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AND PROACCELERIN TO THREE
METHODS FOR MEASURING
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A STUDY OF THE RELATIONSHIP OF CONCENTRATIONS OF PROTHROMBIN, PROCONVERTIN, AND PROACCELERIN TO THREE METHODS FOR MEASURING "PROTHROMBIN TIME"

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THE results of the Quick one-stage prothrombin time depend not only upon prothrombin, but also upon fibrinogen (Alexander and co-workers,^{2, 3} Quick and Hussey²⁰) and upon accessory factors (Alexander and co-workers,² Owen and associates,^{10, 11} Owren,^{12, 13} Quick,¹⁷ Quick and Hussey²⁰) in the test plasma. Modifications of the one-stage technique have been devised, in order to supply fibrinogen and one or more of the accessory factors as reagents, in the attempt to assay more specifically for prothrombin (Owren and Aas¹⁴).

The end point of the "prothrombin time" reaction is the appearance of a fibrin clot, which results from the action of thrombin upon fibrinogen. Fibrinogen is, therefore, a limiting factor in the Quick prothrombin method, in which the test plasma is the only source of fibrinogen. Alexander and associates³ reported a progressive lengthening of the prothrombin time when the concentration of fibrinogen was reduced below 100 mg. per cent.

The present study is a comparison of the effect of serial dilutions of prothrombin and of the accessory factors proconvertin and proaccelerin upon three one-stage methods used for the measurement of prothrombin. The methods under investigation were the prothrombin time of Quick,¹⁸ the prothrombin-proconvertin method of Owren and Aas,¹⁴ and the Russell viper venom-cephalin prothrombin method of Hjort, Rapaport, and Owren.⁸ Normal plasma was diluted with plasma containing all clotting factors except the one under study. This permitted an evaluation of the sensitivity of each method to variations in each clotting factor separately. In addition, the effect of a combined dilution of prothrombin and proconvertin was investigated. Finally, the effect upon the partial thromboplastin (cephalin) time of Langdell, Wagner, and Brinkhous⁹ of varying the concentration of proconvertin and of proaccelerin was investigated.

The terminology of Owren¹⁵ will be used in this paper. Proaccelerin is the labile plasma factor which is present in fresh or fresh-frozen plasma and which remains in the plasma after adsorption with barium sulfate, asbestos, or the usual adsorbents for prothrombin. Proconvertin is the stable plasma factor which is adsorbed onto barium sulfate or an asbestos filter, whose activity is increased by contact with glass,²¹ and which is depressed, along with prothrombin, by administration of Dicumarol and related drugs.

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MATERIALS AND METHODS

Normal Plasma.—Venous blood from normal subjects was mixed 9 parts to 1 with potassium oxalate 0.1 M and centrifuged at 2,000 to 2,500 r.p.m. for 15 to 30 minutes. The plasma was shaken for 5 minutes with Speedex,* 0.4 Gm. per milliliter plasma, and centrifuged at 2,500 r.p.m. for 15 to 30 minutes. This "activated" plasma was used within 6 hours after it was drawn.

Prothrombin-Proconvertin Deficient Plasma.—Normal oxalated plasma was stirred for 5 minutes with barium sulfate (Baker), 100 mg. per milliliter plasma, and centrifuged at 2,500 r.p.m. for 30 minutes. The resulting plasma, free of prothrombin and proconvertin but containing proaccelerin, was used within 6 hours after it was drawn.

Prothrombin Deficient Plasma.—Proconvertin was prepared by coagulating normal blood with thromboplastin and allowing the serum to stand at room temperature for five days. The proconvertin was adsorbed from this serum onto barium sulfate and eluted with sodium citrate; the eluate was dialyzed for 20 hours against sodium chloride and the pH was adjusted to 7.4 (method of Duckert, Koller, and Matter⁷). The solution was stored at -20° C. This preparation (one-sixth the volume of the original plasma) had a proconvertin activity six times that of normal plasma, by the specific proconvertin assay of Aas.¹ In order that an artificial prothrombin deficient plasma might be prepared, normal barium sulfate-adsorbed plasma (5 parts) was mixed with this proconvertin preparation (1 part). The resulting plasma, containing approximately normal concentrations of proconvertin and proaccelerin but deficient in prothrombin, was used within 6 hours after the blood was drawn.

