Blood Coagulation Simplified

F. NOUR-ELDIN

Second Edition

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Preface to the Second Edition

The popularity of the first edition is undoubted; it was well received by haematologists, pathologists, medical laboratory technologists, general practitioners, medical undergraduates and post-graduates and science students. Nevertheless, I am grateful to my colleagues, in Great Britain and abroad, who offered constructive criticism and comments; these are now met despite the difficulty in deciding on where 'to draw the line' for the less initiated.

Since the publication of the first edition, new developments in the field have continued. Apart from the necessary additions, the chapters on haemophilia and fibrinolysis are revised and extended and a new chapter incorporated to deal with thrombosis. The easy-to-understand style is maintained without loss of substance.

I wish to thank the Editor of the *British Medical Journal* and the Canadian Society of Laboratory Technologists for permission to use *Figures 10* and *6* respectively. The skilled secretarial help of my wife has greatly lightened the task of preparing a new edition.

F. NOUR-ELDIN

Preface to the First Edition

In the past few years not only have blood coagulation studies advanced but also the tests are now being routinely used in many hospitals. In addition, one cannot escape the fact that this subject now forms a part of the medical and technical curriculum and is a regular visitor among the written questions and in the practical examinations of the Institute of Medical Laboratory Technology. This gave me the incentive to write this small book in the hope that it will be of help to technicians and students preparing for the final examination in haematology.

Although certain relevant theories and views are presented, it is not my intention here to delve into highly academic points for which many valuable detailed monographs already exist. The text presents up-to-date knowledge in a simplified way, describing the appropriate laboratory tests and their clinical significance for the benefit of beginners, thus introducing them to the already accepted methods as used in many hospitals.

I crave the indulgence of my colleagues in the field who, no doubt, will appreciate the difficulty of presenting their advanced work to the beginner. Commentation on the latest developments in haemostasis at the end of the book is in no way intended as an approach to a new concept in this field.

Finally I shall always be indebted to Dr. John F. Wilkinson, M.D., F.R.C.P., M.Sc., Ph.D., F.R.I.C., and Dr. M. C. G. Israels, F.R.C.P., M.D., M.Sc., University Department of Haematology, Manchester, for their encouragement and Dr. F. J. W. Lewis, Director of the Department of Clinical Pathology, Southmead Hospital, for his continued interest.

I wish to thank the Medical Illustration Department, Manchester Royal Infirmary, and Mr. L. A. F. Warne, for help in the preparation of the illustrations; the Editor of the *British Medical Journal* for permission to use *Figure 8* and Table 11, previously published in one of my articles. My wife, Christine, cheerfully and willingly disentangled and typed my manuscript, and Mr. L. A. F. Warne, F.I.M.L.T., kindly read the typescript.

F. NOUR-ELDIN

Contents

1—Rudiments of Haemostasis

Although for convenience this book is entitled *Blood Coagulation Simplified*, the concise presentation of the basic principles of haemostasis as a whole is fundamentally its subject. To begin with, the fascinating background is portrayed by a short historical account.

Although Malpighi in 1666 showed that strands of fibres remained after a blood clot was washed, Petit (1731) appears to have carried out the first scientific approach to the physiology of haemostasis, concluding that bleeding is stopped by the formation of a blood coagulum, part of which adhered to the internal coat of the vessel. Later, Morand (1736) offered a second explanation, namely, contraction of the artery end. It remained for Jones (1810) to combine the two divergent views, considering the suppression of haemorrhage as a process performed by the concurrent and successive operation of many mechanisms: retraction and contraction of the artery, the formation of a coagulum at its mouth and the inflammation and consolidation of its extremity by an effusion of coagulating lymph.

