NORMAL AND ABNORMAL BLOOD COAGULATION: A REVIEW

BY

R. G. MACFARLANE*

The Radcliffe Infirmary, Oxford

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Introduction

As one of the lesser problems of physiology, the nature of blood coagulation has received more than its share of investigation. There is now an insurmountable mass of literature dealing with its various aspects, contributed over the greater part of a century by some of the foremost experimentalists of their time, and embodying theories, even whole schools of thought, that have produced little but acrimony and confusion. The reasons for this activity are not at once apparent, though no doubt there is something provocative to the curious mind in the spontaneous conversion of fluid blood to solid clot. More attractive, perhaps, is the ease with which experiments in blood coagulation can be devised and carried out. The requirements for such work in technique, apparatus, and even in time are seemingly not exacting. The real or imaginary components of a theoretical mechanism can be separated to the investigator's satisfaction with little difficulty and allowed to interact in endless permutations and combinations. Each experiment suggests another; always the intangible solution seems just within reach and the experimenter is led deeper and deeper into his own, often unjustified, interpretations of his findings. Thus it is that elaborate theories have grown up which, aided by the confusion of rival terminologies, have so obscured and entangled basic facts that these are now scarcely recognizable.

Within the last decade this hitherto rather academic problem has taken on a more practical aspect; the discovery of vitamin K and the use of anticoagulants to reduce the danger of post-operative thrombosis, both advances of fundamental importance, have demanded the reliable quantitative assay of coagulation factors. This development has necessarily clarified

some of the obscurities, but it has thrown a strain on accepted theory, which is now showing signs of undergoing considerable modification. It seems, therefore, that some review or rather "interim report" of the problem might be desirable. Though the present communication is an attempt to provide this, it is no more than an outline and in no way claims to be comprehensive.

The Function of Blood Coagulation

Haemostasis.—Before discussing the mechanism of blood coagulation, it would be well to consider briefly its functional significance. Its most obvious use is concerned with haemostasis. The fact that wounds which have ceased to bleed are filled with blood clot naturally suggests that the clotting of the blood has arrested the haemorrhage. This is supported by the fact that in conditions with defective blood clotting there is a tendency for patients to bleed for long periods from even slight injuries. Coagulation is not, however, the only factor concerned in this tendency, since severe haemorrhagic states may exist despite a normal clotting mechanism. The part played by coagulation has been discussed in detail by the author (Macfarlane, 1941), who concluded that after injury there is a period of bleeding from cut vessels which is succeeded by a period of haemostasis lasting for about an hour and which is due primarily to the contraction of the damaged vessels. These vessels then dilate, but recurrence of the haemorrhage is prevented by the firm coagulation of the blood that had filled the wound during the initial haemorrhage. Normal coagulation, therefore, is the essential complement to the vascular contraction (and perhaps normal platelet function) which initiates haemostasis. Either factor without the other is useless. This "two stage" hypothesis of haemostasis and the two main abnormalities which, in the author's view,

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cause the haemorrhagic states is illustrated diagrammatically in Fig. 1 (Macfarlane, 1945). It will be seen that this hypothesis in no way lessens the supposed importance of coagulation in the mechanism of haemostasis, but regards it as an interlocking part of a more complex system.

FIG. 1.—Diagram illustrating the time relationship of normal capillary contraction, dilatation, and blood coagulation, and the two main defects that may occur. A wound of the skin surface is shown in section, injuring a capillary loop. Fluid blood is represented by the dotted areas, blood clot by solid black, and the detached "drops" indicate active haemorrhage. (Macfarlane, 1945. Reproduced by permission of the publishers of the *Proc. roy. Soc. Med.*)

Resistance to bacterial invasion.—The inflammation accompanying local bacterial infection is usually associated with plasma transudation into the tissue spaces. Coagulation of this oedema fluid takes place and the fibrin so formed serves as a barrier more or less effective in preventing the spread of infection through the adjacent tissues. It has been found experimentally (Thuerer and Angevine, 1947) that impairment of coagulation by administration of dicoumarin greatly reduces the minimal lethal dose of pathogenic organisms injected subcutaneously into rabbits. It may be that the characteristic invasiveness of the haemolytic streptococci as compared with the localization of staphylococcal infection is due to the fibrinolytic action of the former as against the coagulase produced by the latter. It has been pointed out by Robb-Smith (1945) that a feature of gas-gangrene due to *Cl. welchii* is the almost complete absence of local fibrin formation, and in no other infection is tissue invasion so rapid. The fact that in most infections there is a marked

rise in the fibrinogen content of the blood may be related to a defence reaction of this type.

Healing.—The importance of a fibrin network in the process of repair of wounds has often been suggested, but actual proof is not available. It is a fact, however, that in haemophilia the healing of even minute wounds is extremely slow if haemorrhage is taking place. It is no unusual experience for a patient to ooze blood for weeks from a small abrasion that normally would be healed completely in a few days. The continued bleeding itself may prevent the reparative processes from making headway, but it is also possible that these require the presence of preformed fibrin as a scaffold for the new network of collagen and capillaries.

Metabolism.—The mechanism of blood coagulation is extremely elaborate, requiring the interaction of many factors. Some of its protein constituents, such as fibrinogen and prothrombin, are normally present in considerable excess of what is required for haemostatic efficiency. This prodigality has seemed to a number of authorities to suggest that coagulation is a result of a constantly operating metabolic process that has been adapted to fulfil the secondary purpose of haemostasis. In support of this, it appears that both fibrinogen and prothrombin are consumed with considerable rapidity in the absence of any obvious clotting activity, though some of the evidence from which this is inferred is questionable, as will be seen later. The fact that proteolysis appears to be in some way connected with the coagulation mechanism also suggests that the latter may be concerned in normal protein metabolism.

Finally, despite these speculations it can be said that blood coagulation is not essential to life. Several patients have been observed to lead a relatively normal existence though they have no fibrinogen in their blood.

Normal Blood Coagulation

Historical.—While it is outside the scope of this article to give more than an outline of the historical background to the present views on blood coagulation, such a background assists the visualization of the problem in its proper perspective.

Malpighi (1666) was one of the first to consider the nature of the solidity of clotted blood. He found that after a clot was washed in water a mass of fibres remained. In 1735 the French surgeon Petit observed that coagulation was an attribute of the clear, fluid part of the blood, not of the cells, an observation that was extended by Hewson (1770), who called this fluid "coagulable lymph" and showed that clotting could be prevented by cold, or the presence of salts.

Chaptal (1797) applied the appropriate name, "fibrin," to the fibres constituting the clot. The nature of the clotting process then began to evoke considerable discussion. Hunter (1794) maintained that it was a "vital" action of the blood analogous to the contraction of a living muscle. Others considered that it was chemical in nature, and depended on such changes as contact of the blood with the air, cooling, or stasis.

In 1835 Buchanan made a series of observations that anticipated the discovery of thrombin and thromboplastin. He showed that the clotting of "coagulable lymph," in this case hydrocele fluid, was accelerated by the addition of the washings of a blood clot or of leucocytes. He compared the action of these substances to that of rennin on milk, thus founding the concept of a reaction between a precursor of fibrin and a ferment-like agent generated in the blood during clotting; but his work received little recognition until it was resuscitated by Gamgee (1879). The development of a clearer understanding of the coagulation mechanism was hampered for a while by the popular theory, propounded by Richardson (1858), that blood clotted when it left the vessels because the ammonia it contained during life was then able to escape. This tenacious fallacy was finally exploded by Lister (1863), who performed a series of experiments that are a model of ingenuity and precision. A few years before this Denis (1859) had been able to separate the fluid precursor of fibrin, calling it "plasmine," a name changed to the more descriptive "fibrinogen" by Hammarsten (1877).

The scene was now set for Schmidt (1892) to present his conception of a chain of reacting factors culminating in the conversion of fibrinogen into fibrin by a ferment called thrombin, a theory that has held the stage ever since. Schmidt recognized that thrombin was not present in the circulating blood, and he postulated an inactive precursor, prothrombin, that was activated by zymoplastic substance, considered, even at that early stage, to be lipoidal in nature.

The hypothesis of coagulation, as it stood at this time, is illustrated by the following diagram quoted by Quick (1942):

It remained for Arthus and Pagès' (1890) discovery of the essential part played by ionized calcium in the activation of prothrombin to complete the basis for the modern conception of the coagulation mechanism. This was restated in a more simple form by Morawitz

(1905), who considered that clotting involved two stages and four essential components, thus:

1. Prothrombin + Ca + Thromboplastin = Thrombin
2. Fibrinogen + Thrombin = Fibrin

This, the so-called classical theory, has been pre-eminent up to recent times. This is not to say that other theories have not been put forward; many indeed are the spirited, if sometimes bizarre, heresies that have arisen, so that at times the number of theories has exceeded the number of propounders (Wöhlisch, 1929), but, particularly in the United States and in this country, no attack on this "4-factor" theory has been taken seriously.

Two unorthodox views formulated at the beginning of this century must, however, be considered. The first is that produced by Nolf (1908) and still vigorously defended by him (1945). In Nolf's view, coagulation requires, in addition to calcium, the interaction of four protein-like factors, all of which are present in the plasma. These factors are: thrombokinase (thromboplastin); thrombozyme; thrombogen; and fibrinogen. The thrombokinase of the plasma is a lipoprotein, having the same properties as tissue thrombokinase, and accelerating, in the presence of calcium, the reaction between thrombozyme and thrombogen. These two factors together constitute what was usually called "prothrombin" and which is therefore not a simple substance but a mixture. Neither thrombogen or thrombozyme alone is capable of generating thrombin without the presence of the other, and thrombin is regarded as a product of their union, since its formation coincides with their disappearance. The theory can be illustrated diagrammatically thus:

According to Nolf, the thrombozyme-thrombogen complex is responsible not only for the coagulation of fibrinogen but also for fibrinolysis, particularly if the thrombozyme is in excess of its normal ratio to thrombogen. The lifelong work on blood coagulation of this investigator has received far less attention than it deserves. This is due in part to the rather obscure presentation of his admittedly complex conception and the involved experimental evidence on which it is based. It is largely due, however, to the fact that many modern workers have not troubled to read his published material. As will be seen later, some aspects at least of his theory are receiving unsuspected confirmation.

Another supporter of a "5-factor" theory was Bordet (Bordet and Delange, 1912; Bordet, 1920). He postulated an inert precursor (proserozyme) of what is usually termed prothrombin, which was activated to serozyme by contact with a foreign surface

in the presence of calcium. Serozyme then combined with cytozyme (thromboplastin) to form thrombin, thus:

Bordet's theory is based on a number of interesting experiments which may have a significant part in any modern re-orientation of the classical theory, though Bordet's own interpretations may not always be acceptable.

These earlier theories in their preoccupation with clotting did not explain adequately the fluid state of the normal circulating blood. Lister (1863) had emphasized the importance of contact action in the inception of blood coagulation, and it has already been stated that other workers recognized the importance of admixture with tissue fluid containing thromboplastin, or its release following the disruption of cells or platelets when blood is shed. The absence of contact with a foreign surface, however, is not a sufficient explanation of the inherent stability of blood in the circulation, since in a variety of pathological and traumatic conditions both vessels and tissues may be so injured as to permit contact action and the passage of thromboplastin into the blood stream.

It was Howell (1910, 1911, 1935) who first emphasized the possible importance of anticoagulant substances, and who laid the foundations for the idea of an equilibrium between such anticoagulants and the coagulant factors that come most strongly into play when blood is actually shed. He at first postulated an antiprothrombin that was neutralized by thromboplastin. Later he assumed that the newly discovered anticoagulant substance heparin (Howell and Holt, 1918) was identical with this factor. His theory at that time could be illustrated as follows:

Later work suggests that heparin is mainly an antithrombin, but the concept of anticoagulant factors essential for the preservation of normal blood fluidity still maintains its place in present-day explanations of the coagulation mechanism.

Among workers in this country whose investigations advanced the international knowledge of this subject during the early part of the century, Mellanby (1909) and Pickering (1928) are foremost. The former perfected the technique of separation and study of active fractions of the coagulation mechanism, and by his study of their properties found a solid basis for much modern work (Mellanby, 1930, 1933). Pickering contributed his exhaustive monograph on the subject, together with his synthesis of the divergent and discordant views of his predecessors.

The Factors of the Classical Theory

Before discussing the recent events that have resulted in a modification of the earlier acceptance of the classical theory, some of the well-established facts relating to the familiar components of this system should be reviewed.

Fibrin.—The end-result of normal mammalian blood coagulation is the formation of the fine three-dimensional network of fibres that entangles the formed elements of the blood. Under certain circumstances, principally those involving changes of pH, application of heat, or long storage, clotting produces a structureless gel rather than a reticulum, but this, though of theoretical interest, might be regarded as an artifact.

Examination of the minute structure of the fibrin threads by polariscopic (von Dungern, 1937), radiological (Herman and Worschitz, 1935), or electron-microscopical means (Wolpers and Ruska, 1939; Hawn and Porter, 1947) has suggested that they are composed of needle-like micelles packed lengthwise into bundles. When freshly formed the threads are extremely adhesive, sticking to each other, to the platelets and cells of the blood, to the tissues, and to certain foreign surfaces—a property to which the blood clot owes its haemostatic efficiency.