Proconvertin Deficient Plasma.—Citrate plasma (9 parts blood and 1 part sodium citrate dihydrate 3.13 per cent) from a patient having a congenital lack of proconvertin was stored at -20° C. It contained approximately normal amounts of prothrombin and proaccelerin. Proconvertin content was less than 2 per cent.¹

Proaccelerin-Deficient Plasma.—Citrate plasma from a patient having a congenital lack of proaccelerin was stored at -20° C. It contained approximately normal amounts of prothrombin and proconvertin. This plasma is believed to be almost entirely free of proaccelerin, since no activation with thrombin can be demonstrated.

Thromboplastin.—1. Acetone-dried human brain thromboplastin, prepared by the method of Biggs and Macfarlane,⁵ was used in a concentration giving a one-stage prothrombin time of approximately 12 seconds with normal silica-activated plasma and calcium chloride 0.01 M. The activity of this thromboplastin preparation paralleled that of a rabbit brain preparation (Difco†) on serial dilutions of normal plasma in barium sulfate-adsorbed plasma. Eight hundred milligrams of the dried thromboplastin (stored at -20° C.) was incubated with 100 ml. sodium chloride 0.9 per cent at 45 to 50° C. for 30 minutes. This reagent was separated into small lots suitable for one day's use and stored at -20° C. This preparation was used for all estimations of the Quick time.

2. Saline-extracted human brain thromboplastin, prepared according to the method of Owren and Aas¹⁴ and stored at -20° C., was used for estimations of the prothrombin-proconvertin time.

Cephalin.—This reagent was prepared according to the method of Hjort, Rapaport, and Owren.⁸ Cephalin was extracted with ether from acetone-dried human brain, suspended in Veronal buffer, and stored at -20° C.

Russell Viper Venom-Cephalin.—The cephalin suspension was diluted to optimal concentration (1:30) in Veronal buffer. Dried viper venom (Stypven†) was diluted to optimal concentration (1:40,000) in this cephalin suspension.

*Dicalite filteraid: amorphous diatomaceous SiO₂. Dicalite Division, Great Lakes Carbon Corp., New York.

†Rabbit brain thromboplastin. Difco Laboratories, Detroit.

‡Burrhoughs, Wellcome and Co., London.

Bovine Plasma Proaccelerin Reagent.—1. For estimations of the prothrombin-proconvertin time, citrated ox plasma was filtered once through 20 per cent asbestos filter pads and once through 50 per cent asbestos filter pads (Owren and Aas¹⁴). The plasma was stored at -20° C.

2. For estimations of the viper venom-cephalin prothrombin time, oxalated ox plasma was filtered once through 20 per cent asbestos filter pads and then adsorbed with barium sulfate 75 mg. per milliliter plasma (Hjort, Rapaport, and Owren⁸). The plasma was stored at -20° C.

Veronal Buffer.—

Sodium diethyl barbiturate	11.75 Gm.
Sodium chloride	14.67 Gm.
Hydrochloric acid 0.1 N	430 ml.
Distilled water to	2,000 ml.

This buffer has an ionic strength of 0.154 and a pH of 7.35.

*Dilution Fluids.*⁸—

I. Sodium citrate 3.13 per cent	200 ml.
Sodium chloride 0.9 per cent	500 ml.
II. Veronal buffer	200 ml.
Sodium citrate 25.66 mM	200 ml.
Sodium chloride 0.9 per cent	600 ml.

Plasma mixtures were made by diluting normal fresh silica-activated plasma in each of the factor deficient plasmas to concentrations of 1.0, 2.5, 5.0, 7.5, 10, 20, 30, 40, 50, 60, 70, 80, and 90 per cent. In preparing mixtures with prothrombin deficient plasma, 1 part of Veronal buffer was first added to 5 parts of normal plasma, in order to compensate for the dilution of the barium sulfate-adsorbed plasma with proconvertin reagent. The plasma mixtures were tested as follows:

Quick Time.—Two-tenths milliliter acetone-dried human brain thromboplastin (8 mg. per milliliter) was mixed with 0.2 ml. undiluted plasma mixture and incubated at 37° C. for 3 minutes. Then, 0.2 ml. calcium chloride 0.01 M* was added and the clotting time noted. All determinations were done at least in duplicate.

