Thromboplastin Time (Prothrombin Time)

Heinrich Südhof

Principle

By thromboplastin time (prothrombin time) is meant the *time* required for coagulation after the addition of an optimum amount of Ca^{2+} and excess thromboplastin to blood plasma. Contrary to the original idea of *Quick* [1,2] the coagulation time obtained under these conditions estimates not only the prothrombin (Factor II) content of the plasma, but also a series of other blood coagulation factors: Factor V (proaccelerin-accelerin), Factor VII (proconvertin-convertin) and Factor X (Stuart-Prower Factor). A large increase of antithrombin or a decrease of fibrinogen also affect the prothrombin time.

The "Quick *value*" corresponding to the thromboplastin time is read off from a *standard curve*. This value gives no indication of the amounts of the individual coagulation factors contained in the plasma, but merely gives the coagulation activity as a percentage of normal: the Quick value indicates how much normal plasma must be diluted so as to have the same coagulation time as the patient's plasma.

The thromboplastin added to the citrated plasma, in conjunction with the above mentioned factors, acts as "prothrombin conversion factor". In the presence of Ca^{2+} ions, the prothrombin contained in blood plasma is converted to thrombin which initiates clot formation ("Phase I of blood coagulation"). In its turn the thrombin acts on the complicated process of "Phase II of blood coagulation", which starts with the transformation of fibrinogen and ends with the formation of the fibrin clot.

Standardized and stable thromboplastin preparations must be used for the determination of the thromboplastin time. The same plasma can have different thromboplastin times depending on the type of thromboplastin preparation. The reason for this is that particular thromboplastin preparations differ in their reactions to the individual coagulation factors.

Commercially Available Thromboplastin Preparations

Thrombokinase — "Behring-Werke":

Monkey brain thrombokinase, very strongly Factor VII sensitive; obtainable in ampoules, the contents of which should be dissolved in 1 ml. distilled water.

Thrombokinase — "Geigy":

Thrombokinase from lung extracts. Factor II or X sensitive; changes in the content of other factors are less detectable. It is marketed in tablet form. Each tablet contains sufficient calcium so that the suspension prepared from the tablets is 0.1% with respect to $CaCl_2$.

Thromboplastin solution — "Roche":

Extracts from human brain. Factor VII sensitive. Ampoules containing 1 and 5 ml. The solution contains gelatine. If stored in a refrigerator the contents solidify, so that the gel must be carefully liquified before use by allowing it to warm to room temperature or by warming in a water bath at 37°C.

---

[1] *A. J. Quick, M. Stanley-Brown* and *M. Bancroft*, Amer. J. med. Sci. *190*, 501 [1935].
[2] *A. J. Quick*, Amer. J. Physiol. *118*, 260 [1937].

Determination of the Thromboplastin Time According to Quick[3)]

(Laboratory Method)

Reagents

1. Thromboplastin solution "Roche" *)
2. Sodium citrate, $Na_3C_6H_5O_7 \cdot 5^1/_2\ H_2O$
3. Calcium chloride, $CaCl_2$, anhydrous
4. Sodium chloride

Preparation of Solutions

I. Thromboplastin:
Use a commercial preparation undiluted.

II. Sodium citrate (0.1 M):
Dissolve 3.6 g. $Na_3C_6H_5O_7 \cdot 5^1/_2\ H_2O$ in 100 ml. distilled water.

III. Calcium chloride (2.5×10^{-2} M):
Dissolve 0.275 g. $CaCl_2$ in 100 ml. distilled water. 10 ml. of this solution should be equivalent to 5 ml. 0.1 N $HgNO_3$ solution (indicator: potassium chromate).

IV. Sodium chloride (0.85% w/v):
Dissolve 0.85 g. NaCl in 100 ml. distilled water.

Procedure

Experimental material

Draw up

0.2 ml. Na citrate solution (II)

with a 2 ml. injection syringe. With light pressure on the upper arm puncture the cubital vein and withdraw

1.8 ml. blood.

Mix the contents of the syringe by carefully withdrawing the plunger somewhat further and tilting back and forth. Transfer the blood to a centrifuge tube and centrifuge for 15 min. at 3000 r.p.m. Carefully take off the plasma and store at room temperature until ready for the determination (no later than 2 hours after collecting the blood).