A curious reaction of freshly coagulated fibrin is that of retraction or, more properly, contraction. The normal blood clot will contract to about 40 per cent of its original volume, squeezing out serum mixed with a small proportion of red cells. Plasma clots will contract to 10 per cent or less of their original volume (Macfarlane, 1939). This activity is reduced naturally in those conditions in which there is thrombocytopenia and can be inhibited artificially by heating to 47° C. or by removing the platelets (Macfarlane, 1938a). The significance of retraction from the point of view of haemostasis is not clear, but there is no doubt that the contracted clot is more solid and elastic than when it is newly formed.

Fibrin is soluble in acids and alkalis, and is digested by proteolytic enzymes. After such solution it cannot be made to re-form into its original reticulum. If it is kept sterile, the fibrin of whole clotted blood will remain intact for days or weeks under normal conditions, but in certain circumstances it undergoes rapid dissolution or fibrinolysis, a process now known to be due to the activation of a proteolytic enzyme of the plasma, and discussed by Macfarlane and Pilling (1946a).

It has been suggested that fibrin may exist temporarily in a soluble form in the immediate pre-clotting stage. This hypothetical precursor of fibrin has been called profibrin by Apitz (1937), and its

existence is accepted by Astrup (1944) and Owren (1947a). Apitz (1939) believes that a sticky film of profibrin absorbed on to the platelets during coagulation causes their agglutination, an explanation recalling the earlier suggestion of Maltaner and Johnston (1921) that fibrinogen was necessary for the "conglutination" of erythrocytes and bacteria.

Fibrinogen.—Fibrinogen may be described as the fraction of the plasma protein which is coagulable by thrombin. It has the greatest molecular weight of the plasma proteins, a recent estimate being 441,000 (Nanninga, 1946). It is also the most labile, being precipitated by heating to 47° C. or by half saturation with sodium chloride or quarter saturation with ammonium sulphate. Its iso-electric point is pH 5.5 (Nordbö, 1927), and it is precipitated by bringing the pH to this point after reduction of electrolyte by preliminary dialysis or dilution with distilled water.

Wöhlisch and Clamann (1932) and Wöhlisch and Kiesen (1936) have presented evidence that the molecule of fibrinogen is long and thread-like, this conclusion being in accord with the evidence of the miscellar structure of fibrin already mentioned.

It is generally assumed that fibrinogen is formed by the liver. The evidence for this is as follows: Nolf in 1905 showed that exclusion of the liver from the circulation of dogs resulted in a rapid and profound fall in the blood fibrinogen. This observation was supported by other workers, including Jones and Smith (1930), who observed a similar fall after hepatectomy. Destruction of liver function by the administration of chloroform or phosphorus will also produce the same result (Foster and Whipple, 1921) accompanied, in the case of the former agent, by a decrease in prothrombin as well (Smith, Warner, and Brinkhous, 1937).

In such experiments it is assumed that the fall in fibrinogen is due to the cessation of production by the damaged or excluded liver, the normal rate of fibrinogen utilization being presumably very high. Against this, however, must be set certain objections. The most serious is that substances such as chloroform induce intense fibrinolytic activity, a fact well recognized by workers on this subject. Fibrinolysis was also observed by Macfarlane and Biggs (1946) to occur after any operative procedure, and it has also been shown to occur in severe liver damage. It might be argued, therefore, that the disappearance of fibrinogen in these cases was due to an increased rate of destruction rather than to deficient production (Macfarlane and Biggs, 1948). The second objection involves the normal rate of utilization of fibrinogen. Admit-

tedly in the experimental animals described the rate of utilization may have been higher than normal as a result of haemorrhage. In human beings there is little direct evidence of the normal rate of usage apart from the observation of Pinniger and Prunty (1946) that fibrinogen transfused into a patient with congenital absence of fibrinogen was still present in a detectable amount after ninety-six hours. If this reflects the rate of utilization in normal persons, removal of the source of supply would not have resulted in a detectable drop in the course of the few hours' duration of an acute experiment. The whole question thus requires re-examination.

Most investigators consider that fibrinogen is a homogeneous protein, and that they are able to obtain it in a state of considerable purity by fractionation methods. Some, however, have postulated the existence of different forms of fibrinogen, a view which is now receiving increasing experimental support. As long ago as 1889 Wooldridge regarded fibrin as being derived from the combination of two components, fibrinogen A and fibrinogen B, neither being identical with the fibrinogen separated in the laboratory from plasma, which he regarded as an artifact. Recent work by Nolf (1947) suggests, however, that Wooldridge's fibrinogen A is in some respects similar to the classical thromboplastin, but there are interesting points of difference. Wertheimer and others (1944) have shown that on storage the fibrinogen of plasma separates into a more labile fraction that is precipitated by cooling and a less labile fraction remaining in solution. The proportion of less labile fibrinogen is reduced in liver disease and increased in pregnancy or after haemorrhage.

A very interesting contribution is that of Lyons (1945a, b), who suggests, on the basis of coagulability by naphthoquinone, that there are two forms of fibrinogen—fibrinogen A, which exists in fresh plasma and normal blood, and fibrinogen B (these names are not to be identified with those of Wooldridge), which occurs in certain pathological states and develops on storage of blood and during the clotting of fibrinogen with thrombin. The significance of this work will be discussed later.

Thrombin.—Thrombin is the factor which, though absent from the normal circulating blood, develops during the process of coagulation and on separation can be shown to be capable of clotting fibrinogen. Though it is doubtful if pure thrombin has been obtained or is even obtainable, preparations having very great activity relative to dry weight can be produced with little difficulty, and such preparations can clot several hundred times their own weight of fibrinogen (Eagle, 1935a;

Seegers, 1940; Astrup, 1944). Astrup (1944) and his colleagues have shown that thrombin is an albumin-like protein, with a molecular weight of about 75,000. The phosphorus content of Astrup's preparations was too low to be detected by chemical means, a finding that agrees with that of Chargaff and others (1940), who followed the generation of thrombin using a radioactive phosphorus isotope. The calcium content is also negligible (Ferguson, 1937). Thrombin is heat-labile, being destroyed by heating to 60° C., and beginning to lose activity at 40° C.

Lyons (1945b), having found that thrombin digested with trypsin gives a colour reaction characteristic for this substance, maintains that thrombin contains a naphthoquinone group.

Reaction of thrombin with fibrinogen.—The earlier authorities regarded thrombin as an enzyme acting specifically on fibrinogen. Mellanby (1933) called thrombin "thrombase" and thought that it split the fibrinogen molecule into fibrin and a soluble globulin. There was a temporary setback to the enzymatic view, largely due to the work of Howell (1910), from which it appeared that thrombin was consumed quantitatively during the conversion of fibrinogen to fibrin, a fact possibly explained by absorption on to the newly formed fibrin and the presence of antithrombin. Howell's contention was to some extent supported by Hekma (quoted by Pickering, 1928), who thought that thrombin acted as an agglutinin of fibrinogen in the ordinary immunological sense. Pickering (1928) argued against this, pointing out that thrombin is almost inactive at low temperatures, is thermolabile, and soon loses its activity on storage, while agglutinins are more active at low temperatures, are heat stable, and retain their activity for years.

Since more recent work, already quoted, has shown that thrombin can convert at least 600 times its own weight of fibrinogen into fibrin (Astrup, 1944) and that the molecular weight of thrombin is probably not less than one-tenth that of fibrinogen, it seems unlikely that any stoichiometric combination of thrombin and fibrinogen can occur, and it seems as if the enzymatic action of thrombin is more probable.

The nature of this enzymatic action is not known. Mellanby's (1933) view that the fibrinogen molecule was split into a soluble and an insoluble fraction is unlikely to be correct, since Jaques (1938) found that the fibrinogen-nitrogen lost during clotting reappeared quantitatively as fibrin-nitrogen. However, the supposition that the process was, in some way, a proteolytic one had some

circumstantial support in that certain proteolytic agents such as papain (Eagle and Harris, 1937) and the proteolytic venoms of *Echis carinata* (Barratt, 1920) and other snakes (Eagle, 1937) can coagulate fibrinogen. Moreover, it was argued that thrombin preparations were often fibrinolytic as well as fibrin-forming, this suggesting that coagulation was merely the prelude to lysis (Nolf, 1938). More recently, however, there is strong evidence to show that the clotting action of thrombin is distinct from any fibrinolytic action which certain preparations may have and which is almost certainly due to contamination with plasmin, the proteolytic enzyme of the plasma (Seegers, 1940; Ferguson, 1943; Macfarlane and Pilling, 1946a).

In 1941 Baumberger made the fruitful suggestion that thrombin contributed to the formation of fibrin by potentiating a chemical linkage between fibrinogen molecules. He considered that the SH groups known to be present in the fibrinogen molecule might be oxidized by thrombin, so that an S-S linkage between fibrinogen molecules could occur, with consequent construction of the fibrin lattice, thus:

This conception has been extended by Lyons (1945a and b). It has already been stated that he believes that the thrombin molecule contains a naphthoquinone group, and that this substance is able, by itself, to clot a certain form of fibrinogen known as fibrinogen B. He explains the previous observations that ninhydrin will clot fibrinogen (Chargaff and Ziff, 1941) on the basis of the similarity of the structure of this substance to the naphthoquinones (Fig. 2). The failure of others to

FIG. 2.—Structural formulae of certain substances active in the coagulation mechanism.

obtain coagulation of fibrinogen by naphthoquinone is due to the use of fibrinogen A in their experiments. Lyons suggests that in its native form fibrinogen has its thiol groups blocked, this form constituting fibrinogen A. On storage, or in certain pathological states, fibrinogen A may become partly converted to fibrinogen B, in which the SH groups are free. Such fibrinogen is coagulable by ninhydrin and the naphthoquinones. During natural coagulation fibrinogen A is acted upon by a component of thrombin (thrombin A), the thiol groups being freed. The fibrinogen B thus formed is then clotted by the naphthoquinone component of thrombin (thrombin B) thus:

1. Fibrinogen—SH (blocked)+Thrombin A
(Fibrinogen A) ↓
2. Fibrinogen—SH+Thrombin B (or naphthoquinone)
(Fibrinogen B) ↓
3. Fibrinogen—S—S—Fibrinogen (Fibrin gel)

This work, which is supported by a number of points of evidence, is of considerable interest and, if confirmed, of obvious practical and academic importance.

Laki and Mommaerts (1945) have also postulated a two-stage reaction in the conversion of fibrinogen to fibrin, but have been less precise as to the mechanism.

Prothrombin.—Prothrombin is the inactive precursor of thrombin, and, as such, is present in the circulating blood. It has the physical and chemical attributes of a globulin (Astrup, 1944), being precipitated at pH of 5.6 and by half saturation with ammonium sulphate. Like thrombin its activity is destroyed by heating to 60° C. It is strongly adsorbed by such substances as BaSO₄ (Dale and Walpole, 1916), Ca₃(PO₄)₂ (Bordet and Delange, 1914), Mg(OH)₂ (Fuchs, 1929), and Al(OH)₃ (Quick, 1935a). It is also adsorbed by Seitz or Berkfeld filters. It is destroyed by proteolytic enzymes (Eagle and Harris, 1937).

The evidence that prothrombin is produced by the liver is similar to that purporting to show that fibrinogen has the same origin, that is, upon the observation of prothrombin deficiency arising after chloroform poisoning (Smith and others, 1937) or partial hepatectomy (Warner, 1938). Since prothrombin is destroyed by proteolytic enzymes and the experimental procedures described are potent activators of the proteolytic system of the blood, the same objections to these conclusions can be made as were discussed in relation to the site of origin of fibrinogen.

Vitamin K.—The story of the discovery of vitamin K and of its essential part in prothrombin

production by the body now forms a familiar page in the history of fundamental medical advancement. It is instructive and stimulating to study the rapid unravelling of threads leading to an understanding of a whole series of haemorrhagic disorders that resulted from the brilliant and often independent contributions produced in quick succession. In 1934 Dam and Schönheyder reinvestigated the haemorrhagic disease that develops in chicks fed on a synthetic diet, and Dam (1935) decided that it was due to the absence of a factor (coagulation-vitamin or vitamin "K") required for the proper clotting of the blood. In the same year Halbrook (quoted by Quick, 1942) showed that the condition was cured by the administration of alfalfa (lucerne) or its ether extract, a finding confirmed and expanded by Almquist and Stokstad (1935). Further investigations by Dam and others (1936) showed that the haemorrhagic disease was due to a prothrombin deficiency, a conclusion reached independently by Quick (1937), who also showed that the prothrombin level could be restored to normal by feeding alfalfa. Quick (1935b) had already discovered the prothrombin deficiency in obstructive jaundice and he (1937) was not slow to predict that this might be due to a deficiency of the new (fat-soluble) vitamin because of deficient adsorption in the absence of bile salts from the intestine. He was able to substantiate this prediction (1938a) by actual clinical observations, which were confirmed by Warner and others (1939a) and by Butt and others (1938).