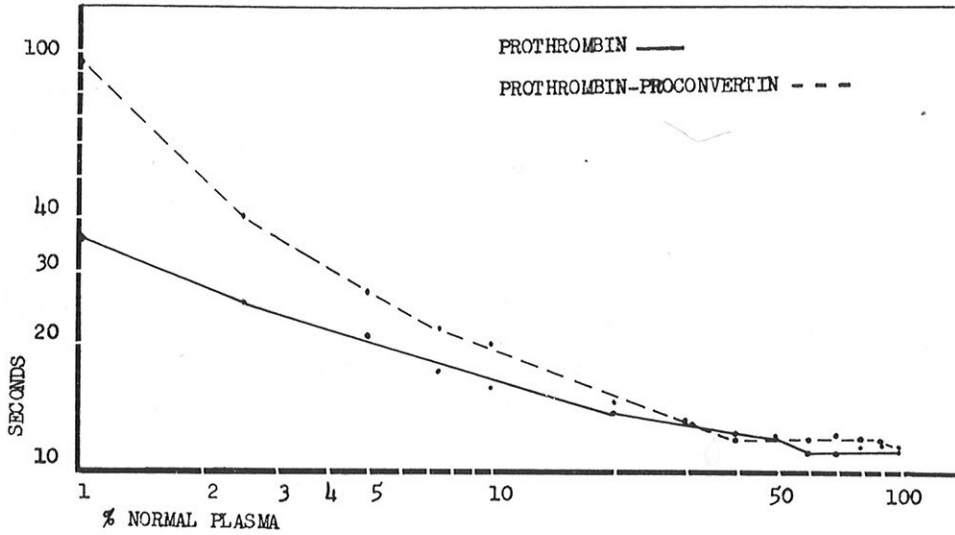
Prothrombin-Proconvertin Time.—The plasma mixtures were diluted 1:10 in fluid II. Two-tenths milliliter saline-extracted human brain thromboplastin, 0.2 ml. ox plasma proaccelerin reagent, and 0.2 ml. diluted plasma mixture were incubated at 37° C. for 3 minutes. The clotting time was noted after the addition of 0.2 ml. calcium chloride 0.035 M.* All determinations were done in duplicate.

Russell Viper Venom-Cephalin Time.—A 1:50 dilution of the plasma mixtures was made by diluting 1:5 in fluid I, and again 1:10 in fluid II. Two-tenths milliliter venom-cephalin reagent, 0.2 ml. ox plasma proaccelerin reagent, and 0.2 ml. diluted plasma mixture were incubated at 37° C. for 3 minutes. The clotting time was noted after the addition of calcium chloride 0.035M.* All determinations were done in duplicate.

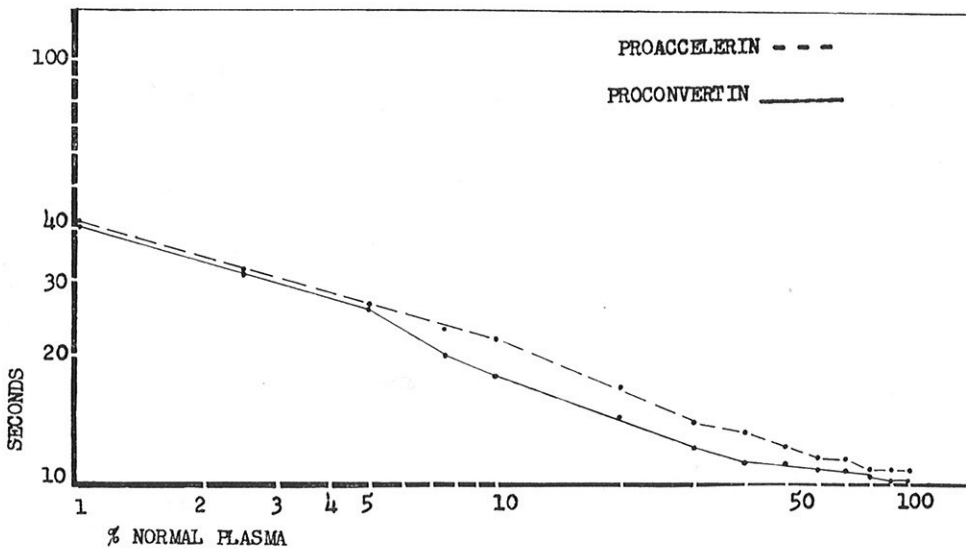
Partial Thromboplastin Time With Cephalin.—Normal citrated plasma (9 parts blood and 1 part sodium citrate 3.13 per cent) which had been stored at -20° C. was used. This normal plasma, as well as the proconvertin deficient and proaccelerin deficient plasmas used in this experiment, was activated with silica before use.

Two-tenths milliliter cephalin 1:50 (in Veronal buffer) was mixed with 0.2 ml. undiluted plasma mixture and immediately placed in a water bath at 37° C. Exactly 6 minutes later, 0.2 ml. calcium chloride 0.02M* was added and the clotting time noted. All determinations were done in duplicate.

