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Prothrombin Estimation: A Procedure and Clinical Interpretations.

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This work deals with a standardized modification of estimating prothrombin concentration (or activity). It places especial emphasis upon the difference between the prothrombin times of whole and diluted plasmas, taking into consideration the respective absolute values of these readings. By these 3 measurements it appears possible to detect certain abnormalities of blood not revealed by the prothrombin time of whole or diluted plasma considered alone.

The technic used for the estimation of prothrombin was that described by Link and his coworkers[1] based on the method of Quick, Stanley-Brown, and Bancroft.[2] In the present study two series of estimations were made: one using the thromboplastin-calcium chloride mixture of Link and his students[1] and the other using Russell snake-viper venom* [3, 4] in place of rabbit brain extract.

Procedure. 4.5 ml freshly drawn venous blood were added to 0.5 ml M/10 sodium oxalate. Clear plasma was obtained by centrifuging. 0.1 ml whole plasma which had been kept at 37°C was quickly added to 0.2 ml thromboplastin-calcium chloride mixture (also at 37°C) and the time which elapsed before the fibrin clot formed was noted with a stop-watch. The identical procedure was then repeated using 0.1 ml of 12.5% plasma in place of whole plasma.

When venom was substituted for the thromboplastin the technic was as follows:

0.1 ml whole plasma was added to 0.1 ml venom and placed in the constant temperature bath at 37°C for 5-10 minutes. To this was quickly added 0.1 ml M/40 calcium chloride which had been kept

1 Campbell, H. A., Smith, W. K., Roberts, W. L., and Link, K. P., *J. B. C.*, 1941, **138**, 1.

2 Quick, A. J., Stanley-Brown, M., and Bancroft, F. W., *Am. J. Med. Sci.*, 1935, **190**, 501.

* The Russell snake-viper venom was supplied through the kindness of Dr. R. C. Page of Burroughs Wellcome Co.

3 Fullerton, H. W., *Lancet*, 1940, **2**, 195.

4 Page, R. C., and Russell, H. K., *J. Lab. and Clin. Med.*, 1941, **26**, 1366.

TABLE I.

	Thromboplastin-calcium chloride			Russell snake-viper venom		
	Whole plasma	12.5% plasma	Difference	Whole plasma	25% plasma	Difference
Range	20-27	36-47	11-22	12-22.5	20-39.5	7-17.5
Mean	24	41.4	18.5	19.5	29	10.5
Standard deviation	±2.3	±3.8	±3.5	±2.9	±4.6	±2.5

at the same temperature and the time which elapsed before the fibrin clot formed was noted with a stop-watch. The same procedure was repeated with 0.1 ml of 25% plasma in place of whole plasma.

The estimations were done in duplicate.

Dilution of one part of plasma with 7 parts of 0.85% saline permits of a clearly detectable and satisfactorily reproducible end-point when the thromboplastin-calcium chloride mixture is used. With snake venom, this dilution often does not give a reliable end-point, especially when the prothrombin time is prolonged. 25% plasma was found to be better suited for this when venom was used.

The data obtained by the use of these two methods of estimating prothrombin time in two groups of 20 normal individuals is compared in Table I.

The difference between the prothrombin time of whole and of diluted plasma increases as the concentration (or activity) of the prothrombin decreases. This inverse relationship was observed in

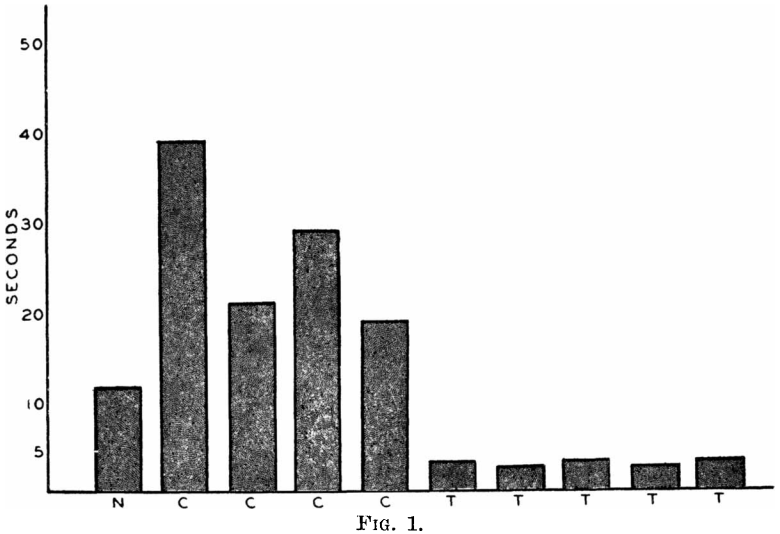

FIG. 1.