Efforts were now made to isolate the new substance. It was found that it is widely distributed in plants, particularly in green leaves (Dam and Glavind, 1938), and that bacteria can synthesize the vitamin, so that its production in the animal intestine may be an important factor in its natural assimilation (Almquist and others, 1938). Scientific methods of assay (Almquist and Klose, 1939a) based on Quick's (1935b) prothrombin-time method greatly assisted this progress in the study of the distribution and synthesis of vitamin K. As regards the chemistry of vitamin K, the principal work was carried out by Dam and his co-workers on the one hand and Almquist's group on the other (see review by Almquist, 1941). The structure of two naturally occurring substances with vitamin-K activity were quickly determined, both being derivatives of 1,4-naphthoquinone. Almquist and Klose (1939b) found that phthiocol, a compound isolated from tubercle bacilli, had vitamin-K activity and was also a 1,4-naphthoquinone derivative. It was then almost simultaneously discovered by four separate groups of workers (Almquist and

Klose, 1939b; Ansbacher and Fernholz, 1939; Fieser and others, 1939; Thayer and others, 1939) that the comparatively simple synthetic substance 2-methyl-1,4-naphthoquinone had even greater vitamin-K activity than the naturally occurring compounds. Since that time a number of derivatives of this substance with the added advantage of solubility in water have been produced commercially under different trade names, and vitamin K has taken its place among the factors essential for life.

Its mode of action in the production of prothrombin is at present unknown, though the observations of Lyons (1945b) suggest that it is an integral part of the thrombin molecule. It has no effect on prothrombin activity *in vitro* and its administration in cases of hypoprothrombinaemia is effective only if the function of the liver is relatively normal. In the latter case the rise in prothrombin following vitamin-K administration is very rapid, a tenfold increase occurring within twelve hours in some patients (Quick, 1942).

Calcium.—It is now undisputed that ionized calcium is required for the conversion of prothrombin to thrombin, but that the latter can act on fibrinogen with unimpaired effect in the absence of calcium or in the presence of decalcifying agents such as oxalate or citrate.

The calcium of the blood-plasma is present in two forms; the diffusible ionized calcium and the non-diffusible protein-bound fraction, which is usually thought not to take part in the coagulation process. Removal of free calcium by precipitating it as the oxalate, or suppression of ionization by the addition of citrate, will inhibit the normal clotting process indefinitely. It is significant, however, that if clotting is to be prevented at least three times as much oxalate must be added to plasma as the calculated amount necessary to precipitate all the available calcium (Vines, 1921; Scott and Chamberlain, 1934; Quick 1940). This finding is difficult to understand unless it is supposed that there is a competition for available calcium by the coagulation factors on the one hand and the decalcifying agent on the other, it being necessary to have an excess of the latter to deprive the former of calcium by mass action.

Ferguson (1937) supposed that an intermediate prothrombin-calcium-thromboplastin complex was formed during clotting and that this complex could be inactivated by decalcification. Quick (1940) also postulates a prothrombin-calcium complex, from which calcium has to be removed before the decalcifying agent can prevent coagulation. He has

also provided evidence (1947b) that there is a calcium co-factor associated with, but distinct from, prothrombin. Greville and Lehman (1944) have studied the interesting cation antagonism affecting this part of the clotting mechanism, in which certain ions, particularly those of magnesium, antagonize the effect of calcium.

The amount of calcium required for effective coagulation has a wide optimum value, ranging from 0.025 to 0.0025 molar concentration. Above and below this level coagulation is depressed and finally inhibited (Quick, 1942).

Thromboplastin.—Although blood which is collected without contamination with tissue fluid and which is then kept in a container with a "neutral" (non-water-wettable) surface will not clot, it can be made to do so with great rapidity by the addition of a small quantity of the watery extract of almost any tissue. It can therefore be assumed that prothrombin, even in the presence of calcium, is inactive, but that it can be activated by a factor or complex of factors present in the tissues. This agent has received various names, such as cytozyme, thrombokinese, thromboplastin, zymoplastic substance, or simply kinase. It occurs in most tissues, but brain, lung, thymus, testes, and platelets yield the most active preparations, and it can be demonstrated in human saliva (Bellis and Scott, 1932).

In discussing the nature, properties, and modes of action of thromboplastin a major difficulty arises which is by no means limited to this particular aspect of the coagulation problem. This is the probability that "thromboplastin" has more than one component, and that different substances, claimed by various workers to be thromboplastin in a state of relative purity, are in fact different fractions of the whole.

The original zymoplastic substance used by Schmidt (1895) and his associates was an alcohol extract of tissue, and was thought to contain lecithin as the active factor. Morawitz (1905) showed that aqueous tissue solutions had the same effect and were, moreover, heat-labile; he concluded that the active factor was a protein, and called it "thrombokinese." Howell (1912) showed that both extraction by water or by lipid solvents yielded active material, and he applied the now popular term "thromboplastin" to the water-extractable fraction. He was able to show that the ether-soluble fraction was cephalin, which itself was active as a thromboplastin, though, when in combination with an unknown protein factor, the activity was greatly increased. The cephalin was heat-stable as regards activity, but the cephalin-protein complex was heat-labile. Confirmation for

the suggestion that cephalin was an active component of thromboplastin came from a number of investigators, including Gratia and Levene (1922), McLean (1916), and Quick (1936a), while lecithin was shown to be inactive (Clowes, 1917; Gratia and Levene, 1922). A particularly active cephalin preparation has been isolated from platelets by Chargaff and others (1936). Cohen and Chargaff (1940) supported Howell's belief that thrombokinase was a lipoprotein and suggested that the protein component was effective in orientating the cephalin at its surface. Chargaff and others (1944) have studied the chemistry of a lung preparation that is homogeneous in the ultra-centrifuge. They could not separate an active protein component. The lung preparations are more stable to heat than the brain extracts, and Lenggenhager (1936) has shown that even boiling does not greatly impair their activity. Quick (1942) has made similar observations.

Further indications that thromboplastin is a complex factor can be quoted. Though prothrombin can be activated by calcium and pure cephalin, the process is very slow compared with the reaction when crude tissue preparations are used (Seegers and others, 1938). A potent thromboplastin has been prepared from lung that contains no significant amount of cephalin, a finding of obvious importance (Charles and others, 1934).

It is possible that the action of Russell's viper venom may eventually throw some light on this problem. The fact that certain snake venoms were potent coagulants of blood was recognized by Martin in 1894, and their action was investigated by Lamb (1903) and Mellanby (1909). The prevalent use of oxalated or citrated blood as an indicator of activity, however, obscured the extreme potency of the venom of Russell's viper, which was pointed out by Macfarlane and Barnet (1934) in their search for a local haemostatic agent that might be effective in haemophilia. They found that haemophilic blood was rapidly clotted by extremely small amounts of venom, a dilution of one in many million still having a marked effect. The venom was incapable of clotting oxalated or citrated blood and had no effect on fibrinogen. It behaved in fact as if it were a thromboplastin.

Further work by Trevan and Macfarlane (1936) demonstrated the fact that the action of the venom was greatly increased by the presence of tissue lipoids, or lecithin, suggesting that the latter acted as a co-factor with the venom in its thromboplastic role. Macfarlane (1937, unpublished observations) found that the venom alone was unable to clot plasma from which the lipid had been removed

by extraction with petroleum ether, but that rapid coagulation occurred if the extracted lipid or lecithin were mixed with the venom before addition to the plasma. He also observed that the coagulant extractable from human saliva behaved in the same way, since it would only clot the extracted plasma in the presence of added lipid. These experiments extended the observations of Zak (1912) that plasma extracted with petroleum ether will not clot on recalcification unless the lipid fraction is returned. Macfarlane and others (1941) obtained similar results with samples of plasma from which lipid had been removed by high-speed centrifugalization, and suggested, as had Leathes and Mellanby (1939), that the venom was analogous to an enzyme (protein) component of natural thromboplastin, the lecithin being analogous to the natural lipid co-factor.

The complex nature of thromboplastin has been emphasized by Feissly (1945a), who has extracted a thermostable phospholipoid from platelets and a thermolabile protolipoid from plasma. Lenggenhager (1946) also considers that more than one factor is involved, since he supposes that prothrombokin, a precursor of thrombokin (? thromboplastin), is activated by proteolysis.

The findings of Zondek and others (1945) that thromboplastic substances derived from placental tissue were heat-stable when fresh but heat-labile after some days' storage emphasizes the difficulty of interpretation of published observations. Nevertheless it is clear that further experiments along the lines indicated may clarify the problem of the nature of thromboplastin, and recent work with haemophilic blood is already showing promising results.

Thrombin formation.—The nature of the reaction between thromboplastin, calcium, and prothrombin has given rise to endless discussion, fostered by uncertainty as to the "purity" of the various investigators' preparations of these factors, and the possibility that other undefined agents were involved.

The original view of Bordet and Delange (1912), supported in more modern times by Lenggenhager (1935), was that the three factors combined chemically to form a new compound, which was thrombin. It now seems most unlikely that this can be the case. It has already been said that the thrombin molecule appears to be smaller than the prothrombin from which it is derived, so that any simple combination of thromboplastin and prothrombin is, on the face of it, impossible. Moreover, it has been shown that thrombin contains no

demonstrable phosphorus (a considerable component of thromboplastin), and no calcium.

It would seem most likely, therefore, that thrombokinase activates prothrombin by removing some part of its molecule. Howell (1935) contended that this process consisted of the removal of heparin, which normally formed an inert compound with prothrombin. Quick (1936b), however, was not able to demonstrate the neutralization of heparin by thromboplastin, and, in any case, heparin exerts its main anticoagulant effect on the action of thrombin. There is, on the other hand, some evidence to favour the idea that one component of thromboplastin is an enzyme. The objection that thromboplastin has been stated to be heat-stable may well apply only to the lipoid co-factor which has been mistaken for the whole complex.

The significance of the activity of Russell's viper venom, which is almost certainly enzymatic, and its co-factor lecithin, has already been mentioned. There is additional support for proteolysis in the action of trypsin. Douglas and Colebrook (1916) observed that trypsin hastened the clotting of blood, a finding confirmed by Heard (1917), who found, moreover, that the enzyme was capable of coagulating oxalated blood. Mellanby (1935) and Mellanby and Pratt (1938), investigating this finding, came to the conclusion that trypsin activated the thromboplastin of the blood, and that the calcium present in the trypsin afforded sufficient to allow clotting. Eagle and Harris (1937), however, obtained the same result with calcium-free crystalline trypsin, and concluded that the prothrombin molecule was split proteolytically to form thrombin. Ferguson and Erickson (1939) concluded that the action of trypsin was to free a cephalin-calcium complex. It is of interest that a number of proteolytic snake venoms have a coagulant action similar to trypsin (Eagle, 1937).

It may be significant that substances with an anti-proteolytic effect have also been shown to be anticoagulant. The pancreatic trypsin inhibitor of Kunitz and Northrop (1936) is strongly anticoagulant (Ferguson, 1942), and the soya bean trypsin inhibitor isolated by Kunitz (1945) has been shown by Macfarlane and Pilling (1946b) and Macfarlane (1947) to act as an antithromboplastin.

The evidence seems, therefore, to favour the existence of a thromboplastic enzyme (kinase) which, in the presence of a lipoid activator and calcium, splits the prothrombin molecule. This enzyme is present with its activator in most tissues, and extracts of such tissues are spontaneously

active. It is also probable that the enzyme exists in the plasma as an inert precursor which is activated by contact with a foreign surface or by some other factor resulting from such contact. This precursor is possibly identical with Lenggenhager's "proplasmakinin," Feissly's (1945b) "plasma thrombokinase," Macfarlane's (1945) "prokinase," and the so-called "antihaemophilic globulin" first described by Patek and Taylor (1937). Since the systems used by most investigators to test thromboplastic activity may be deficient in lipoid co-factor, but contain unsuspected and significant amounts of prokinase, it follows that the addition of lipoid may produce an acceleration of coagulation which may be taken to indicate the thromboplastic activity of the added lipoid.

An argument against the enzymatic, possibly proteolytic, activation of prothrombin is the apparent consumption of thrombokinase during thrombin formation. Though Eagle (1935a) considered that the amount of thrombin generated in a mixture of prothrombin, calcium, and thrombokinase was independent of the amount of thromboplastin, the work of Mertz and others (1939) suggests that, for low concentrations, a given amount of thromboplastin causes the conversion of a given amount of prothrombin, a finding taken to disprove the enzymatic nature of the reaction. Such an observation, however, might merely mean that it is the lipoid factor that is consumed during the process of activation.

The Inception of Blood Clotting

For many years the search for the prime mover of the coagulation mechanism has been undertaken without very convincing results. It has been recognized for more than a century that the introduction of tissue emulsions or extracts into the blood stream will result in intravascular coagulation in the living subject (de Blainville, 1834; Wooldridge, 1886). The coagulation of blood escaping from the body and mixed with the products of damaged tissue would seem, therefore, to be due to this admixture, and it has already been shown that such tissue fluids contain active thrombokinase. It can be argued, therefore, that active thromboplastin is capable of clotting blood without the intervention of any other agency; yet it is a familiar observation that blood removed with the greatest care to avoid admixture with tissue juice still clots in a short time on contact with glass or other foreign surfaces. The larger the surface area with which it is in contact, the more rapid the clotting, hence the coagulant effect of substances like cotton-wool or bandages and

their practical use in haemostasis, and the fact that the coagulation time of blood samples is faster in small tubes than large ones.