*The optimal concentration of calcium as determined for this technique.



A.



B.

Fig. 1.—A, Quick method. Relationship of clotting times to concentrations of prothrombin (—) and of prothrombin and proconvertin (-----). Varying concentrations of prothrombin were obtained by diluting normal plasma in prothrombin deficient plasma. Varying concentrations of prothrombin-proconvertin were obtained by diluting normal plasma in prothrombin-proconvertin deficient plasma.

Zero per cent normal plasma (100 per cent factor-deficient plasma) gave clotting times as follows:

Prothrombin deficient ----- 232 seconds.
Prothrombin-proconvertin deficient ----- not clotted in 19 hours.

B, Quick method. Relationship of clotting times to concentrations of normal plasma diluted in proaccelerin deficient (-----), and proconvertin deficient (—) plasmas.

Zero per cent normal plasma (100 per cent factor-deficient plasma) gave clotting times as follows:

Proaccelerin deficient ----- 100 seconds.
Proconvertin deficient ----- 73 seconds.

RESULTS

The prothrombin-proconvertin estimations¹⁴ and the viper venom-cephalin estimations⁸ were carried out as described in the original methods and as they are done in the routine laboratory. The Quick prothrombin estimation was done according to the method of Quick¹⁸ as closely as was practicable in our laboratory. In order to obtain maximum activity of the proconvertin, and in

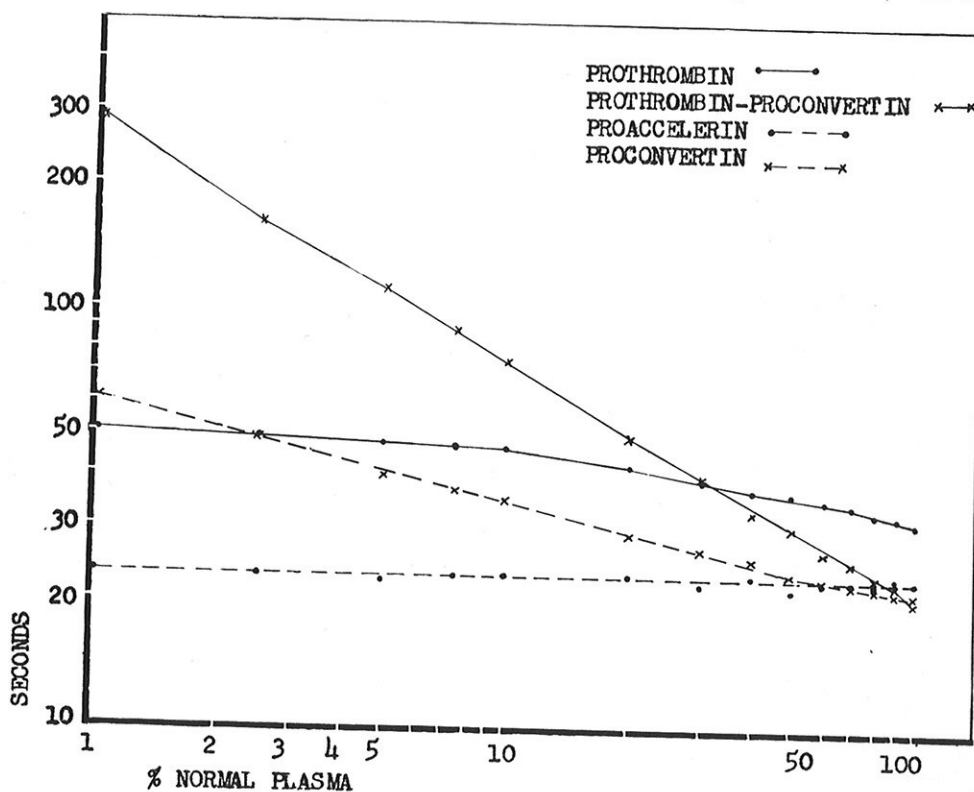


Fig. 2.—Prothrombin-proconvertin method. Relationship of clotting times to concentrations of normal plasma diluted in prothrombin deficient (—●—), prothrombin-proconvertin deficient (X—X), proaccelerin deficient (---), and proconvertin deficient (X---X) plasmas. Zero per cent normal plasma (100 per cent factor-deficient plasma) gave clotting times as follows:

Prothrombin deficient	51.8 seconds.
Prothrombin-proconvertin deficient	840 seconds.
Proaccelerin deficient	23.5 seconds.
Proconvertin deficient	104 seconds.

a simple way eliminate variations in proconvertin activity due to exposure to glass, the normal plasma and the proaccelerin deficient plasma were activated with silica. In the case of the partial thromboplastin time, the proconvertin deficient plasma was also activated with silica, in order to eliminate similar variations in the activity of antihemophilia B factor. By using as diluent a plasma in which only the factors under investigation were absent or had been removed, fibrinogen and all other factors were maintained at approximately normal levels.