The difference between the prothrombin times of whole and dilute plasmas of 10 individuals is shown in stippled columns. N—a normal individual, C—cases of hepatic cirrhosis, T—cases of embolization. Note reduction of difference to below normal in thromboembolization.

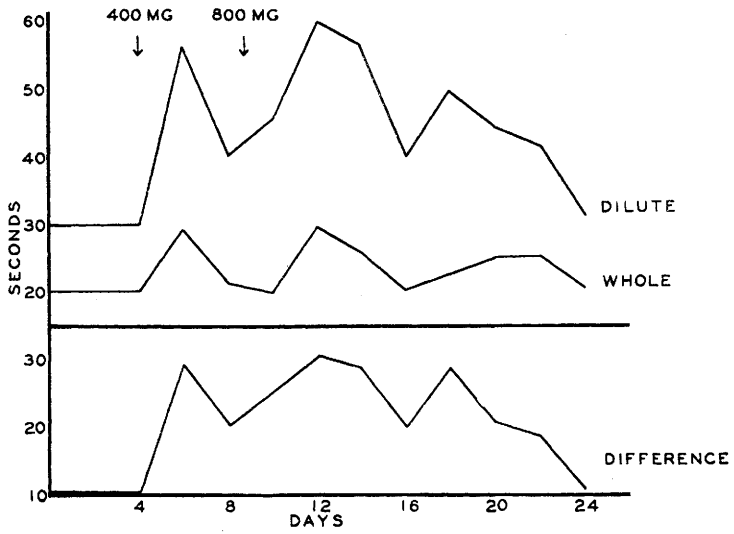

FIG. 2.

The response to different dosages of 3,3′methylenebis (4-hydroxycoumarin) of simultaneously estimated whole and diluted plasma prothrombin times and of the difference is shown. The difference increases considerably more than the whole plasma prothrombin time and is less variable than the dilute.

four cases of cirrhosis of the liver believed to have hypoprothrombinemia (Fig. 1). It is also illustrated in serial estimations following the administration of 3,3′-methylenebis (4-hydroxycoumarin)† which reduces the level (or activity) of prothrombin (Fig. 2). The point at which the dilute plasma prothrombin time becomes substantially prolonged while the whole plasma prothrombin time is only moderately extended becomes an excellent guide to the therapeutic use of this substance.

A state of hyperprothrombinemia is theoretically a possibility. To be able to detect this condition, should it occur, is obviously desirable. If the differences between the prothrombin times of whole and diluted plasma are an expression of the prothrombin concentration (or activity) then it is clear that hyperprothrombinemia is one of the factors responsible for a reduction of this figure to a point lower than that observed in normal plasmas.‡ Such reduction was observed in 5 cases (Fig. 1).

† Commonly designated as "Dicumarol." The compound was supplied through the courtesy of Dr. K. K. Chen of Eli Lilly and Co.

‡ In 2 such cases the difference between whole and 8% plasma was also estimated. In one, the difference (using venom) was 24 sec. while a normal control showed no end point in this dilution. In the second, this difference was 16.8 sec. and that of a normal control 30 sec. (Thromboplastin-calcium chloride mixture used.) This is to be studied further by the method of Warner *et al.*[5]

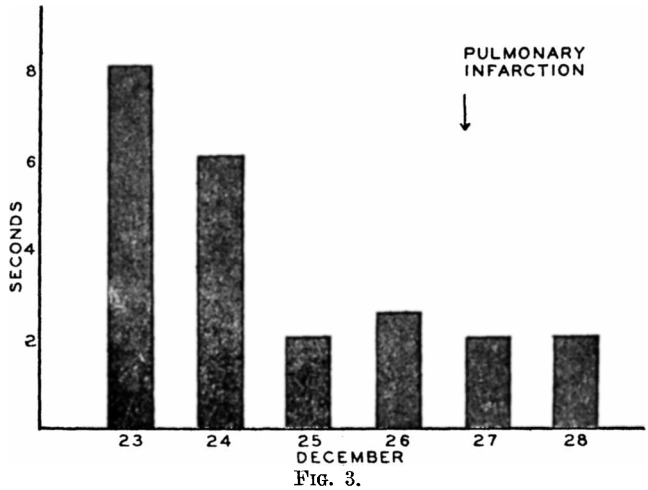

FIG. 3.