Lister (1863) was one of the first to point out the importance of such contact and showed that certain surfaces such as rubber delayed blood clotting, as compared with others such as glass. It soon became established that certain neutral substances such as paraffin (Freund, 1888), collo-dion, lusteroid (Lozner and others, 1942), and most recently silicone (Jaques and others, 1946), which are water-repellent and are not wetted by blood, have very little stimulating effect on coagulation, so that blood collected without tissue fluid contamination will remain fluid for long periods in vessels made of, or coated with, these materials.

The most familiar explanation of these observations is that originally put forward by Morawitz (1905), who supposed that active thrombokinas was liberated by the platelets which rapidly disintegrated when blood was shed. Calcium is necessary for such disintegration (Cramer and Pringle, 1912), and it has been shown by Tait and Green (1926) and Lampert (1930) that the surfaces active in promoting coagulation are those which cause disintegration of platelets.

In favour of this hypothesis it has been established that platelets contain thromboplastin, and significant changes in their morphology have been observed by Nygaard (1941) at the beginning of the clotting process.

It seems, however, that this is not the whole explanation. It is known, for instance, that the clotting time of whole blood as measured by the method of Lee and White (1913) is considerably influenced by small changes in thromboplastin (Quick, 1942), yet in severe thrombocytopenic purpura, in which the platelets may be almost entirely absent from the blood, a significant increase in the clotting time of the whole blood does not usually occur. An important finding of Lozner and others (1942) is that the effect of foreign surfaces on blood coagulation is largely independent of the platelets, there being acceleration or delay in glass or lusteroid respectively of the clotting time of plasma previously deprived of platelets by centrifuging. These authors, therefore, favour the view, already put forward by a number of others from Mellanby (1909) onwards, that there exists an inactive precursor of thrombokinas in the plasma and that this is activated in some way by contact with a foreign surface.

Recent observations by Brinkhous (1947) and Quick (1947b), in relation to the clotting effect in haemophilia, may have an important bearing on

the normal first stage of coagulation. With the use of silicone, plasma can be prepared free of platelets and without surface contact action. On transfer to a glass surface such plasma will not clot without the addition of platelets. Brinkhous (1947) infers the existence of a "thrombocytolysin" that disrupts the platelets on contact with a foreign surface with release of thromboplastin. Quick (1947b) postulates a factor released by platelets on contact that activates "thromboplastinogen." It might equally be argued, from what has already been said, that platelets on rupture supply the "lipoid factor" which reacts with the contact-activated "prokinase" or thrombokinas precursor.

The nature of such contact action is almost certainly physical. As Pickering (1928) writes, "Electrical changes produced by contact of blood with a surface which it wets seems sufficient for the inauguration of clotting. A wetted surface becomes electro-negative and the presence of a negative charge implies an equal positive charge in the liquid in contact with it . . . a change of electrical conditions is thus a feature of normal clotting." What other factors intervene between such contact and the activation of prokinase (or thromboplastinogen) are not known. It has already been stated that Lenggenger considers that proteolysis plays a part, a view favoured in principle by Wöhlisch (1940) and Ferguson and others (1947). If this is so, the whole fibrinolytic system, which results in the activation of the proteolytic enzyme plasmin (see Macfarlane and Biggs, 1948), may be concerned with the inception of blood clotting.

The Maintenance of Normal Blood Fluidity

Many workers on the subject of blood coagulation have been so preoccupied with an explanation of the change from fluid to solid that their theories hardly explain why the blood is fluid in the body. Yet it is clearly as important to the individual that his blood should not clot in his vessels as that it should clot outside them.

Normally the blood has an inherent stability in the body. The main reason why intravascular coagulation does not occur is the unbroken continuity of the vascular endothelial surface, which is inactive as regards clotting. Nevertheless, it is not only on this absence of foreign contact that the body relies. Disease may alter the vascular endothelium, but no more than a local deposit of platelets and fibrin may form. Trauma may cause extensive damage to tissues and vessels, with inevitable absorption of thromboplastin and

thrombin, but only local thrombosis of the affected vessels occurs in the majority of cases. Though intravascular clotting can, as has been said, be produced by the injection of tissue extracts, relatively large amounts have to be given to produce it, and even thrombin given intravenously may have no apparent effect (Davis, 1911).

The classical theory does not explain this reluctance of the mass of circulating blood to clot in the presence of active coagulants. It is almost certainly due to the presence of anticoagulant factors, which, though demonstrable, have received comparatively little attention and which probably maintain a dynamic equilibrium with coagulant factors that are slowly produced in even normal circulating blood.

It was early recognized that thrombin rapidly disappeared from serum after coagulation was complete. Schmidt (1892), in studying this problem, paid particular attention to the fact that after such spontaneous inactivation the thrombin could be regenerated by the addition of acid or alkali. Morawitz (1924) came to the conclusion that thrombin was converted into an inactive form which he named "metathrombin," and Weymouth (1913) and Gasser (1917) concluded that there is a substance, or antithrombin, present in serum with which thrombin combines and by which it is therefore inactivated. No further progress was made until 1935, when Lenggenhager stated that this antithrombic factor was associated with the albumin fraction of the serum, a finding confirmed by Quick (1938b), who later called it "albumin X" (Quick, 1942) and showed that it had a lesser affinity for thrombin than had fibrinogen. Glazko and Ferguson (1940) have called it the "progressive antithrombin" to distinguish it from heparin and its co-factor, which they called the "immediate antithrombin." It has been studied by Astrup (1944), who calls it simply "antithrombin." Wilson (1944) believes that the thrombin is adsorbed directly by the albumin, but Wöhlisch and Köhler (1942) and Grüning (1943) find that the antithrombin activity of serum albumin is removed by extraction with chloroform or ether. The ether extract is itself antithrombic and appears to be a lipid. Antithrombin has, therefore, similarities to the serum antitrypsin, or antiplasmin, which is also a labile factor associated with serum albumin and extractable with lipid solvents (see Macfarlane and Biggs, 1948), and it is of interest that proteolytic enzymes such as trypsin are capable of regenerating from a thrombin-antithrombin mixture (Wöhlisch and Köhler, 1942).

An anticoagulant which has been more intensively investigated is heparin. Discovered by McLean (1916), it was thought by Howell (Howell and Holt, 1918) to be the antiprothrombin required by his theory. Mellanby (1934) and Quick (1936b), however, were able to show that the main action of heparin was against thrombin and not prothrombin or thromboplastin, though Brinkhous (1939) later showed that there was some inhibition of prothrombin conversion in the presence of heparin.

The action of heparin on thrombin, however, is not direct. Howell and Holt (1918) showed that it had little effect on thrombin and fibrinogen, and Quick (1936b) showed that this was due to the absence of a co-factor present in the albumin fraction of whole plasma without which heparin loses its effect. This co-factor has also been studied by Astrup (1944), who finds that it is more labile than the serum antithrombin, being destroyed by heating to 56° C., and that it disappears during the process of coagulation. The heparin co-factor complex has been called "immediate antithrombin" by Glazko and Ferguson (1940), and "thrombin inhibitor" by Astrup (1944), the co-factor being called "thrombin co-inhibitor."

Heparin was purified by Charles and Scott in 1933 and found to occur in liver, lung, muscle, heart, and blood. The chemistry of the substance was studied by Jorpes and Bergström (1937), who established the fact that it is a mucosin polysulphuric acid ester. Hölmgren and Wilander (1937) showed that the basophil granules of tissue mast cells are probably composed of heparin, a supposition recently confirmed by Oliver and others (1947).

It is likely (Astrup, 1944) that heparin exerts its effect by virtue of the strongly charged sulphuric acid group. Bergström (1936) has synthesized sulphuric acid esters of different polysaccharides, and some, including those of chitin, cellulose, and pectin acid, have an anticoagulant action like that of heparin. It is significant that the anticoagulant azo dyes also contain a sulphuric acid group.

Jaques and others (1942) have shown that the heparins isolated from different animal species have different anticoagulant activity.

The importance of heparin as a physiological anticoagulant is still uncertain. If it is present in normal blood, it is in small amounts, but certain conditions as, for example, anaphylactic shock (Howell, 1925; Jaques and Waters, 1941) or heavy exposure to irradiation (Allen and Jacobson, 1947)

may cause such a rise in blood heparin that partial or complete incoagulability results.

Other anticoagulant agents in the normal clotting mechanism, though postulated, have not been definitely established. Collingwood and MacMahon (1912) have suggested an antithromboplastin, and Tocantins (1944a, b; 1945) has produced evidence that such a factor may exist, being, he thinks, increased in amount in haemophilia.

Quantitative Studies on the Coagulation Factors

It is not until the study of a particular reaction has reached the stage at which the factors concerned can be measured quantitatively that it can be termed a scientific investigation. Though certain of the more stable clotting factors have been the subjects of quantitative study, it is only in the past few years that the main developments along this line have taken place. The stimulus has undoubtedly been the discovery of vitamin K and the condition of hypoprothrombinaemia, both being dependent on the determination of prothrombin concentration; and the use of anticoagulants in the control of thrombosis must itself be controlled if the treatment is not to be more dangerous than the disease. It is obvious that a general change in attitude towards the problem of coagulation has sprung from these necessities, and its study from being a pleasant pastime for argumentative academicians has grown up overnight into a practical science with vital applications.

The effect of the increased precision of thought and technique which this new development has demanded has not, however, been to simplify the general picture of the coagulation process. On the contrary, it has been found that new factors and new and complicated interrelationships must be postulated if the new quantitative results are to be intelligible. These have, for the moment, been wedged into the already rather rickety framework of the "classical theory." Whether it will continue to bear this increased weight, or whether it will collapse to be replaced by a new structure, remains to be seen. These forebodings can be illustrated best by considering the various quantitative procedures and the complications that have arisen from them.

Simple blood coagulation time.—The time taken for blood to clot after its removal from the body provides the oldest and simplest of the quantitative approaches to the problem of coagulation and was estimated by most of the early workers on the subject. Many of them devised their own methods, some of considerable ingenuity, and it would be impossible to embark on even a brief survey of the instruments

constructed, varying in complexity as they do from an inch or so of capillary glass tubing to a room full of electrical machinery.

In general the simplest methods are the best for ordinary purposes, and the methods in common use to-day require no special apparatus. Blood obtained by venepuncture is preferable to that from a skin puncture, because in the former case, assuming reasonable technical skill, there is a minimum admixture with tissue juice, which in capillary blood may be present in variable amounts, thus adding an uncontrollable variable to the estimation. For venous blood samples the method of Lee and White (1913) is usually employed. In patients in whom venepuncture is contraindicated capillary blood samples are usually tested by the methods of Dale and Laidlaw (1911), Wright (1893), Săbrăzes (1904), or Bürker (1907).

Prolongation of the simple coagulation time gives evidence of gross impairment of the coagulability of the blood, such as is met with in haemophilia, fibrinopenia, or severe hypoprothrombinaemia. It does not, however, give a significant indication of the level of fibrinogen until this is almost completely absent (Schmitz, 1933), prothrombin until it is less than 10 per cent of the normal value (Quick, 1945a), and calcium unless this is below the level at which tetany usually occurs (personal observation). In the majority of cases the main factor controlling the clotting time of whole blood seems, therefore, to be the concentration of thromboplastin (Quick, 1942). All other factors are normally present in considerable excess for the production of fibrin within the time of this estimation.

As such estimations are commonly performed there are a number of factors which may influence the results. Small amounts of tissue juice may greatly shorten the clotting time of haemophilic blood. Dirty tubes or syringes may introduce significant variations. Temperature must be controlled carefully, since it has a considerable effect. Fig. 3 illustrates the effect of varying temperature on the clotting time of normal blood in a mechanical coagulometer of the author's design (Macfarlane, 1938a). At the temperatures above 47° C. it was observed that clotting was poor, part of the fibrinogen being precipitated before coagulation could occur.

Fibrinogen and calcium.—The two substances which have been susceptible to ordinary biochemical assay methods are calcium and fibrinogen. The former has not involved any serious difficulty from the point of view of coagulation theory, though its exact part in the mechanism is still not known.

Fibrinogen.—The estimation of fibrinogen usually involves the clotting of a given amount of oxalated plasma by recalcification, removal of the fibrin, washing it free of soluble protein, and the determination of its mass by either drying and weighing or by nitrogen determination. Apart from technical errors and difficulties, this method assumes that all the available fibrinogen is converted into fibrin by this process, and

FIG. 3.—Curve showing the effect of temperature on the coagulation time of normal whole blood. (Macfarlane, 1938a.)

that one milligramme of fibrin is derived from one milligramme of fibrinogen. The latter assumption is probably correct, but the former is not necessarily so. In any condition in which thrombin production is defective only a part of the fibrinogen may be converted, and subsequent addition of thrombin may produce another clot. It is also doubtful if even added thrombin removes all the protein which should strictly be regarded as fibrinogen. It has been found in the writer's laboratory that after a given amount of fibrinogen has been clotted by thrombin there is still 10 per cent left in solution that can be precipitated by heating to 47° C. or by quarter-saturation with ammonium sulphate.