The basis underlying the actual process of clot formation was laid down by Hewson (1772) who having succeeded in keeping the blood fluid outside the body, established that the coagulation elements resided in the non-cellular part of the blood. Two further steps were achieved by Buchanan (1835) who postulated that clotting occurs as a result of a ferment converting a soluble protein to a coagulum and by

Hammersten (1877) who stated that only thrombin was necessary to coagulate fibrinogen. Subsequently, the work of Schmidt (1893) postulating prothrombin as a precursor to thrombin and the investigations by Arthus and Pages (1890) providing irrefutable evidence that calcium was essential in the coagulation process, paved the way to the well-known classical theory of Morawitz (1904) and Fuld and Spiro (1904), which may be summarized as follows.

$$\text{Prothrombin} + Ca^{++} + \text{Thrombokinase} = \text{Thrombin}$$
$$\text{Fibrinogen} + \text{Thrombin} = \text{Fibrin}$$

In the following years, multiple theories with different variations on the theme developed and various discords appeared with no real progress. Of significance, however, were the introduction of sodium citrate as an anticoagulant in 1914 and 1915 independently by Hustin, Agate and Lewisohn, the estimation of the bleeding time by Duke in 1912 and the recognition that adhesion of the vascular wall may participate in the stoppage of bleeding (Stegemann, 1922). This relative lull, broken by the discovery of vitamin K, the development of Quick's prothrombin time (1935) and the two-stage prothrombin estimation by Warner, Brinkhous and Smith (1934, 1936) has never recurred. Factors V and VII were recognized soon afterwards as activators of tissue extracts. The platelets, their very existence questioned by some workers and their function in haemostasis denied by others since first described in 1842 by Donne, became the centre of many investigations which revealed their distinct and fundamental role in this respect.

The wide use of tissue extract in blood-clotting investigations, revived the suggestion of early workers that the extravascular coagulation of blood was due to the clotting activity of tissues. However, following the differentiation between Factors VIII and IX defects in 1952 and the introduction of the thromboplastin generation test (Biggs and Douglas, 1953), it became clear that the blood is capable of forming

its own thromboplastin and that tissue clotting activity gradually develops only after severe trauma (Nour-Eldin, 1962a, 1964), death or disease (Nour-Eldin, 1966a, 1968a).

In the last decade new blood-clotting factors have been identified and great strides taken towards our understanding of their interaction. The events leading to these discoveries are summarized in Table 1.

Contributions to haemostasis will no doubt continue, and it is hoped that with the combined efforts of world-wide workers, more precise knowledge will eventually solve this enigma. Until then, it will be appreciated that haemostasis is a complex process in which at least the following mechanisms are known to participate.

(1) The blood coagulation mechanism.
(2) Blood platelets.
(3) The vascular wall.
(4) Clot stability.
(5) Biochemical and biophysical factors including anticoagulants.

Although the exact interrelation between these different mechanisms is not yet completely solved, there are certain findings which indicate that each plays an important role in sustaining normal haemostasis. In order to facilitate reading, each mechanism is dealt with in a separate section.

THE BLOOD COAGULATION MECHANISM

When blood from a normal individual is drawn outside the body into a glass tube, it clots in about 4–7 minutes. During this period, various reactions and interactions occur in the plasma, culminating in the formation of a fibrin gel or clot. The precise mechanism responsible for this coagulation is still unknown. Nevertheless, there are certain fundamental facts which are now almost universally accepted. Based on these, Table 2 gives a simplified account of the processes

TABLE 1

Internationally Accepted Blood-clotting Factors

*Factor**	*Synonyms*	*Observations leading to discovery*	*References*
I	Fibrinogen		
II	Prothrombin	See text for classical theory.	
III	Thromboplastin		See text
IV	Calcium		
V	Labile factor	Deficiency other than prothrombin causing	Quick, 1943
	Proaccelerin	prolonged prothrombin time.	Owren, 1947
	Ac globulin	Stored blood contained a labile factor affecting prothrombin time.	
VII	Proconvertin	Serum incubated with brain extract increases	Jacox, 1949
	Serum prothrombin conversion accelerator (SPCA)	its activity. Serum (not $Al(OH)_3$ treated plasma) corrected deficiency of plasma from patients receiving oral anticoagulants. A haemorrhagic defect corrected by serum and not by Factor V (adsorbed plasma).	Koller, Loeliger and Duckert, 1951 Alexander and colleagues, 1951
VIII	Antihaemophilic globulin (AHG) Antihaemophilic factor (AHF)	Globulin in normal plasma correcting haemophilic defect.	Lozner and Taylor, 1939 Lewis and colleagues, 1946