Assay

Duplicate determinations are recommended. Pre-warm the thromboplastin and calcium chloride solution to 37°C (water bath). Pipette into two test tubes (length: 8–10 cm.; diameter: 0.9–1.3 cm.)

0.1 ml. plasma

and place in a water bath at 37°C. After equilibration, mix into each tube

0.1 ml. thromboplastin solution (I)

0.1 ml. calcium chloride solution (III).

On mixing in the $CaCl_2$ solution (blow out the pipette) start a stopwatch. Draw a sterile, cold platinum loop once or twice every second through the reaction mixture. As soon as threads of fibrin hang on the loop stop the stopwatch. The time recorded is the thromboplastin time. The Quick value is obtained from this by means of a standard curve.

*) or any other commercially available thrombokinase preparation. In which case the procedure given by the manufacturer should be used.

3) *A. J. Quick:* The Physiology and Pathology of Hemostasis. Henry Klimpton, London 1951, p. 125.

Standard curve

Dilute normal plasma (obtained as described in the Section "Experimental material") with 0.85% NaCl solution (IV) in the ratios of 20:100, 40:100, 60:100 and 80:100 *). Determine the thromboplastin times for these dilutions and for the original plasma (see under "Assay"). Plot the measured coagulation times (ordinate) against the % by volume of plasma in the dilute solutions (= Quick value; abscissa) on double logarithmic graph paper. This permits easier reading and the detection of errors in the method on drawing the standard curve (see Fig. 1a and b).

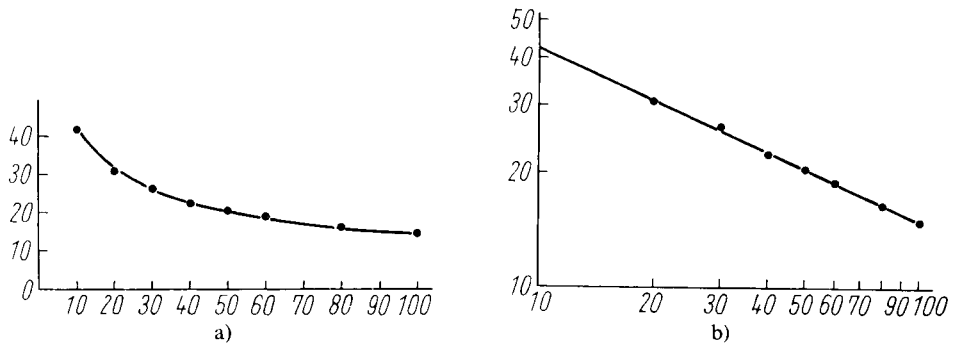

Fig. 1. Standard curve (relation between the thromboplastin time and the Quick value).
a) Linear graph paper
b) Double logarithmic graph paper
Ordinates: Thromboplastin time [sec.]
Abscissa: Quick value (= plasma dilution) [% by volume plasma in the dilute solutions]

"Geigy" Micromethod [5]

(Whole blood method, also suitable as a bedside method)

Reagents

1. Thrombokinase, "Geigy"
2. Sodium citrate, $Na_3C_6H_5O_7 \cdot 5^1/_2\ H_2O$
3. Sodium chloride

Preparation of Solutions

I. Thrombokinase suspension:
Grind a "Geigy" thrombokinase tablet in a thick-walled test tube with a glass rod. Stir the powder with a few drops of distilled water to form a homogeneous paste. Suspend this paste in 2.5 ml. distilled water and incubate for 15 min. in a water bath at 37°C (constant). Use this warm suspension.

II. Sodium citrate (3.8% w/v):
Dissolve 3.8 g. $Na_3C_6H_5O_7 \cdot 5^1/_2\ H_2O$ in 100 ml. distilled water.

III. Sodium chloride (0.85% w/v):
Dissolve 0.85 g. NaCl in 100 ml. distilled water.

*) This procedure is satisfactory for routine studies. For research purposes prepare the plasma dilutions with fibrinogen solution or better still with barium adsorptive plasma [4] instead of NaCl solution or veronal buffer.

[4] *P. A. Owren* and *K. Aas*, Scand. J. clin. Lab. Invest. *3*, 201 [1951].

[5] *C. Montigel*, Therap. Umschau *9*, 17 [1952].