The estimation of thrombin activity.—The estimation of the activity of a given solution of thrombin is the essential basis of quantitative assay of any of the other coagulant factors, since, of all the reactions involved in clotting it is only the reaction between thrombin and fibrinogen that gives a visible effect. In principle, the thrombin to be tested is added to either oxalated plasma (Mellanby, 1933) or fibrinogen solution (Warner and others, 1936), and the clotting time determined. Mellanby (1933) defined a thrombin unit as that amount required to clot 1 ml. of oxalated plasma in 30 seconds at 37° C. For actual assay, Quick (1942) prepares a "standard thrombin" from a given volume of normal plasma, makes serial dilutions of this from full strength to 1/300, and adds

0.1 ml. of each dilution to 0.2 ml. of normal oxalated plasma at 37° C., taking the time of coagulation for each dilution. From the curve of clotting times plotted against dilutions, the strength of an unknown thrombin is read off by estimating the coagulation time with the same plasma. Such a method, of course, merely gives the value of the unknown in terms of the arbitrary standard, and, as has been shown by Owren (1947a) and Astrup (1944), oxalated plasma is apt to give variable results even with the same thrombin dilutions. These difficulties are almost certainly due, in part, to the presence of antithrombin which will affect the longer clotting times of weak thrombin dilutions more than the shorter times (Fig. 4).

The use of fibrinogen solutions, prepared by removing prothrombin by adsorption with magnesium hydroxide and subsequent salt precipitation, allowed Warner and others (1936) to define a "thrombin unit" as that amount of thrombin that would clot 1 ml. of 0.08–0.1 per cent fibrinogen in 15 seconds at 28° C. There are, however, difficulties even with this refinement of the method. The reaction time is considerably influenced by such variables as pH, protein and salt concentration, and osmotic pressure (Quick, 1942; Owren, 1947a), and Astrup (1944) was forced to use a dried preparation of thrombin as a reference standard in his precise quantitative investigations. Some of these difficulties have been explained by Owren (1947a), who has shown that fibrinogen solutions prepared in the ordinary way

FIG. 4.—Curves showing the relationship of thrombin concentration to clotting time of oxalated plasma (A) and fibrinogen solution (B). (After Owren, 1947a.)

contain variable amounts of profibrin, which react more rapidly with thrombin than ordinary fibrinogen. Profibrin-free fibrinogen, which is therefore essential for accurate work, can be prepared by centrifuging off the bulk of the profibrin in the cold from freshly thawed frozen fibrinogen, and adsorbing the rest with kaolin. The resulting clear solutions of fibrinogen give regular results.

The estimation of thromboplastin.—The estimation of thromboplastic activity is one of great technical difficulty, since it requires adequate standardization and control of all the other known (and unknown) factors involved in the coagulation process. Fischer (1935a) used bird plasma as an indication of thrombokinase activity, but in view of the known species specificity of the clotting factors (Quick, 1942) such a system may not be applicable to mammalian thromboplastin. Mills (1921) deduced a formula for the assay of thromboplastin which has been also used by Astrup and Darling (1942). Astrup (1944), however, has found that there are differences in the qualitative reactivity of thromboplastins prepared from different organs which introduced uncertainty, brain thromboplastin being less reliable than lung preparations. Other fallacies have been discussed by Owren (1947a), who illustrates the inhibitory effect on coagulation of excessive concentrations of thromboplastin. Quick (1936a) and Aggeler and Lucia (1938) have used oxalated plasma for assay of thromboplastin.

The estimation of prothrombin.—The development of practical methods for the estimation of prothrombin was essential to the most fruitful research in blood coagulation, the discovery of vitamin K, the hypoprothrombinaemic states, and dicoumarin.

FIG. 5.—Curve showing the relationship of prothrombin concentration to coagulation time of recalcified oxalated plasma in the presence of optimum thromboplastin. (After Quick, 1942.)

Quick (1935b), during an investigation of the haemorrhagic tendency in jaundice, developed his method of prothrombin estimation which has proved so useful. In principle, it depends on the assumption that there are four reactants in the coagulation mechanism—thromboplastin, prothrombin, calcium, and fibrinogen. If any three of these are fixed, variations in the clotting time of the mixture must, it is assumed, be due to variations in the fourth factor. To determine the prothrombin concentration the concentration of thrombokinase, calcium, and fibrinogen should be controlled, preferably at their optimum concentration for coagulation, so that the clotting time of such a system should then be proportional to the concentration of prothrombin. In practice it is found (Quick, 1942) that fibrinogen variations in human plasma are not usually of such magnitude as to introduce a significant error, and the calcium concentration can be controlled by using oxalated plasma and a known amount of calcium chloride.

The remaining variable to be controlled is thromboplastin. In ordinary whole blood this is present in amounts so far below the optimum that variations in other factors have little effect on the clotting time. Quick therefore added "excess" thromboplastin—in the form of rabbit brain emulsion (later changed to acetone-extracted dried rabbit brain made up in saline (Quick, 1942)—to the oxalated plasma to be tested, together with the optimum amount of calcium chloride, and recorded the coagulation time. At first the results of this estimation were expressed in terms of the clotting time of the mixture, a normal control plasma being used. In 1938, however, Quick published a curve relating the clotted time by this method to the prothrombin concentration. This curve

was constructed by making a number of dilutions of normal and abnormal plasma samples with saline, and from it percentage of prothrombin can be read off, assuming that 100 per cent is an average normal content, and that the thromboplastin used gives the clotting time with normal plasma that is equivalent to 100 per cent on the curve (Fig. 5). The theoretical basis of this curve is, of course, open to several objections. In the first place, as pointed out by Aggeler and others (1946) it was not constructed from a sufficiently large number of normal individuals, and the 100 per cent figure is quite arbitrary. More serious is the assumption that plasma diluted with saline is equivalent to undiluted plasma with a reduced prothrombin content. It is clear that dilution reduces all the clotting factors equally, including fibrinogen, while the naturally occurring hypoprothrombinaemia presumably affects only prothrombin. Many attempts

have been made to circumvent this difficulty, mainly by the use as a diluent of "prothrombin free" plasma, this being prepared by adsorbing normal plasma with $\text{Al}(\text{OH})_3$ or by Seitz filtration. The results of such attempts to reproduce ranges of plasma dilution corresponding to natural prothrombin-deficient plasma have, however, been difficult to interpret. Quick (1945b) has emphasized the difference from saline-diluted plasma. Other workers (for example, Nitshe and others, 1947) have used fibrinogen solution as a diluent; and the whole complex problem has been discussed by Conley and Morse (1948) in relation to the reliability of thromboplastin preparations.

Many modifications of this so-called one-stage method have appeared. Smith and others (1939) introduced what is known as a "bedside" technique, which is carried out with whole blood and with much the same technique as that used for the Lee and White method, except that thromboplastin is added to the blood; and Kato (1940) has devised a micro-method also using whole blood. Fullerton (1940) suggested using Russell's viper venom as thromboplastin, the satisfactory results he reported having been confirmed by Page and Russell (1941). This material has the advantage of very high activity combined with almost complete stability when the venom is dried. On the supposition that the minimum clotting time indicates the optimum thromboplastin concentration, Witts and Hobson (1942) added lecithin to the venom, thereby so greatly increasing its activity that the normal prothrombin time was about 5 seconds. The use of Russell's viper venom, however, may give dangerously misleading results in patients receiving dicoumarin.

Several workers (Quick, 1942; Shapiro and others, 1942; Link, 1943; Allen and others, 1940; Aggeler and others, 1946) have recommended the practice of making one or more dilutions of the unknown plasma in order to obtain a longer clotting time which is more critically related to prothrombin concentration. It is true that the first part of the curve is flat and that little change in clotting time is observed until the prothrombin is 50 per cent or less, but the use of dilutions obviously introduces errors and fallacies of its own, as discussed by Fisher (1947).

Other complications appear when the prothrombin concentration is so low that long clotting times are obtained by this method. It has been found by Jaques and Dunlop (1945a, b) that the calcium concen-

tration becomes very critical when low prothrombin concentrations are produced *in vivo* by the use of dicoumarol, or by adsorption of prothrombin by alumina *in vitro*, so that a slight divergence from the optimum has a considerable anticoagulant effect. Observations made by Dr. Rosemary Biggs (unpublished), extending those of Aggeler and Lucia (1938), show that thromboplastin concentration is also an increasingly critical factor as prothrombin concentration is reduced and that in such cases a slight excess of thromboplastin may actually inhibit clotting (Fig. 6).

Despite these theoretical and practical disadvantages Quick's original method provides a most valuable indication of the patient's liability to bleed, which is the main object of the majority of prothrombin estimations. The procedure has been standardized and its reliability investigated statistically by Aggeler and others (1946), but a few comments on the expression of the results might be added. In many laboratories it is the practice to express the results of a prothrombin

FIG. 6.—Curves showing the relationship of thromboplastin concentration to coagulation time for a number of different prothrombin concentrations ("100 per cent" thromboplastin = 1 g. acetone dried brain in 10 ml. saline). Curve A is for 5 per cent prothrombin, B for 10 per cent, C for 20 per cent, D for 50 per cent, and E for 100 per cent prothrombin. (Dr. Rosemary Biggs's data.)

determination as an "index" by means of the formula

$$\frac{\text{Normal Prothrombin Time} \times 100}{\text{Patient's Prothrombin Time}}$$

This, while convenient, gives a very misleading impression of the prothrombin concentration actually present in the patient's blood as calculated from Quick's curve. For instance, a "prothrombin index" of 33 per cent (i.e., patient's prothrombin time = 36 seconds; normal time = 12 seconds) is equivalent to 10 per cent prothrombin, and an index of 80 per cent is equivalent to 50 per cent prothrombin. Quick (1939) has suggested the use of the formula

$$\text{Prothrombin \%} = \frac{302}{\text{Prothrombin time} - 8.7}$$

assuming that the normal prothrombin time with the thromboplastin used is about 12 seconds. The use of this is preferable to the index, if the curve itself is not available. A difficulty in the use of both curve and formula is that of standardizing the thromboplastin to give a normal time of 12 seconds. The definition of the term "normal" in this respect obviously requires further investigation. In the writer's laboratory a number of curves have been constructed relating prothrombin concentration to clotting time for different levels of thromboplastin activity. By

FIG. 7.—The relationship of clotting time to prothrombin concentration as it applies to Quick's method (determined by means of plasma dilutions) for 4 thromboplastin preparations of different potency, A, B, C, and D. Approximate linearity has been obtained by plotting the reciprocal of the clotting time (vertical axis) against the logarithm of the percentage prothrombin concentration (horizontal axis). (Dr. Rosemary Biggs's data.)

plotting the logarithm of the prothrombin concentration against the reciprocal of the clotting time, almost straight lines are obtained (Fig. 7). By the use of these, a given prothrombin time can be read off as concentration by interpolation, the activity of the thromboplastin used being known by testing against normal plasma. Rapoport (1947) has discussed other methods of calculating results.

It has already been pointed out that Quick's method depends upon the assumption that the *amount* of prothrombin available controls the *rate* at which thrombin is formed, this rate in turn determining the clotting time of the plasma. In fact, only about 1 per cent of the prothrombin has been converted by the time that clotting occurs in normal plasma, full conversion, even in the presence of optimum thrombokinase, taking some minutes.

It would appear more scientific to use, instead of this dynamic method, dependent as it is on unknown factors controlling the rate of thrombin formation, a static method in which all the available prothrombin is converted into thrombin, which is then assayed by means of standard fibrinogen. Such a principle is the basis of the 2-stage method originated by Warner and others (1936). In this, the unknown plasma is first clotted by a small amount of thrombin (equivalent to about 1 thrombin unit), and the fibrin so formed is removed after 15 minutes when the added thrombin has been inactivated by the antithrombin of the plasma. The resulting fluid, containing all the original prothrombin but no fibrinogen, is then diluted to 1 in 20 with oxalated saline and activated with a mixture of buffered calcium chloride and thromboplastin. Samples of this reacting mixture in which thrombin is generated are then transferred to standard fibrinogen solutions and the clotting time of this is recorded. Dilutions of the reacting mixture are made to give a clotting time of about 15 seconds, this dilution therefore containing 1 unit of thrombin per unit volume as originally defined. The number of units per ml. of original plasma is then calculated from the final dilution of the plasma, and it is assumed that one unit of thrombin is derived from one unit of prothrombin.