1. The *Quick method* (Fig. 1, A and B) reflected decreases in the amounts of prothrombin, proconvertin, and proaccelerin to a roughly parallel degree. With a reduction of any one of these factors from 100 per cent of normal to 40 per cent, no change greater than 2.5 seconds occurred in the clotting times. The method was, therefore, practically insensitive in this range of concentrations. When both prothrombin and proconvertin were varied in the same mixture, the method was again sensitive only between 0 and 40 per cent. In concentrations of these two factors lower than 5 per cent, longer clotting times were observed than with deficiencies of prothrombin, proconvertin, or proaccelerin alone.

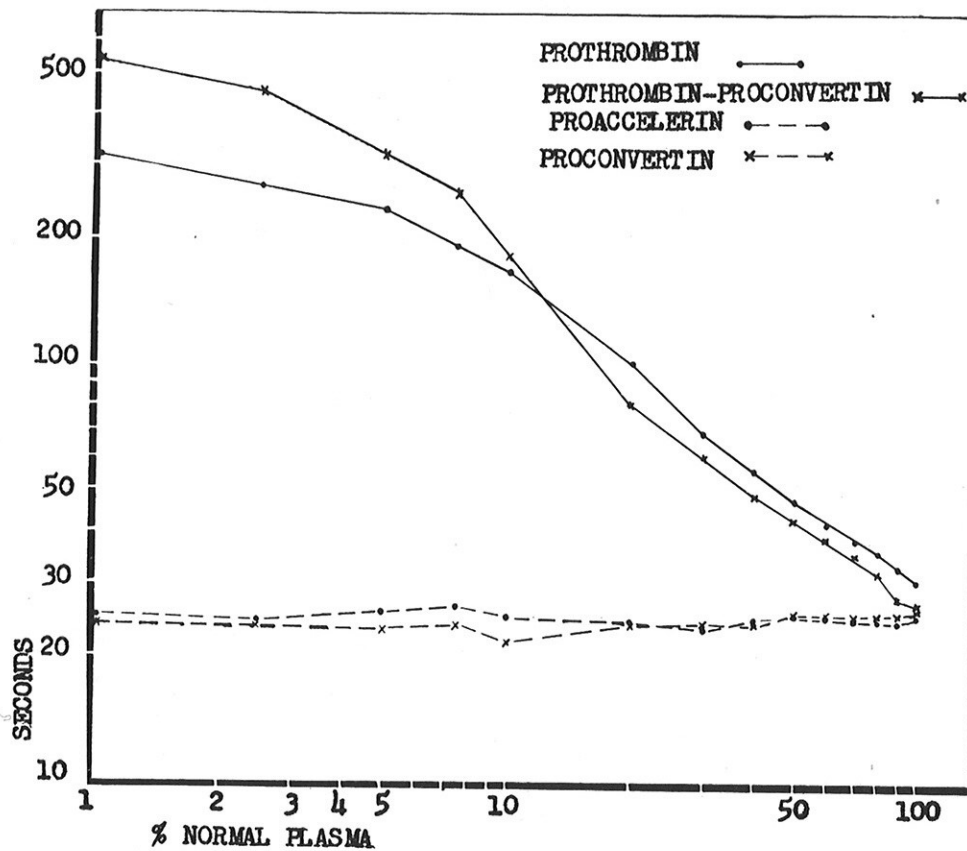


Fig. 3.—Russell viper venom-cephalin method. Relationship of clotting times to concentrations of normal plasma diluted in prothrombin deficient (—●—), prothrombin-proconvertin deficient (X—X), proaccelerin deficient (----), and proconvertin deficient (X---X) plasmas.

Zero per cent normal plasma (100 per cent factor-deficient plasma) gave clotting times as follows:

Prothrombin deficient	-----	409 seconds.
Prothrombin-proconvertin deficient	-----	573 seconds.
Proaccelerin deficient	-----	24.8 seconds.
Proconvertin deficient	-----	24.8 seconds.