Procedure

Collection of blood

In a blood sugar pipette containing

0.01 ml. Na citrate solution (II)

suck up

capillary blood to the 0.05 ml. mark

(from the finger, or with babies from the heel). Rapidly blow out the contents of the pipette into a small test tube.

Assay

Triplicate determinations are advisable. Pre-warm citrated blood for 2 min. in a water bath at 37°C, add

0.08 ml. of the throughly mixed and pre-warmed (37°C) thrombokinase suspension (I)

and start a stopwatch. Incubate the test tube in the water bath at 37°C with continual gentle tipping until the appearance of coagulation is visible to the naked eye. Stop the stopwatch. The time recorded is the thromboplastin time. To obtain the corresponding Quick value, see p. 910.

Standard curve

Obtaining plasma: Draw up 1 ml. Na citrate solution (II) with a 5 ml. injection syringe and with light pressure on the upper arm withdraw blood to the 5 ml. mark. Mix the contents of the syringe, centrifuge for 10 min. at *ca.* 2000 r.p.m., siphon off the plasma and dilute as described on p. 910 under "Standard curve".

Proceed as described on p. 909 under "Assay", but instead of the thromboplastin and $CaCl_2$ solution add only 0.1 ml. thrombokinase suspension to 0.1 ml. plasma. Plot the coagulation time against the dilution of the plasma (see p. 910 and Fig. 1).

For routine studies the prothrombin scale provided by Geigy can be used instead of a standard curve[6].

Other Information

Apart from the two procedures described here there are several well-tried, slightly modified methods[7]. These include the so-called bedside method which enables the thromboplastin time to be determined directly at the bedside of the patient[8,9].

Coagulation of the mixture can also be detected by adding a small glass bead (35 to 40 mg.) to the reaction mixture and carefully turning the sloping test tube on its longituidinal axis until the glass bead is caught by the fibrin clot. The paper strip method described by *Studer* and *Winterstein* has the advantage that the reaction mixture is not moved[10]. For large laboratories the automatic electric apparatus (Coagulometer)[11] manufactured by Heinrich Schnittger, Bracke/Lippe, Germany is suitable. With this apparatus several determinations can be carried out simultaneously and the personal error is eliminated.

---

[6] *F. K. Beller* and *Th. Deggelmann*, Die Medizinische *1954*, 395.
[7] *H. E. Schultze* and *G. Schwick*, Laboratoriumsblätter der Behring-Werke, Heft 2, Oct. 1953.
[8] *N. Fiechter*, Schweiz. med. Wschr. *21*, 259 [1940].
[9] *F. K. Beller:* Überwachung gerinnungshemmender Maßnahmen. In *Th. Naegli* et al.: Die thrombembolischen Erkrankungen. 2nd Edition, Verlag Schattauer, Stuttgart 1960, p. 404.
[10] See the instructions enclosed with the "Roche" thromboplastin solution.
[11] *H. Schnittger* and *R. Gross*, Klin. Wschr. *32*, 1011 [1954].

In medical literature many other names have been used for thromboplastin time, *e.g.* one stage coagulation index, coagulation power, coagulation index, coagulation time, prothrombin content, prothrombin index, prothrombin level, prothrombin value, prothrombin time, Percentage Quick. It is now generally accepted that the values are expressed as percentage activity (Quick value).

It is important that the experimental conditions should be adhered to exactly. In particular, the pipetting of the solutions must be exact and the temperature of the water bath must be kept constant. A new standard curve is required for each change of the experimental conditions and for each thromboplastin preparation from another source. The plasma for the standard curve must be obtained in the same way as for the routine determinations.

The determination of the thromboplastin time is used to follow the rate of clotting of blood from patients on anticoagulant therapy (anticoagulants, *e.g.* in myocardial infarction and thrombosis). In addition, it is a routine determination in the diagnosis of haemorrhagic diathesis (haemophilic diseases). In the *interpretation* of the Quick value both the *special sensitivity* of the particular thrombokinase and the *different half-life times* of the reacting coagulation factors must be taken into account. For example, 25% Quick for Geigy and Roche thrombokinase when read off from the respective standard curves need not necessarily give the same coagulation time. Factor VII sensitive thrombokinase gives lower Quick values earlier than Factor II or X sensitive[12].

[12] *P. Matis*, *W. Mayer* and *W. Nagel*, Med. Welt 1961, 891.