The process of thrombin formation under these conditions in relation to time is illustrated in Fig. 8. It will be seen that the process starts slowly, and increases in rate until the thrombin concentration reaches a maximum which, instead of remaining constant, declines. The general shape of the initial part of the curve suggests an autocatalytic reaction (see Astrup, 1944), which is of considerable theoretical interest. The important practical points are the sharply defined maximum, necessitating repeated sampling during activation for its recognition and, even more important, the destruction of thrombin by antithrombin, indicated by the falling off of the curve. The authors of the method assumed that this antithrombin action was not significant at a dilution of 1 in 20 or over, but there is little doubt that it may be, and that it constitutes a serious drawback to a method already handicapped by the practical dis-

FIG. 8.—Curves illustrating the concentration of thrombin in a reacting mixture of thromboplastin, calcium, and diluted oxalated plasma, plotted against time, and for different dilutions of plasma. Curve A is for plasma diluted 1/25, curve B for plasma diluted 1/50, and curve C for plasma diluted 1/100. (Author's data.)

advantages of elaborate technique and reagents. Attempts have been made to overcome some of these disadvantages. The technical modification of Herbert (1940) provides a much simpler procedure, suitable for routine laboratory use. She makes an initial dilution of the test plasma, activates with thromboplastin and calcium, and takes sub-samples for thrombin assay, finding that the fine web of fibrin forming in the dilute reaction mixture does not interfere with the sampling. The thrombin units are read off by a calibration curve so that serial dilutions are unnecessary. Sternberger (1947) has also introduced a practical and possibly important modification by suppressing the antithrombin by the addition of alcohol. This, if successful, would eliminate the danger of loss of thrombin in slowly activating mixtures, but it has been found (personal observation) that the alcohol makes the end-point difficult to read, and it may have other unknown effects.

The 2-stage method has, of course, the academic advantage that it gives an estimate of prothrombin in absolute units and, when suitably modified, is not outside the scope of a pathological laboratory. It does not provide, however, the clinical information that the 1-stage test gives on the patient's liability to bleed.

Discrepancies Arising out of Prothrombin Estimation

The use of these two different principles for determining prothrombin on clinical and experimental material soon revealed a number of disturbing discrepancies in the results obtained. The first to appear was the relative normal prothrombin levels of different species. Quick found, for instance (1936b, 1941a), that rabbit plasma contained five times as much prothrombin as human blood, and cat blood about three times as much. Using the 2-stage technique, however, Warner and others (1939a, b) found human, cat, and rabbit plasma to have about the same prothrombin content of about 300 units per ml. It was also found by the 2-stage technique that stored blood maintained its prothrombin content for months (Warner and others, 1940) whereas by the Quick method it lost up to 90 per cent in the first week (Quick, 1943). Other discrepancies involving the prothrombin concentration in the newborn (Owen and others, 1939; Brinkhous and others, 1937; Quick and Grossman, 1939), and in jaundice, and also the level at which haemorrhage occurs (Brinkhous, 1940; Quick, 1938a) are listed in Table I.

TABLE I
DIFFERENCES BETWEEN RESULTS OF 1- AND 2-STAGE METHODS FOR ESTIMATING PROTHROMBIN

Source of prothrombin	1-stage	2-stage
Blood during storage	Rapid fall	Slow fall
Newborn babies	Sometimes normal	Consistently low
Obstructive jaundice	Low in some cases	Low in most cases
Cases beginning to bleed from prothrombin deficiency	10% prothrombin	30-40% prothrombin
Rabbit blood ..	500% of man	100% of man
Cat blood ..	300% of man	110% of man

It will be seen from this that the difference is not in the same direction in each case, the 2-stage giving lower results than the 1-stage in animals, jaundice cases, and babies, but higher on stored blood and at the haemorrhagic level. It is clear that the two methods are measuring different things, though both claim to measure prothrombin.

At first these discrepancies were considered to be due to differences in the "activity" or reaction rate of prothrombin (Warner and others, 1939b),

but later Quick (1943) made a fundamental observation relating to the discrepancy in the stored-blood estimation. He found that if a volume of plasma from which "prothrombin" had been removed by adsorption by alumina were mixed with a volume of stored plasma with a prothrombin time of 37 seconds (indicating 10 per cent prothrombin content), the prothrombin time of the mixture was 10 seconds, so that the mixture appeared to contain 100 per cent prothrombin and not the expected 5 per cent. Further work established the fact that plasma with a low "prothrombin" content following dicoumarol administration also had the effect of reducing the prothrombin time of stored plasma. From this and other evidence Quick concluded that "prothrombin" is made up of two components, a labile factor destroyed by slight degrees of heat, not absorbed by $\text{Al}(\text{OH})_3$, and becoming inactive after a few days' storage (called at first component A), and a stable factor (component B) which retains its activity for long periods, is relatively heat-stable, is strongly adsorbed by $\text{Al}(\text{OH})_3$, and is reduced by the *in vivo* effect of dicoumarol. In normal and dicoumarol plasma the labile factor is in excess.

Confirmation of the existence of such hitherto unrecognized factors required for thrombin production came rapidly and in some cases independently. Quick's observations were substantiated by Oneal and Lam (1945) and Munro and others (1945), but were disputed by Loomis and Seegers (1947), whose contribution will be discussed later. Feissly (1945c) and Fantl and Nance (1946a) obtained evidence of an accessory factor not included in the classical theory and which they concluded was required for the activation of prothrombin. Zondek and Finkelstein (1945) concluded that there was a thromboplastin co-factor which had been overlooked.

Meanwhile, Owren (1947a, b), working in enemy-occupied Norway and largely cut off from events in the United States, carried out an investigation which has brilliantly illuminated the problem of additional clotting factors. This work began in 1943, when a patient with a curious haemorrhagic diathesis came to his attention. She was a woman of 29, who from infancy had suffered from a severe haemorrhagic diathesis, clinically almost indistinguishable from haemophilia except for the absence of haemarthroses. There was no significant family history, no detectable physical abnormality not attributable to the effects of haemorrhage, and no abnormal blood findings apart from a long clotting time of 25 minutes. It was found, however, that,

unlike true haemophilia, the prothrombin time by Quick's method was prolonged (70 seconds), indicating gross prothrombin deficiency. There was no reason to suspect vitamin-K deficiency, and the administration of vitamin K had no effect. Since all other clotting factors appeared to be normal and no anticoagulant could be demonstrated, the diagnosis appeared to be "idiopathic hypoprothrombinaemia." Many investigators would have been content (as were Rhoads and FitzHugh, 1941; Austin and Quastler, 1945) to accept this diagnosis and do no more. Owren, however, was more tenacious, and soon found that the addition of normal human plasma to the patient's plasma, even in as small a concentration as 10 per cent, would reduce the prothrombin time of the mixture to normal. Since this could not be explained by the simple addition of prothrombin, Owren tried the effect of adding normal plasma from which the prothrombin had been removed by adsorption with $\text{Al}(\text{OH})_3$; 20 per cent of such plasma, itself incoagulable, reduced the prothrombin time of the patient's plasma to normal, thus demonstrating the presence of some factor that was not fibrinogen, since this was normal in the patient's plasma, nor prothrombin, nor calcium or thromboplastin, both of which had been ineffective in the prothrombin test. Owren called this factor which was deficient in his patient "factor 5," being the fifth clotting component. He went on to isolate factor 5 in a state of relative purity, and in an extensive series of experiments he showed that it is very labile, is not absorbed by $\text{Al}(\text{OH})_3$, and is essential for the conversion of prothrombin to thrombin by thromboplastin and calcium. He showed that the injection of the factor into his patient reduced her coagulation time to normal. The failure by previous workers to recognize factor 5 seems to be due to the fact that, by ordinary methods of preparation, the fibrinogen and prothrombin fractions are heavily contaminated with it, so that its detection by virtue of its absence is difficult.

Quick (1947c) has not been slow to recognize Owren's factor 5 as being probably identical with his own "labile factor." Indeed, it seems probable that most of the discrepancies between the 1- and 2-stage methods are due to unsuspected variations in factor 5. The 1-stage method, being dependent as it is on the rate of conversion of prothrombin, is very sensitive to variations in the concentration of such an accelerator, while the 2-stage method, dependent upon the amount of thrombin generated, is much less so. This is one of the reasons why, for practical purposes, Quick's method gives a better indication of the patient's

liability to bleed. In the rare event of a factor-5 deficiency being suspected in a patient, factor 5 can be estimated by the method of Owren (1947a), which depends on the addition of dilutions of the test plasma to factor-5-free prothrombin, fibrinogen, thromboplastin, and calcium.

It seems to be established by weight of evidence, therefore, that what is usually regarded as prothrombin is a complex of two or more components. The objection of Loomis and Seegers (1947) was based on the fact that the prothrombin time of old plasma could be restored by the addition of fresh fibrinogen so that they considered Quick's labile factor to be a fibrinogen defect, but it is probable that their fibrinogen was contaminated with factor 5, which might thus be the active agent. The finding of Chak and Giri (1948) that the prothrombin time of stored plasma can likewise be restored by the passage of CO_2 suggests, however, the possible existence of a labile inhibitor that is developed during storage, and it is not, at the moment, clear in its implications.

Factor 5 or its equivalent has been accepted as an entity by some workers with such enthusiasm that they have discussed the priority of its discovery with considerable energy (*Lancet* correspondence, 1947). It must not be forgotten, however, that it is Nolf who has always maintained (Nolf, 1908, 1938, 1945) that five factors take part in coagulation. It is most probable that the factor called "thrombogen" by Nolf is identical with the newly described "factor 5" of Owren and "labile factor" of Quick. All three descriptions portray a relatively labile substance not adsorbed by such agents as $\text{Al}(\text{OH})_3$, $\text{Ca}_3(\text{PO}_4)_2$, or Seitz filtration, and which is necessary for the coagulation, by thromboplastin and calcium, of a mixture of fibrinogen and the conventional "prothrombin." The credit for original recognition of this factor would thus appear to be due to Nolf.

Other factors.—Unfortunately factor 5 is not the only addition which is likely to be made to the coagulation theory. Owren (1947a) considers it necessary to postulate a derivative of factor 5 which autocatalytically activates prothrombin in the presence of thromboplastin and calcium. This is based on the observation that small subsamples of a reacting mixture of thromboplastin, prothrombin, factor 5, and calcium will clot oxalated plasma faster than it will clot an equivalent solution of fibrinogen, whereas a given amount of thrombin will clot the fibrinogen faster than the plasma. It is argued that something must be produced during the reaction that activates the

prothrombin of the oxalated plasma, so that there is more available thrombin in this system than in the fibrinogen mixture. It can be shown that the thromboplastin and calcium are not active in this way in the absence of prothrombin. It is supposed, therefore, that a "factor 6" is generated that explains this autocatalytic reaction. These results are of interest, but their interpretation is not yet quite clear. Astrup (1944) obtained substantially similar results, and discussed the autocatalytic reaction at some length, being of the opinion that it is due to the activation of a plasma thrombokinase. Fischer (1935b) had made similar observations, and Bertrand and Quivy (1946)—using a photometric method for estimating clotting times—and Laki (1943) also support the necessity for postulating autocatalysis in the normal generation of thrombin, but further and carefully controlled experiments are required before the significance of this phenomenon is clear.

Quick (1947c), from studies of two families with inherited haemorrhagic diathesis associated with prolonged 1-stage prothrombin times, comes to the conclusion that in one of them the defect is due to a deficiency of prothrombin component B, already described. The other family, however, shows the deficiency of another prothrombin factor which is neither B nor the labile factor (previously called component A, very probably identical with factor 5). Quick, therefore, postulates the existence of a third prothrombin component deficient in this family, and proposed "to avoid confusion" by calling his original component A "labile factor," and the new component "component A." He suggests that it is the new component A which is reduced in vitamin-K deficiency.

Quick (1947a) has also—by means of an ingenious experiment involving the decalcification of plasma in silicone-treated tubes, by "amberlite"—shown that there is probably a co-factor required for calcium action in thrombin generation, this being independent of the other components of the prothrombin complex.

Abnormal Blood Coagulation

Artificial anticoagulants.—Of the anticoagulants used either *in vivo* or *in vitro* for therapeutic or experimental purposes, heparin has already been considered, since technically it is a naturally occurring anticoagulant. The various decalcifying agents such as citrates, oxalates, phosphates, and fluorides have been discussed in relation to the effect of calcium on the coagulation mechanism;

and the pancreatic and soya bean trypsin inhibitors have been considered from the point of view of their powerful and interesting antagonism to the action of thromboplastin.

An important group of anticoagulants are certain azo-dyes, including Chicago blue and chlorazol pink studied by Rous and others (1930), Huggett and Rowe (1933), and Huggett (1934), the latter worker considering that their action was on thrombokinas. Recent work by Astrup (1944) suggests that they are similar to heparin in their action, though they do not require the heparin co-inhibitor. Germanin, the anticoagulant action of which was noted by Mayer and Zeiss (1920), was found by Lenggenhager (1936) to act by stabilizing fibrinogen. Astrup (1944) states that this drug, and liquoid (sodium polyanethosulphonate), inhibits the thrombin-fibrinogen reaction possibly by reacting directly with fibrinogen. It has been observed by Fleming and Fish (1947) that penicillin has an anticoagulant effect, but its mode of action has not yet been investigated.

Hirudin, the anticoagulant secreted by the leech, was thought by Mellanby (1909) to be an anti-thrombin. This, and many other similar anticoagulant secretions produced by blood-sucking animals, have been largely neglected in modern times.

Dicoumarin.—The discovery of dicoumarin ranks, in the recent history of research on the haemorrhagic diatheses, second only in importance to the discovery of vitamin K. In 1929 and 1931 Roderick recorded that there appeared to be a prothrombin deficiency in animals suffering from toxic sweet-clover disease. Quick (1937) showed that the toxic clover hay produced a significant fall in the prothrombin level of animals to which it was fed, and, with the aid of prothrombin-time estimations as an assay method, Link and his co-workers (Campbell and others, 1941; Campbell and Link, 1941; Stahmann and others, 1941) were able to identify the toxic principle as 3,3'-methylenebis-(4-hydroxycoumarin) and were then able to synthesize it in the laboratory.