2. The *prothrombin-proconvertin method* (Fig. 2) was not affected by variations in the concentration of proaccelerin, which is supplied in the ox

plasma reagent. Decreasing the amount of proconvertin resulted in a steady prolongation of the clotting times. Reductions in prothrombin produced a similar, although markedly lesser, effect. As with the Quick method, the most marked effect was seen when both prothrombin and proconvertin were reduced simultaneously. The range of sensitivity to both prothrombin and proconvertin is between the extremes of 0 and 100 per cent.

3. The *Russell viper venom-cephalin method* (Fig. 3) was not influenced by variations in either proconvertin or proaccelerin. The curves obtained with varied prothrombin and with varied prothrombin plus proconvertin concentrations were similar in shape and height; the slight differences can be accounted for in part by the dilution of prothrombin deficient plasma (5 parts) with proconvertin reagent and buffer (1 part). The range of sensitivity for prothrombin is between 0 and 100 per cent.

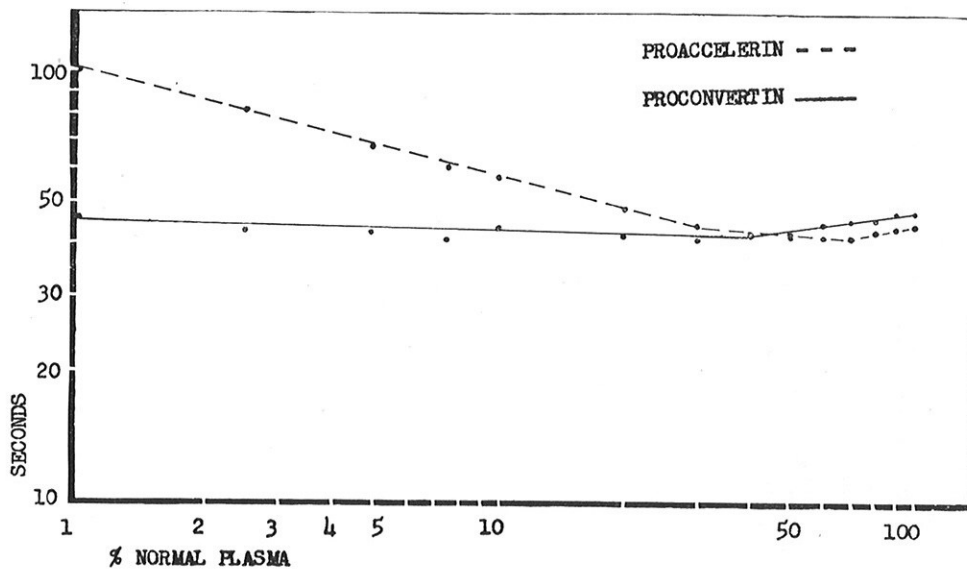


Fig. 4.—Partial thromboplastin (cephalin) time. Relationship of clotting times to concentrations of normal plasma diluted in proaccelerin deficient (-----) and proconvertin deficient (——) plasmas.

Zero per cent normal plasma (100 per cent factor-deficient plasma) gave clotting times as follows:

Proaccelerin deficient	-----	149.6 seconds.
Proconvertin deficient	-----	49.8 seconds.

4. The *partial thromboplastin (cephalin) time* is used primarily to evaluate another phase of coagulation, the formation of "plasma thromboplastin." The effect upon this test of varying the concentrations of proaccelerin and of proconvertin was determined. Normal citrated plasma which had been stored at -20° C. was used in this experiment, in order to compare in this regard with the proaccelerin deficient and proconvertin deficient plasmas, both of which had also been stored at -20° C. (marked differences in the results of the partial thromboplastin time were found between normal fresh and normal stored

plasmas). The results (Fig. 4) indicated that the proaccelerin concentration could be lowered to about 30 per cent before the clotting time was prolonged. Below 30 per cent, a decreased proaccelerin concentration resulted in a steady prolongation of the clotting time. Variations in the concentration of proconvertin had no measurable influence upon the partial thromboplastin time.

DISCUSSION

Our purpose in this study has not been to determine the suitability of one method over others for the measurements of prothrombin conversion or prothrombin concentration, but rather to emphasize the specificities and limitations of methods used in clinical practice. Comparisons between methods have been made by others (Owen and Bollman,¹¹ Soulier and Larrieu²²) in order to analyze suitability or specificity.

The "prothrombin time" can be defined as the interval of time necessary for prothrombin, in the presence of accessory factors and an excess of thromboplastin and calcium, to form thrombin, and for thrombin then to convert fibrinogen to fibrin. Factors other than prothrombin, proaccelerin, and proconvertin have not been considered here, although it is possible that other factors, accelerators, and/or inhibitors, might be concerned.

