AN IMPROVED METHOD FOR THE DETERMINATION OF "PROTHROMBIN TIME"

QUICK[1] has described a method for evaluating 'prothrombin time' by noting the time-interval for the formation of a clot in the plasma when it is mixed with a solution of thromboplastin and calcium. The procedure may be briefly represented as follows:—[See (A).]

(A) 0·1 c.c. Plasma (oxalated) + 0·1- c.c. Thrombo-plastin sol. (prepared from rabbit's brain) + 0·1 c.c., 0·1 M. $CaCl_2$ ——— > Appearance of clot (Prothrombin time) = 12 to 13 seconds.

(B) 0·2 c.c. Plasma (oxalated) + 0·2 c.c. Russel Viper Venom (1–10,000) + 0·2 c.c., 0·025 M. $CaCl_2$ ——— > Appearance of clot (Prothrombin time) = 18 to 25 seconds.

'Prothrombin time' obtained by this method has often yielded divergent results and minor modifications in the details of the technique have therefore been recommended by Dam,[2] and Kato and Poncher,[3] with a view to obtain more uniform and dependable results. However, apart from the technical difficulty of observing accurately the end-point (formation of the fibrin web in a rather opaque solution provided by the rabbit-brain extract), the chief disadvantage of Quick's technique in routine determinations lies in the fact that a fresh thromboplastin preparation has to be obtained every time that a determination is made —a procedure which is not only inconvenient and time-consuming, but is open to the objection that the thromboplastin preparation employed in the reaction, being freshly made each time, may and actually does, vary in potency from batch to batch. In a standard technique, on which the interpretation of data of diagnostic and prognostic value will depend, such variation in potency of a reaction component is obviously not desirable, and may give misleading results.

Taking advantage of the thromboplastic property of Russel Viper Venom, Fullerton[4] has recommended a modified method wherein the thromboplastin preparation from rabbit's brain extract is replaced by a solution of Russel Viper Venom of standard strength. [See (B).]

The author recommends that every time a prothrombin test is performed, an ampoule

containing viper venom (0·1 mg.) in dry form (commercially available as "Stypven" or "Russven", B.W. & Co., or Boots Pure Drug Co.) should be dissolved in 1 c.c. distilled water *immediately before use.* The viper venom in solution, according to the author, is liable to rapid deterioration.

Hobson and Witts[5] have recently reported that the Fullerton technique, while an improvement on the original Quick procedure, is still not quite satisfactory. When viper venom *alone,* even in its optimum concentration of 1–20,000, is used as the thrombokinase in the reaction, the clotting time obtained is often very much delayed (18 to 25 seconds instead of 12 to 13 seconds by the Quick method) and the range of variation is also liable to be wider than usual. To obviate these difficulties, the authors have suggested the addition of *lecithin* to the venom solution. [See (C).]

(C) 0·1 c.c. Plasma + 0·1 c.c. R.V. Venom–lecithin reagent + 0·1 c.c. 0·025 M. $CaCl_2$
(lecithin 5 mg./c.c. venom)
———> Appearance of clot (Prothrombin time) = 8 to 11 seconds.

(D) 0·2 c.c. Oxalated plasma + 0·2 c.c. R.V. Venom (1–20,000) in 0·025 M. $CaCl_2$ ———
(kept at a temp. of 37°–38° C.) (from stock solution)
———> Appearance of clot (Prothrombin time) = 8 seconds.

While working on the relationship between plasma trypsin and blood coagulation, it was necessary to determine the 'prothrombin time' in a number of physiological and pathological conditions as a preliminary to further work. After many trials with both the Quick (A) and the Fullerton (B) techniques, it was realised that there is considerable room for the improvement of this useful clinical test. Experience with the Fullerton technique indicated that the method would yield quite satisfactory results, if the dilution of the prothrombin in the plasma could be reduced and the speed of the thromboplastin—prothrombin—calcium reaction accelerated. It was found that both these problems could be solved by adding *directly* to the plasma 0·2 c.c. of 1 in 20,000 venom solution in 0·025 M. $CaCl_2$ as shown below:—[See (D).]

The total volume of the reaction mixture was thus reduced to 0·4 c.c. [instead of 0·6 c.c. when the thromboplastin and calcium solutions were added separately as in (B)], thereby resulting in an increase in concentration of prothrombin in the reaction mixture by about 33 per cent. A further improvement was rendered possible when it was discovered that, contrary to previous conceptions, a solution of viper venom (1 in 20,000) in water or in 0·025 M. $CaCl_2$ can be maintained under stable conditions under toluene at a temperature of about 5° C. A stock solution of venom can therefore be used in prothrombin determinations instead of a fresh solution prepared from dry venom for each test, as is demanded by the Fullerton (B) technique. The simultaneous addition of thromboplastin and calcium further brings down the clotting time to 8 seconds, thus making it unnecessary to add lecithin to the venom solution, as recommended by Hobson and Witts (C).

The advantages of our method may be briefly stated as follows:—

(*a*) It permits the employment of a stable stock solution of thromboplastin of constant potency. In routine testing of a large number of samples, the availability of a ready-made standardised thromboplastin solution is often a real advantage.
(*b*) The addition of thromboplastin and calcium in one solution abolishes the time-interval of the thromboplastin—prothrombin reaction, which can take place only in the presence of calcium.
(*c*) The 'Prothrombin time' is speeded up on account of the increased concentration of prothrombin in the reaction mixture.
(*d*) The clot formed is well marked, as the fibrinogen is not diluted to the same extent as in Fullerton's method.

Since the isolation of Vitamin K and its therapeutic utilisation in certain hæmorrhagic conditions, the determination of the prothrombin level in blood has attracted considerable attention. The improved method for the determination of 'Prothrombin time' outlined here will, it is hoped, be found of particular interest

to clinical hematologists and other laboratory workers.

The details of this investigation will appear elsewhere. Our thanks are due to Colonel Sir Ramnath Chopra for his encouragement and guidance.

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[1] Quick, *J. Amer. Med. Assn.*, 1938, **110,** 1658.
[2] Dam, Tage-Hausen and Plum, *Lancet*, **1939, 2,** 1157.
[3] Kato and Poncher, *J. Amer. Med. Assn.*, 1940, **112,** 749.
[4] Fullerton, *Lancet*, August 17, 1940, **195.**
[5] Hobson and Witts, *Lancet*, Aug. 24, 1940, **247.**