Dicoumarin acts when given by mouth by reducing the prothrombin available for coagulation. There is a lag period after administration of from 24 to 48 hours before the reduction in prothrombin (Allen and others, 1942), suggesting an interference with production rather than a direct effect on existing prothrombin. Vitamin K given with dicoumarin prevents the fall in prothrombin (Shapiro and others, 1943a; Davidson and others, 1945), but once the hypoprothrombinaemia is estab-

lished vitamin K has little effect (Allen and others, 1942; Davidson and Macdonald, 1943; Meyer and others, 1942).

Quick (1947c) considers that dicoumarin causes a reduction of component B of prothrombin, but its mode of action is unknown. Its relation to the salicylates has been put forward by Lester (1944), and there are points of similarity between its structure and that of vitamin K which might suggest a possible competition in which the inactive dicoumarin displaces vitamin K (Fig. 2). A number of workers (Link and others, 1943; Meyer and Howard, 1943; Shapiro and others, 1943b; Rapoport and others, 1943) have reported an effect similar to that of dicoumarin resulting from the prolonged administration of salicylates in man and animals.

Naturally occurring coagulation defects.—There is a group of haemorrhagic diatheses in which the primary cause of the abnormality is inefficient blood coagulation. This may be a result of an inborn, often hereditary, defect, or of an acquired deficiency of some necessary factor, or it may be secondary to a disease process. In general, the clinical manifestations of the different clotting defects are similar. The patient is liable to bleed for long periods of time from, or into, any tissue of the body in which the vessels are damaged by trauma or disease. In consequence, not only is there a danger of death from haemorrhage, but the effects of large effusions of blood into superficial or deep tissues, into joints, the nervous system, or internal organs, may be crippling or even fatal. There is thus a *generalized* haemorrhagic diathesis, in distinction to that of the purpuras, which is usually localized to the skin and mucous membranes (Macfarlane, 1941). As regards pathological investigations, the platelet count, the bleeding time, and the capillary resistance tests are typically normal, the coagulation defect being the only positive finding. Table II shows a classification of these conditions based on the coagulation defects thought to be responsible.

Haemophilia.—Many attempts have been made in the last 50 years to settle the problem of the slow coagulation of haemophilic blood, and to many of the investigators concerned it seemed that a solution had been found only to be refuted by others. The earliest conclusions, curiously, were nearer the most recent views than those that intervened. Sahli (1905) thought that thromboplastin was lacking in this condition, a view supported by the observation of Morawitz and Lossen (1908) that haemophilic blood clotted rapidly on

TABLE II

A CLASSIFICATION OF THE HAEMORRHAGIC DIATHESSES DUE TO DEFECTIVE COAGULATION

Condition	Causation		Coagulation defect
Haemophilia	Hereditary		Thromboplastinogen deficiency
Pseudo-haemophilia	Idiopathic or secondary		
Hypoprothrombinaemia	Idiopathic or hereditary		Component A deficiency
	Vitamin-K deficiency :		
	1. Mal-absorption	Jaundice : spruc	
	2. Congenital	Neonatal	
	3. Mal-utilization	Liver disease	
	Hereditary: idiopathic		Component B deficiency
	Dicoumarin poisoning		
	Idiopathic		Factor 5 deficiency
Afibrinogenaemia	Hereditary		Fibrinogen deficiency
	Liver disease		

the addition of tissue extract, suggesting that the rest of the clotting mechanism was intact.

These observations were extended by Fonio (1914), who showed that normal platelets clotted haemophilic blood in a normal time. Attention was thus directed towards the platelet function in haemophilia, and it was suggested by Minot and Lee (1916) that they were functionally defective in this condition, a conclusion supported by Birch (1932).

It was soon pointed out, however, that these experiments, involving the transfer of normal platelets to haemophilic plasma and *vice versa*, might have other interpretations, and that a plasma factor contaminating the platelets might be the active agent (Eagle, 1935b; Patek and Stetson, 1936). The latter workers, and later Patek and Taylor (1936), showed that small amounts of platelet-free normal plasma were effective in clotting haemophilic blood, even in high dilutions and after filtration, thus explaining the familiar beneficial effect of transfusion of normal blood in haemophilia. Fractionation of the plasma showed that the active factor was associated with a globulin, a conclusion reached independently by Bendien and Van Creveld (1937). Considerable progress has been made along this line by a large group of workers in the United States, and it has been found that the

factor in normal plasma effective to clot haemophilic blood is present in Cohn's Fraction 1 (Cohn, 1946), which contains also fibrinogen and some prothrombin. The factor is relatively heat-stable, so that fibrinogen and prothrombin can be destroyed without loss of activity (Lewis and others, 1946a). The administration of small quantities of this material to haemophilic subjects materially, if temporarily, reduces their clotting time (Minot and others, 1945; Lewis and others, 1946b) without inducing a refractory state sometimes seen as a result of repeated blood transfusions (Munro and Jones, 1943). It may therefore be assumed that in haemophilia there is a lack of a soluble plasma factor, associated with the globulin fraction, and probably related to, or identical with, the plasma thromboplastins already discussed. Reduced activity of this factor could explain the slow clotting of haemophilic blood which is due to the very small amount of prothrombin converted during the clotting process (Brinkhous, 1939), and the normal "prothrombin time" observed by Quick, Stanley-Brown, and Bancroft (1935) when thromboplastin is added.

The platelets, however, have once more assumed some indirect importance in the understanding of this problem. In 1941(b) Quick introduced a diagnostic test for haemophilia which depended on the

fact that centrifuging oxalated haemophilic blood increases the clotting time of the recalcified plasma to a much greater proportionate degree than does the similar treatment of normal blood. Though the exact implication of this reaction is not clear, it suggests that haemophilic plasma is abnormally dependent on the presence of platelets. This particular type of investigation has now been extended by Brinkhous (1947). Using platelet-free plasma samples obtained without any contact action by centrifuging in silicone-lined tubes, he made the following observations:

1. Neither normal nor haemophilic platelet-free plasma will clot on contact with glass, either separately or mixed together.
2. The addition of normal or haemophilic platelets to the haemophilic plasma did not result in clotting in glass.
3. The addition of *both* platelets (normal or haemophilic) and a small amount of normal plasma to the haemophilic plasma resulted in rapid coagulation in glass.

Brinkhous therefore concludes that both platelets and a plasma factor are essential for thromboplastic activity, and that the plasma factor, which he thinks is a "thrombocytolysin" that normally disrupts the platelets to furnish thromboplastin, is lacking in haemophilia. Quick (1947b) has made essentially similar observations by a similar technique, but concludes that the plasma factor is a thromboplastin-precursor (thromboplastinogen) normally activated by a platelet factor, the former being deficient in haemophilia. The writer has already suggested that the platelet factor may not be an activator but a lipoid co-factor.

Whether this defect is due to deficiency of "thromboplastinogen" or to the presence of an inhibitor is not yet clear. Tocantins (1943a, b; 1946) favours the presence of an "anticephalin" in excess. Feissly (1944) also finds an anticoagulant excess in haemophilic blood, associated with the albumin. On the other hand it is curious that there are persistent reports of some defect in the fibrinolytic system of haemophilic blood (Tagnon and others, 1942; Feissly, 1942; Macfarlane and Biggs, 1948), and the possibility that plasmin may be an activator of the thrombokinase complex cannot be ruled out. It may be mentioned that factor 5 is normal in haemophilia (Owren, 1947a).

Pseudohaemophilia.—The term pseudohaemophilia should be reserved to describe the states in which there is a clotting defect indistinguishable from that found in haemophilia, but which, for genetic reasons, cannot be regarded as the true condition. Joules and Macfarlane (1938) described such a diathesis acquired by a woman in late middle life. In no way could

the clotting defect be shown to vary from that of true haemophilia, and apart from the history the clinical resemblance was complete. A similar case has been described by Maddison and Quick (1945). It has also been observed by the writer (unpublished) to occur as a complication of conditions with abnormal globulins, such as multiple myelomatosis, and as Hodgkin's disease. The use of the term pseudohaemophilia to describe cases of athrombocytopenic purpura, a condition which has no clinical, genetic, or haematological resemblance to haemophilia but which is still so described by recent writers (Estrer and others, 1946; Perkins, 1946), is to be deplored.

Hypoprothrombinaemia.—The historical background to the recognition of hypoprothrombinaemia and the existence of vitamin K has already been briefly outlined, and an admirable and detailed account of the conditions in which it occurs will be found in Quick's monograph (Quick, 1942).

The most important cause of hypoprothrombinaemia is vitamin-K deficiency, which may arise in several ways. In obstructive jaundice and biliary fistula the absence of bile salts reduces the absorption of the natural fat-soluble vitamin K from the intestine, with consequent lowering of the prothrombin content of the patient's blood and the development of the characteristic bleeding tendency that so often complicated this type of jaundice in the past. In haemorrhagic disease of the newborn there is, apparently, a congenital deficiency of vitamin K which can be prevented by the prophylactic use of the vitamin (see Lehmann, 1944). A less familiar cause of vitamin-K deficiency is the intestinal absorption abnormality associated with sprue, steatorrhoea, or chronic diarrhoea (Kark and others, 1940; Collins and Hoffmann, 1943). The haemorrhagic diathesis in such cases may occasionally develop rapidly and lead to diagnostic confusion. In liver disease with impaired function the prothrombin-deficiency is apparently due to failure to utilize vitamin K, and, unlike the other conditions mentioned, there is little response to its administration.

In animals, prothrombin deficiency may occur as a result of poisoning by feeding stuffs containing dicoumarin, a condition that has already been mentioned in relation to the discovery of the toxic factor. The now prevalent use of this material in human beings for therapeutic or prophylactic purposes is an important source of hypoprothrombinaemia, though, strictly, it cannot be called a "naturally occurring" condition. The effect of dicoumarin, being delayed and to some extent cumulative, is not always easy to control. In consequence there are many instances of the development of alarming haemorrhagic manifestations,

such as bleeding from operation sites, from mucous membranes, into the tissues, or from the kidneys (Allen and others, 1942; Crawford and Nassim, 1944; Shlevin and Lederer, 1944). Allen and others (1942) found that vitamin K did not reverse the effect of dicoumarin in such cases, though blood transfusion was more effective.

In controlling dicoumarin therapy, and in the detection of hypoprothrombinaemia in general, it is the writer's experience that Quick's prothrombin method performed with a brain thromboplastin gives clinically the most useful results. Prothrombin concentration is expressed as a percentage of normal by the use of a number of the curves already described. It is important to remember that with low concentrations of prothrombin such as are met with in dicoumarin poisoning, the concentration of calcium and thromboplastin becomes very critical, and that occasionally an excess of either may so delay the clotting that Quick's test has a longer time than the simple coagulation time of the same blood sample (Macfarlane, 1942; Fantl and Nance, 1947). Russell's viper venom has been found unreliable as a thromboplastin in assaying the effect of dicoumarin, since the prothrombin time may be considerably shorter than with brain extract and a dangerous overdosage of the drug may be given, an effect also observed by Lempert (1948). It must also be remembered that patients with a moderate hypoprothrombinaemia which is above the level at which bleeding occurs may be precipitated into a severe haemorrhagic diathesis by blood loss. The prothrombin concentration of the blood, already deficient, may be lowered disastrously by the dilution of the blood volume that follows severe bleeding, such as that occurring at operation or delivery.

The cases of so-called idiopathic hypoprothrombinaemia as described by Rhoads and FitzHugh (1941) and Austin and Quastler (1945) are probably examples of a deficiency of one of the newly recognized "prothrombin" components such as factor 5. Owren's (1947b) use of the term "parahaemophilia" for this condition seems to cause confusion, in particular since he has identified the precise deficiency in his own case. Quick's (1947a) two families with component-A and component-B deficiency respectively seem to be examples of inherited defects corresponding to those produced by vitamin-K deficiency, which apparently reduces component A, and dicoumarin poisoning which reduces component B.

Fibrinopenia.—Fibrinopenia may occur as a congenital absence of fibrinogen, possibly inherited as a recessive character (Macfarlane, 1937a) or secondarily to severe liver disease. Both are rare conditions, only 3 cases of the congenital form having been described in this country (Macfarlane, 1937a; Witts, 1942; Henderson and others, 1945). The subject has been recently reviewed by Yeager and others (1947).

When the absence of fibrinogen is complete, as was the case with the English patients, the blood is, of course, incoagulable by any means except the addition of fibrinogen. The rest of the clotting system appears to be intact, however (Macfarlane, 1937a), though thrombocytopenia may be associated with the disorder. It is curious that these patients are less severely incapacitated by their abnormality than is usually the case with haemophilia, despite the fact that any participation of blood coagulation in whatever mechanism prevents fatal bleeding is impossible.

The fibrinopenia secondary to some disease process is usually not complete so that diagnosis may be more difficult, since the clotting time is normal. The poor quality of the clots should be obvious, however, and a fibrinogen determination will reveal the defect.