The reagents used for the Quick method are thromboplastin and calcium chloride; all other factors involved in and necessary for the conversion of prothrombin to thrombin and of fibrinogen to fibrin must be supplied by the test plasma. Therefore, the results of the Quick time represent a summation of their concentration and activity. In the prothrombin-proconvertin method of Owren, an adsorbed ox plasma reagent is used which supplies fibrinogen and proaccelerin, and the result is presumably a measure of the levels in the test plasma of prothrombin and proconvertin. In the Russell viper venom-cephalin method, fibrinogen and proaccelerin are supplied in an adsorbed ox plasma reagent, and the venom-cephalin reagent presumably circumvents the action of proconvertin and the thromboplastin components; therefore, insofar as the known factors are concerned, this method is a measure of prothrombin alone. These suppositions are supported by our experiments.

These experiments also indicate that considerable error may be introduced in preparing dilution curves without controlling the accessory factors. Figs. 1, *A* and *B*, and 2 show the greater effect upon the Quick and the prothrombin-proconvertin times of a combined deficiency of prothrombin and proconvertin over a deficiency of either factor alone. This summation effect is not seen with the viper venom-cephalin method (Fig. 3), which is not sensitive to a deficiency of proconvertin.

The actual clotting of normal blood occurs after only a fraction of the content of prothrombin has been converted to thrombin (Quick and Favre-Gilly¹⁹). Therefore, the speed of prothrombin conversion is of greater significance in the process of hemostasis than the concentration of prothrombin. The determining variable in the Quick prothrombin time is the rate of prothrombin conversion (Owen and Bollman¹¹), except in the case of extreme deficiencies of prothrombin. The method has value as a basic test. For a

quantitative assay of a defect in prothrombin conversion or concentration, however, other methods are superior. The different specificities of the three methods considered here provide a tool for differentiation of coagulation defects caused by deficiencies in prothrombin, proconvertin, and proaccelerin.

The sensitivity of any given method, as well as its specificity, contributes to its suitability for clinical use. Dicumarol and related anticoagulants depress the concentration of both prothrombin and proconvertin. The Quick method and the prothrombin-proconvertin method will reflect this depression, but the Russell viper venom-cephalin method is insensitive to reductions in proconvertin and should not be used in following anticoagulant therapy. The more sensitive methods for prothrombin determination should be preferred over the Quick method when subclinical states of hypoprothrombinemia are in question. Biggs and Douglas⁶ reported a patient having an uncomplicated prothrombin deficiency, in whom a prothrombin concentration of about 11 per cent (two-stage method) gave a Quick time of 18 to 22 seconds (normal 14 seconds). This is in agreement with our experiments (Fig. 1, A in which a prothrombin concentration of approximately 11 per cent gave a Quick time of 16 seconds (100 per cent = 11 seconds).

The partial thromboplastin test with cephaline measures a number of reactions: the formation of plasma thromboplastin, the conversion of prothrombin to thrombin, and the conversion of fibrinogen to fibrin. The data presented here are incomplete with regard to the factors affecting the cephalin time. Nonetheless, it is demonstrated that proconvertin is not essential to the reactions measured by the cephalin time, and thus it is suggested that plasma thromboplastin generation is independent of this factor. This is in agreement with the observation that the thromboplastin generation test is normal in patients having a congenital deficiency of proconvertin (Bergsagel⁴).

SUMMARY AND CONCLUSIONS

1. Three one-stage methods for measuring the "prothrombin time" (Quick method, prothrombin-proconvertin method, and Russell viper venom-cephalin method) were investigated with the use of serial dilutions of prothrombin, proaccelerin, and proconvertin.

2. The *Quick* method was similarly sensitive to variations in each of the three factors. At concentrations above approximately 40 per cent, no significant difference from 100 per cent could be detected for any of the deficiencies studied.

3. The *prothrombin-proconvertin* method of Owren was insensitive to variations in proaccelerin. A marked effect was exhibited when both prothrombin and proconvertin were deficient, and a definite but relatively lesser effect was seen when either prothrombin or proconvertin was deficient. There was a gradual shortening of the clotting time with increasing concentrations, which persisted to a concentration of 100 per cent.

4. The *Russell viper venom-cephalin* method was insensitive to variations in proaccelerin and proconvertin. A combined deficiency of prothrombin and

proconvertin was similar to a deficiency of prothrombin alone. There was a gradual shortening of the clotting time with increasing concentrations of prothrombin, which persisted to a concentration of 100 per cent.