IX	Plasma thromboplastin component (PTC) Christmas factor	Certain 'haemophilic' patients correcting defect of other 'haemophilic' patients.	Aggeler and colleagues, 1952 Biggs and colleagues, 1952
X	Prower-Stuart factor	Mutual correction between patient's serum and Christmas disease serum. A patient with prolonged prothrombin time showing defective thromboplastin generation and abnormal stypven time, suggesting a deficiency of a factor other than VII.	Hicks, 1955; Koller, 1955; Duckert, Fluckiger and Koller, 1954; Duckert and colleagues, 1955; Telfer, Denson and Wright, 1956; Hougie, Barrow and Graham, 1957
XI	Plasma thromboplastin antecedent (PTA)	Defective thromboplastin generation when the patient's serum and plasma are mixed.	Rosenthal, Dreskin and Rosenthal, 1953
XII	Hageman factor Contact factor	Blood clotting not accelerated by contact with glass surface as in normal blood.	Ratnoff and Margolis, 1955
XIII	Fibrin-stabilizing factor (FSF) Fibrinase	Clot soluble in 5M urea when deficient. Normal plasma formed insoluble clots, but purified fibrinogen gave soluble fibrin.	Laki and Lorand, 1948 Duckert, Jung and Shmerling, 1960

* The author personally prefers to use the full names (synonyms) rather than the Roman numerals when referring to the first four factors. Thromboplastin should refer to plasma thromboplastin and not to tissue extract.
To avoid confusion, Factor VI is no longer in use, having been given to a hypothetical substance thought to accelerate the conversion of prothrombin (Owren, 1947).

leading to the formation of a fibrin clot. Although perhaps this is over-simplified, it illustrates the essential steps. Its special construction serves two purposes as follows.

(1) It is arranged in such a way that the right-hand side shows the factors normally present in plasma, while on the left-hand side are those resulting from their interaction.

(2) From the horizontal line therein, the significance of each laboratory test is quickly and easily appreciated as will be described in detail in the appropriate sections.

By studying Table 1 and Table 2, the steps leading to the final formation of a fibrin clot can be described as follows.

TABLE 2

Simplified Analysis of Stages of Blood Coagulation

Glass surface, etc.	*acting on*	Hageman factor (XII)
to form: Activated Hageman factor	*combining with* (Ca)	Plasma thromboplastin antecedent (XI), Christmas factor (IX), antihaemophilic globulin (VIII) and platelets
to form: Intermediate product	*combining with*	Factor V and Prower-Stuart factor (X)
to form: Plasma thromboplastin	*acting on* (Ca)	Prothrombin (II)
to form: Thrombin	*acting on*	Fibrinogen (I)

PRODUCING FIBRIN CLOT

A simple scheme showing *in vitro* interactions of blood-clotting factors leading to the formation of a fibrin clot. The horizontal line facilitates for the student the interpretation of the laboratory tests (*see* text)

As mentioned in the Preface, this personal presentation is not intended as a comprehensive cover to different views, or as a new concept of blood coagulation. Nevertheless, it is worth mentioning that there is some evidence suggesting that Factors XII and XI react together to form a contact activation product. However, there are still some unexplained inconsistencies in this connection. Accordingly, the students are advised, for the time being, to leave this in abeyance (*see* also Chapter 3)

(1) The change in the environment of the blood seems to activate or bring forward the action of a certain clotting factor. This is known as the contact factor (activated on

being in contact with glass or a foreign surface) or the Hageman factor (being the name of the first patient described with this deficiency) or Factor XII. No calcium ions are required for this process.

(2) Activated Factor XII interacts with Factor XI (plasma thromboplastin antecedent), Factor IX (Christmas factor or plasma thromboplastin component), Factor VIII (antihaemophilic globulin) and platelets, in the presence of calcium ions, to form an intermediate product, the thromboplastic action of which is equivalent but not identical to that of tissue extract.

