100-mm test tubes, in the amounts listed in Table 9. Do not add the patient's plasma (or normal reference plasma) until immediately be-

TABLE 9. DILUTIONS FOR FACTOR VIII AND IX ASSAY

DILUTING FLUID (ml)	PLASMA (ml)	DILUTION	PLASMA CONCENTRATION
0.4	0.1	1:5	20 %
0.9	0.1	1:10	10 %
1.9	0.1	1:20	5 %
3.9	0.1	1:40	2.5%

fore each test is performed. (The patient's plasma is tested first and then, immediately before use, the reference plasma is prepared.)

6. Place eight 13 × 100-mm test tubes in the 37°C water bath. (All plasma samples are to be run in duplicate.)
7. Prepare the 1:5 dilution of patient's plasma, using a 0.1-ml blow-out pipet for the plasma.
8. To one of the 13 × 100-mm test tubes in the water bath, add:
   A. 0.1 ml of partial thromboplastin (with activator).
   B. 0.1 ml of factor VIII-(or factor IX-) deficient substrate.
   C. 0.1 ml of diluted patient's plasma (1:5 dilution).
9. Quickly mix the contents of the tube and set clock #1 for 3 minutes.
10. When 1 minute has elapsed on clock #1, prepare the duplicate 1:5 dilution of patient's plasma by repeating step 8.
11. Mix contents of the tube and set clock #2 for 3 minutes.
12. When 3 minutes have elapsed on clock #1, quickly pipet 0.1 ml of 0.025 M calcium chloride into the first tube, simultaneously starting a stopwatch. Leave the tube in the 37°C water bath for 30 seconds.
13. After 30 seconds have elapsed on the stopwatch, remove the tube from the water bath and gently tilt the tube at a rate no faster than once per second.
14. When clotting occurs, stop the watch. This is the end point.
15. When 3 minutes have elapsed on clock #2, quickly pipet 0.1 ml of 0.025 M calcium chloride into the second tube, simultaneously starting a stopwatch. Leave the tube in the incubator for 30 seconds.
16. After 30 seconds have elapsed on the stopwatch, remove the tube from the water bath and gently tilt the tube at a rate no faster than once per second.
17. When clotting occurs, stop the watch.
18. Average the preceding two results and record the clotting time for that dilution.
19. Prepare the 1:10 dilution of patient's plasma and repeat steps 8 through 18.
20. Prepare the 1:20 dilution of patient's plasma and repeat steps 8 through 18.
21. Prepare the 1:40 dilution of patient's plasma and repeat steps 8 through 18.
22. Using the reference plasma in place of patient's plasma, repeat steps 5 through 21.
23. Calculation of results (Fig. 130).
    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, plotting 1 to 10% on the first cycle and 20 to 100% on the second cycle. There will be eight points plotted.
    B. Draw a straight line that best connects the four 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

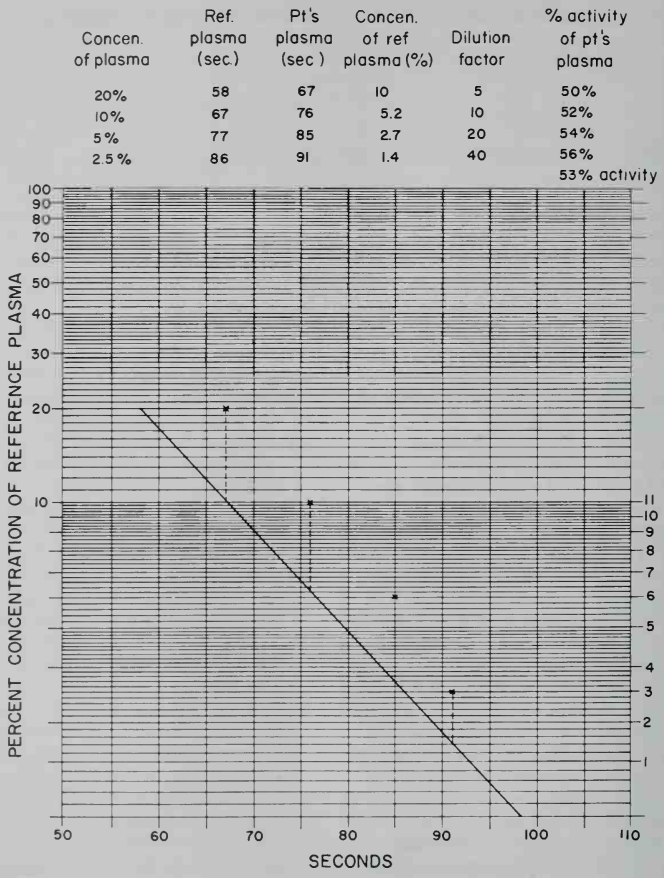

FIG. 130. Calculation of results for factor VIII and IX assay.

same clotting times as the different dilutions of patient's plasma. Multiply the resulting reference plasma dilutions by the patient plasma dilution factors. See Figure 130 for an example.

D. Average the four values received for the percent activity of patient's plasma. This result represents the percent of normal activity of factor VIII (or factor IX) present in the patient's plasma.

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.