proceed with the following dilutions, performing an activated PTT on:

F. Patient's plasma diluted 1:1 with adsorbed plasma.
G. Normal control plasma diluted 1:1 with adsorbed plasma.
H. Patient's plasma diluted 1:1 with aged serum.
I. Normal control plasma diluted 1:1 with aged serum.

6. For simplicity, record the results on a chart similar to the one shown.

	Normal control plasma, 0.1 ml	Patient's plasma, 0.1 ml
Normal control plasma, 0.1 ml		
Patient's plasma, 0.1 ml		
0.85% sodium chloride, 0.1 ml		
Adsorbed plasma, 0.1 ml		
Aged serum, 0.1 ml		

7. Interpretation of results. See Table 7.

DISCUSSION

1. If a factor deficiency is noted, the activated PTT should be performed, using the specific factor-deficient plasma indicated, in a 1:1 dilution with the patient's plasma. When the deficient factor(s) has been positively identified, appropriate specific factor assays may then be performed.
2. In order for the patient's activated PTT to be considered as corrected, the corrected values must fall close to the normal plasma control value.

PROTHROMBIN TIME WITH SUBSTITUTIONS

The prothrombin time substitution test may be performed, along with the activated PTT, when the prothrombin time is prolonged, in order to detect a possible factor VII deficiency.

REFERENCE

Dade Reagents, Inc.: *Coagulation Procedures*, Dade Reagents, Inc., Miami, Fl., 1966.

REAGENTS AND EQUIPMENT

1. Water bath, 37°C.
2. Thromboplastin-calcium chloride mixture.
3. Citrated normal control plasma.
4. Adsorbed plasma. (See Partial Thromboplastin Substitution Test, Reagents and Equipment.)
5. Aged serum. (See Partial Thromboplastin Substitution Test, Reagents and Equipment.)
6. Sodium chloride, 0.85% (w/v).
7. Test tubes, 13 × 100 mm.
8. Stopwatch.

TABLE 7. PROBABLE COAGULATION DEFICIENCIES BASED ON PROTHROMBIN TIME AND ACTIVATED PTT TEST RESULTS

APTT	PT	ADSORBED PLASMA APTT	AGED SERUM APTT	PROBABLE DEFICIENCY
N	N	N	N	No deficiency found
A	N	C	C	XI or XII
A	N	NC	C	IX
A	A	NC	C	X
A	A	C	NC	V
A	N	C	NC	VIII
A	A	NC	NC	II

APTT = Activated partial thromboplastin time. PT = Prothrombin time. N = Normal result. A = Abnormal (prolonged) result. C = Corrected. NC = Not corrected.

SPECIMEN

Citrated plasma: one part 0.11 M sodium citrate to nine parts whole blood.

PRINCIPLE

A prothrombin time is performed on the patient's plasma diluted 1:1 with aged serum, and 1:1 with adsorbed plasma. Together with the results obtained from the activated PTT, it is possible to detect a factor VII deficiency.

PROCEDURE

1. Centrifuge patient's citrated blood at 2500 RPM for 10 minutes immediately after collection.
2. Remove the plasma from the cells immediately and place on ice.
3. Pipet 0.2 ml of thromboplastin-calcium mixture into the appropriate number of 13 × 100-mm test tubes. Warm tubes in the 37°C water bath.
4. Perform the prothrombin time on the following plasmas using the dilutions indicated, and record the results. Each plasma must be tested in duplicate, and the two results averaged.
   A. Patient's plasma.
   B. Normal control plasma.

   If the prothrombin time on the patient's plasma is abnormal, perform a prothrombin time on the following dilutions:
   C. Patient's plasma diluted 1:1 with 0.85% sodium chloride.
   D. Normal control plasma diluted 1:1 with 0.85% sodium chloride.
   E. Patient's plasma diluted 1:1 with normal control plasma.
   F. Patient's plasma diluted 1:1 with adsorbed plasma.
   G. Normal control plasma diluted 1:1 with adsorbed plasma.
   H. Patient's plasma diluted 1:1 with aged serum.
   I. Normal control plasma diluted 1:1 with aged serum.
5. For simplicity, record the results on a chart similar to the one shown following:

	Normal control plasma (1 part)	Patient's plasma (1 part)
Normal control plasma (1 part)		
Patient's plasma (1 part)		
0.85% sodium chloride (1 part)		
Adsorbed plasma (1 part)		
Aged serum (1 part)		

TABLE 8. PROBABLE COAGULATION DEFICIENCIES BASED ON PROTHROMBIN TIME SUBSTITUTION TEST AND ACTIVATED PTT SUBSTITUTION TEST RESULTS

		PROTHROMBIN TIME		APTT		
APTT	PT	ADSORBED PLASMA	AGED SERUM	ADSORBED PLASMA	AGED SERUM	PROBABLE DEFICIENCY
N	N	—	—	—	—	No deficiency found
A	N	—	—	C	NC	VIII
A	N	—	—	C	C	XI or XII
A	N	—	—	NC	C	IX
N	A	NC	C	—	—	VII
A	A	C	NC	C	NC	V
A	A	NC	C	NC	C	X
A	A	NC	NC	NC	NC	II

APTT = Activated partial thromboplastin time. PT = Prothrombin time. N = Normal result. A = Abnormal (prolonged) result. C = Corrected. NC = Not corrected.

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-