(3) This intermediate product is activated with Factors V and X (Prower-Stuart factor, named after two patients) in the presence of calcium ions to form active plasma thromboplastin.

(4) Plasma thromboplastin converts prothrombin to thrombin. Calcium ions are required for this purpose.

(5) Thrombin changes fibrinogen to fibrin. Calcium ions accelerate but are not essential for this process.

Factor VII is not involved in the formation of plasma thromboplastin, but is important in the activation of tissue extracts.

Various tests have been designed to investigate the activity of these blood-clotting factors. Apart from the specific tests for Factors XII and XIII, these fall into three main groups: determining the speed by which blood or plasma coagulates *in vitro*, estimating prothrombin and factors affecting its accelerated conversion and, measuring the generation of plasma thromboplastin. Before describing these in detail certain elementary and basic requirements essential for all blood-clotting investigations are mentioned in the following section.

Laboratory requirements

A 'corner' for routine tests on blood coagulation may be set aside on 6–9 feet of a laboratory bench.

Apparatus

(1) Water bath at 37°C. Lower temperature hinders coagulation by delaying thromboplastin/prothrombin and thrombin/fibrinogen interactions (Nour-Eldin, 1963a).

(2) Tubes 12 × 75 mm referred to hereinafter as incubation tubes. Size 8 × 75 mm referred to hereinafter as clotting tubes.

(3) Tube rack preferably to accommodate different sizes.

(4) Two stop-watches.

(5) Pipettes. Apart from different sizes of ordinary graduated pipettes, 0·1 ml size, enamel backed, with teats are required.

(6) A vessel containing diluted Dettol for used tubes and another for used pipettes. These should be washed and boiled in ordinary soapy water, the use of detergents may affect the results of certain tests.

(7) Small centrifuge.

(8) Syringes (20 ml) and needles size 1 and 2.

Other special apparatus will be mentioned in the appropriate sections.

Siliconed glassware

In order to render the surface of glassware water-repellent, it is treated with certain chemicals. Two main types are available.

(1) Silicone fluids. These are chemically inert colourless compounds with alternating silicon and oxygen atoms, organic groups such as methyl or phenyl being attached to the silicon atom. Water-repellent films are formed on surfaces treated with them. They are usually supplied in a volatile organic solvent and are applied by covering the surface to be treated, draining the excess. Air drying at room temperature is sufficient for most of the silicones now being used in the laboratory (for example, Repelcote). In certain cases (MS200 and MS1107) baking at high tem-

perature is required, apparently producing a more permanent water-repellent surface. In both cases, thorough washing in distilled water before use is advisable.

Detergents and strong acids and alkalis cause deterioration in the surface quality. Although these are not used in cleaning glassware for blood-clotting tests, reliable results in the tests requiring siliconed surfaces necessitate the reapplication of silicone fluid after each use.

(2) Dimethyl-dichlorosilane. One application is sufficient for a permanent water-repellent surface. The manipulations required for siliconing with this corrosive substance are performed in a fume cupboard whilst wearing gloves and mask. The vessel to be treated is filled with the liquid and emptied. After draining any excess, washing continuously in tap water for at least 3 days is carried out before finally rinsing with distilled water.

With the exception of work on Factor XII and platelet agglutinins, there is absolutely no need for siliconed apparatus for routine investigations.

Anticoagulants

(1) Trisodium citrate solution (3·8 g/100 ml). One volume mixed with nine volumes of blood. It appears to de-ionize calcium.

(2) Sodium oxalate solution (1·34 g/100 ml). One volume added to nine volumes of blood. Calcium is precipitated.

(3) Chelating agents. These compounds possess groups which bind metallic ions. EDTA or disodium ethylene-diamine tetra-acetic acid (1 mg/1 ml of blood) forms a calcium complex.

(4) Heparin. This has a complex and multiple effect on the stages of blood coagulation. 1 mg per 5–10 ml of blood is usually used.

Only the first two reagents are suitable for routine blood-clotting tests; the first is preferred in Great Britain while the second is popular in the United States of America.