5. The *partial thromboplastin time* with cephaline was prolonged when the concentration of proaccelerin was lower than 30 per cent, but the results were not influenced by a deficiency of proconvertin. This finding suggests that proconvertin may not be involved in the formation of the final prothrombin conversion principle from blood ("plasma thromboplastin"), whereas proconvertin is essential for the formation of this principle from tissue thromboplastin.

REFERENCES

1. Aas, Knut: Prokonvertin og Konvertin, Thesis, Oslo, 1952, Akademisk Trykningsentral, p. 21.
2. Alexander, B., de Vries, A., and Goldstein, R.: Prothrombin: a Critique of Methods for Its Determination and Their Clinical Significance, New England J. Med. 240: 403-413, 1949.
3. Alexander, B., Goldstein, R., Rich, L., Le Bolloc'h, A. G., Diamond, L. K., and Borges, W.: Congenital Afibrinogenemia. A Study of Some Basic Aspects of Coagulation, Blood 9: 843-865, 1954.
4. Bergsagel, D. E.: Stages in the Formation of Blood Thromboplastin, Thesis, 1955, University College of Oxford, 203 pp.
5. Biggs, R., and Macfarlane, R. G.: Human Blood Coagulation. Oxford, 1953, Blackwell Scientific Publications, p. 344.
6. Biggs, R., and Douglas, A. S.: The Measurement of Prothrombin in Plasma. A Case of Prothrombin Deficiency, J. Clin. Path. 6: 15-22, 1953.
7. Duckert, F., Koller, F., and Matter, M.: Purification and Physiological Properties of Factor VII From Plasma and Serum. Separation From Prothrombin, Proc. Soc. Exper. Biol. & Med. 82: 259-261, 1953.
8. Hjort, P., Rapaport, S. I., and Owren, P. A.: A Simple, Specific One-Stage Prothrombin Assay Using Russell's Viper Venom in Cephalin Suspension, J. LAB. & CLIN. MED. 46: 86-97, 1955.
9. Langdell, R. D., Wagner, R. H., and Brinkhous, K. M.: Effect of Antihemophilic Factor on One-Stage Clotting Tests, J. LAB. & CLIN. MED. 41: 637-647, 1953.
10. Owen, C. A., Hoffman, G. R., Ziffren, S. E., and Smith, H. P.: Blood Coagulation During Infancy, Proc. Soc. Exper. Biol. & Med. 41: 181-185, 1939.
11. Owen, C. A., and Bollman, J. L.: Analysis of the One-Stage Test for Prothrombin Time, J. Appl. Physiol. 5: 515-525, 1953.
12. Owren, P. A.: The Coagulation of Blood. Investigations on a New Clotting Factors. Oslo, 1947, J. Chr. Gundersen, pp. 19-42.
13. Owren, P. A.: Proconvertin, the New Clotting Factor, Scandnav. J. Clin. & Lab. Invest. 3: 168, 1951.
14. Owren, P. A., and Aas, K.: The Control of Dicumarol Therapy and the Quantitative Determination of Prothrombin and Proconvertin, Scandnav. J. Clin. & Lab. Invest. 3: 201-208, 1951.
15. Owren, P. A., Rapaport, S. I., Hjort, P., and Aas, K.: The Biochemistry of Thromboplastin, Its Formation and Action, Sang 25: 752-765, 1954.
16. Quick, A. J., Stanley-Brown, M., and Bancroft, F. W.: A Study of the Coagulation Defect in Hemophilia and in Jaundice, Am. J. M. Sc. 190: 501-511, 1935.
17. Quick, A. J.: On the Constitution of Prothrombin, Am. J. Physiol. 140: 212-220, 1943.
18. Quick, A. J.: The Physiology and Pathology of Hemostasis, London, 1951, Henry Kimpton, pp. 125-134.
19. Quick, A. J., and Favre-Gilly, J. E.: The Prothrombin Consumption Test: Its Clinical and Theoretical Implications, Blood 4: 1281-1289, 1949.
20. Quick, A. J., and Hussey, C. V.: Interpretation of the One-Stage Method for Determining Prothrombin Time, New England J. Med. 248: 624-628, 1953.
21. Rapaport, S. I., Aas, K., and Owren, P. A.: The Effect of Glass Upon the Activity of the Various Plasma Clotting Factors, J. Clin. Invest. 34: 9-19, 1955.
22. Soulier, J. P., and Larrieu, M. J.: Etude Analytique des Temp de Quick Allongés. Dosages de Prothrombine, de Proconvertine et de Proaccélérine, Sang 23: 549-559, 1952.