Progressive decrease in difference between prothrombin time of whole and diluted plasma (height of stippled columns) in a case of post-operative thromboembolization is illustrated.

In 3 cases of thromboembolization (2 after surgical operations and one postpartum) it was observed that simultaneously with, or one or 2 days prior to, the appearance of signs of pulmonary infarc-

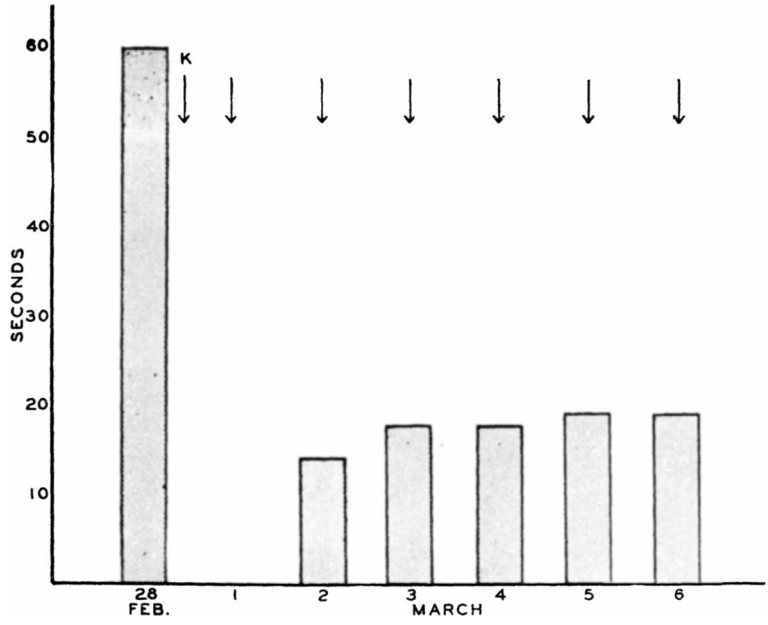

FIG. 4.

The effect of vitamin K in a case of hypoprothrombinemia upon the difference between prothrombin time of whole and 12½% plasma. Therapy was commenced on 2/28/42, after the original estimations. Arrows indicate successive daily injections of 3.2 mg intramuscularly.

tion, the difference between the prothrombin time of whole and diluted plasma became reduced (Fig. 3). The present procedure may, therefore, be of use in selecting patients who are likely to experience thromboembolization (if the observation proves to be constant) and in instituting appropriate prophylactic therapy.

That the extent of the difference between the prothrombin time of whole and diluted plasma is a fairly accurate expression of the prothrombin level (or activity) was further indicated in following the response of hypoprothrombinemic blood to vitamin K therapy (Fig. 4).

The presence of an anticoagulant in the circulating blood has been demonstrated.[6, 7] The possible liberation, under certain pathologic conditions of an excess of this or similar substances into the blood consequently exists. If dilution of these anticoagulant substances renders them ineffectual, it is possible that the prothrombin time of diluted plasma may become as short or even shorter than that of whole plasma at a time when that of whole plasma is lengthened by the activity of the anticoagulant substances. Under these circumstances, the detection of such a condition might be possible by the procedure just described. A case exhibiting a tendency to bleeding suggests that this is so. The prothrombin time of the whole plasma was 51 seconds and that of 12.5% plasma 40 seconds. The estimation of diluted plasma prothrombin time, which would be relatively independent of anticoagulant effect, indicated a normal prothrombin content (or activity), a fact which was verified by the Warner, Brinkhous, Smith method, the result of which was 80% of normal. The reversal of the normal relationship may then be interpreted as excess anticoagulant substances in the blood.

Summary. The difference between prothrombin times of whole and diluted plasma is a more reliable guide to prothrombin activity of the blood than is the prothrombin time of either alone. The difference has been found to be fairly constant in normal individuals. When prolonged, it indicates hypoprothrombinemia. When reduced, it may signify hyperprothrombinemia or an excess of anticoagulants in the blood. The procedure recommended is well suited for following the therapeutic effects of agents which influence the prothrombin level (or activity) such as dicumarol or vitamin K.

[5] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

[6] Howell, W. H., *Am. J. Physiol.*, 1910, **26**, 453; 1911, **29**, 187.

[7] Eagle, H., *J. Gen. Physiol.*, 1934-35, **18**, 531, 547, 818.