Increased fibrinolysis.—It has been suggested (Reimann, 1941) that inherent fibrinolysis of the clots formed in certain cases may be the cause of a haemorrhagic diathesis. The evidence put forward is not convincing, and even in cases with active fibrinolysis no haemorrhagic diathesis has been observed by the writer that could be attributed directly to its action. Bacterial contamination, however, may activate plasmin to an extreme degree and produce considerable proteolysis that may be one cause of so-called secondary haemorrhage.

Circulating anticoagulants.—It has already been stated that anaphylactic shock will cause an outpouring of heparin in animals and possibly in man. A similar effect has been observed by Allen and Jacobson (1947) in animals exposed to irradiation, an observation of importance in view of the increasing risk of such exposure in modern life.

Naturally occurring anticoagulants in demonstrable form as a cause of bleeding are of extremely rare occurrence, though cases have been described by Lozner and others (1940) and Fantl and Nance (1946b).

Hypercoagulability of the Blood

1. Artificial coagulants.—It is understandable that in the past, when the specific deficiencies now recognized to be responsible for coagulation defects were not understood, recourse should have been had to many strange substances thought to promote coagulation. Comparatively few agents have in fact the effect of hastening true coagulation other than those already partaking in the natural process. In recognition of this, attempts were made to extract these natural coagulants, and many proprietary preparations were derived from platelets, leucocytes, brain, muscle, lung, glands, milk, and so on. Since these were often intended to be given parenterally it is perhaps fortunate that few of them possessed the powerful coagulant effect claimed for them, so that if no benefit was derived from their use little harm came either. It soon came to be recognized that in haemophilia,

to the treatment of which these efforts were mainly directed, only the transfusion of fresh normal blood, or the globulin fraction derived from it, is effective in lowering the clotting time.

The use of calcium for diminishing the risk of haemorrhage is still prevalent, however. It is apparently based on the misleading statement by Wright (1893) that calcium reduced the clotting time of haemophilic blood, and there seems to be little other evidence to support its use.

For local application, two natural products, thrombin and fibrin, have proved themselves to be useful. There are now several preparations of thrombin which are extremely active and will produce coagulation within a few seconds. One of these is derived from the interesting "rabbit-clotting globulin" described by Parfentjev (1941) and Taylor and others (1941), and subsequently produced commercially. It must be stressed, however, that no matter how powerful the coagulant its mere surface application to a bleeding point is useless for haemostatic purposes. The flow of blood merely washes the coagulant away to form ineffective clots at some point removed from the site of haemorrhage. It is essential in addition to control the bleeding by temporary pressure to give the mixture of blood and coagulant time to clot *in situ*.

The haemostatic efficiency of a blood clot is increased if it is reinforced by a dressing or light plug. The disadvantage of such foreign material is that it has eventually to be removed, a process often resulting in a recurrence of haemorrhage. The use of preformed fibrin in the form of sheets or "foam" is a considerable advantage in this respect, as it serves as a dressing that is naturally disposed of during healing (Macfarlane, 1943). Such fibrin preparations have now been greatly improved by American workers and have been used with success in a number of conditions (Bering, 1944; Ferry and Morrison, 1944; Woodhall, 1944). Other digestible haemostatic dressings of artificial nature, such as gelatine sponge (Light and Prentice, 1945) and soluble cellulose (Seegers and Doub, 1944), have also been reported to be efficient.

Of the artificial coagulants, some, such as citrate, egg albumin, peptone, pectin, gum arabic, and others, have been given parenterally. Citrate, paradoxically, seems to have some slight and transitory coagulant effect (Neuhof and Hirschfeld, 1922), but very large transfusions of citrated blood to exsanguinated patients have, in the writer's experience, been associated with depressed coagulation. An egg albumin preparation was

developed by Timperley and others (1936) for the treatment of haemophilia, but their early results have not been substantiated.

Of artificial coagulants for local use, the venom of Russell's viper, physiologically resembling a thromboplastin, has been found effective (Macfarlane and Barnett, 1934; Macfarlane, 1935) with the same reservations that apply to the use of thrombin. Other snake venoms have a coagulant action (Barnett and Macfarlane, 1934), and Rosenfeld and Lenke (1935) have used that of the Australian tiger snake as a local haemostatic.

Many other substances, mainly used out of respect for tradition, have been employed as local haemostatics, and it would be laborious and out of place to consider them here. An account of many of them is given by Pickering (1928). Some, such as ferric chloride and turpentine, are not true coagulants but precipitants of plasma protein, and they have a dangerously necrotizing effect on the tissues, so that their use is absolutely contraindicated in haemophilic patients in whom healing must be encouraged by all possible means. The cautery has a similar effect and should be avoided in haemophilic patients for the same reason.

2. Intravascular coagulation.—The clotting of blood within damaged vessels and those immediately adjacent to a traumatized area is, of course, a physiological response to injury and a safeguard against blood loss. Coagulation in such vessels occurs because the local production of coagulant factors overwhelms the local concentration of anticoagulant factors. This process extends until the junction of the affected vessel with one carrying flowing blood is reached, when the clotting factors are so diluted as to be ineffective. Similarly, on isolated areas of damaged endothelium there will be deposition of platelets followed by fibrin to build up the familiar white thrombus, but so long as the blood flow is maintained massive intravascular coagulation will not occur.

The uncontrolled extension of this physiological process or its appearance in remote parts of the body is a familiar hazard of post-traumatic and post-operational states, and of certain disease processes. Such extensive thrombosis may restrict the blood supply of a limb, or of a vital tissue, as in mesenteric, coronary, or cerebral thrombosis, and detachment of a clot may cause pulmonary or other embolism.

Since it is true to say that many more patients die of thrombosis and its effects than of inefficient coagulation, it is clear that the problem is of greater social importance than the haemorrhagic diatheses and would seem to deserve greater attention in this review. However, this mysterious and

unpredictable occurrence apparently is not primarily due to an abnormality of the blood-clotting mechanism, but to the combination of tissue damage and circulatory stasis that may displace the clotting-anticlotting equilibrium in favour of coagulation.

A further cause of thrombosis is infection by certain organisms. It is well known that most pathogenic strains of *Staph. aureus*, for instance, produce a toxin capable of rapidly clotting human blood. Since it is equally effective if the blood is oxalated, it was assumed to act as a thrombin. Smith and Hale (1944) have investigated the action of this toxin and have shown that it requires the presence of a co-factor, at the moment unidentified, that exists in most, but not all, samples of human plasma.

Many of the snake venoms already mentioned are powerful blood coagulants, and the rapidly lethal effect in small animals and the local gangrene in man following bites by such snakes is probably related to intravascular coagulation (Macfarlane, 1937b).

So far, no abnormality of the blood has been established as a reliable indication of impending pathological thrombosis. It is known that trauma, as such, increases the clotting efficiency of the blood. It is well known, for instance, that the platelet count increases (Tocantins, 1938) and that there is a rise in fibrinogen and the sedimentation rate, and a fall in the coagulation time. These changes, however, are physiological rather than pathological, and no quantitative estimate of their magnitude seems to have been related to the incidence of thrombosis.

Platelets and thrombosis.—There are, however, certain lines along which progress is being made. The importance of the behaviour of the platelets in thrombus formation has been stressed since the time of Bizzozero (1882). Recently the "stickiness" of platelets, obviously an attribute that controls their tendency to adhere to foreign surfaces, has been studied quantitatively by Wright (1941, 1942, 1944). She has found that in conditions associated with an increased platelet count, such as parturition and surgical operation, there is an increase in the adhesiveness. This may be related to the fact that young platelets are more adhesive than old ones and that rapid production from the marrow increases the proportion of young platelets. She has also found that anticoagulants, such as heparin and chlorazol-fast pink, decrease the adhesion of platelets as measured by her method. A similar decrease of platelet adhesion following dicoumarin adminis-

tration has been observed by Spooner and Meyer (1944). The nature of platelet adhesion is at present unknown. It was supposed by some authors (Lenggenhager, 1936; Wright, 1946) to be the result, rather than the cause, of coagulation, being due to a film of fibrin forming on the platelet surface. The demonstration of platelet adhesion by Pinniger and Prunty (1946) in the blood of a case of congenital total absence of fibrinogen appears to dispose of this explanation.

Other blood changes in thrombosis.—Hirschboeck and Coffey (1943) have utilized a clot retraction-time estimation which they claim gives results related to the danger of thrombophlebitis and pulmonary embolism, and which, subject to confirmation, are of interest.

Waugh and Ruddick (1944a, b) have made an interesting study of the anti-heparin effect they have observed in the blood of patients kept at rest in bed and in acute infection. They find that heparin has less anticoagulant effect on the blood of such patients *in vitro* than in normal blood, an observation that is worth extending.

The work of Lyons (1945a, b) on the existence of a more reactive fibrinogen (fibrinogen B) that may be increased in conditions associated with thrombosis appears to be of considerable promise and should be followed up without delay.

Finally, it may be said that there are many factors possibly related to the problem of thrombosis that have received very little attention. Fibrinolysis may be a process that normally limits the extension of intravascular clots, and it would be instructive to follow the development of the fibrinolytic reaction in relation to thrombosis. A systematic assay of the normal anticoagulant factors might also yield information of considerable value.

The prevention of thrombosis.—Once thrombosis is established very little can be done apart from attempts to prevent its extension. In this respect, and more particularly as a prophylactic measure in post-operative and post-partum cases, the use of the anticoagulants heparin and dicoumarin has been extensive and apparently successful. The pioneer work of Murray and others (1937), Best and others (1938), and Solandt and Best (1938) established that heparin was an agent preventing platelet agglutination and thrombus formation on damaged intimal surfaces, both active and non-toxic *in vivo*. Thereafter, heparin has been used on a large scale (Murray, 1940; Crafoord, 1939; Crafoord and Jorpes, 1941) as a therapeutic and prophylactic measure, and in vascular surgery. Heparin has a rapid effect which is of short duration, and in consequence it is usually advisable to give it by continuous intravenous drip (Quick, 1942) or by four, five, or more intravenous

injections daily (Crafoord, 1939), if the clotting time is to be maintained at a reasonably high level. This practical disadvantage might be overcome by combining heparin with some slowly absorbed base. Attempts in this direction have been made by Loewe and Rosenblatt (1944) and Evans and Boller (1946), but the local injection of such preparations has its own disadvantages. As regards dosage, this should be controlled by Lee and White clotting-time estimations, a time of about 20 or 30 minutes being desirable. "Units" of heparin are unfortunately still variable quantities, though it has been recommended by the Toronto workers (Best, 1940) that it should be fixed at 1/100 mg. of their pure barium salt of heparin. An initial dose of about 10,000 units is usual for an adult, subsequent doses being regulated by the patient's response.

Since ordinary preparations of heparin have a transitory effect, untoward haemorrhage can usually be controlled by merely stopping administration. Cerebral haemorrhage, however, is a real

danger in patients with infective endocarditis treated with heparin (Witts, 1940).

Dicoumarin.—The difficulty of maintaining the effect of heparin, which is a relatively safe drug, has led to the use of the slowly acting, but more dangerous, dicoumarin. Introduced by Butt and others (1941) and Bingham and others (1941), it has had an extensive clinical application with apparently satisfactory results from the therapeutic point of view (Allen, 1947; Zilliacus, 1946). It must be mentioned that the incidence of thrombosis in the treated cases cannot be compared legitimately with the incidence of thrombosis in cases treated in the days before anticoagulant therapy was instituted, since other factors, such as avoidance of immobilization, reduction of infection, and other improvements may also have reduced the liability to thrombosis. Such comparisons, however, are made by the authors quoted. Not all authors agree, moreover, as to the therapeutic value of dicoumarin (Wasserman and Stats, 1943) or as to the theoretical basis for its use (Moses, 1945).

FIG. 9.—A diagrammatic synthesis of the factors probably concerned in coagulation, and their interrelationship. A single arrow signifies "reacts with." A double arrow signifies "produces." A line joining two factors signifies "in conjunction with," more precise information in this case being not available. Anticoagulant factors are outlined with a dotted line.

As regards dosage, it is usual to begin with 5 mg./kilo body weight on the first day, followed by 1.5 mg./kilo daily (Quick, 1942) until the prothrombin concentration as measured by Quick's method is between 20 per cent and 30 per cent. Below 10 per cent there is a serious risk of bleeding, which once established may be difficult to stop even by large doses of vitamin K and blood transfusion. Control of the action of the drug is not always easy. There is a variable lag period, so that there may be a long delay before a significant fall in prothrombin, and continuing depression after cessation of administration, both complicated by variations in the susceptibility of individual patients. The technical difficulties of prothrombin determination in patients receiving the drug have already been noted.

Conclusion

From the rather confused collection of facts, observations, and opinions discussed in the foregoing review it is possible by a (perhaps unjustified) selection to build up a composite picture of the coagulation mechanism. This, unfortunately, is considerably more complicated than the older conceptions had visualized. It is illustrated diagrammatically in Fig. 9, which is a synthesis embodying those factors which seem reasonably well established. It will be seen that coagulation is initiated by admixture with tissue fluid, which provides active thromboplastin, or, what is probably more important, by contact with a foreign surface. This causes rupture of platelets with the consequent availability of some activating factor, or co-factor, which reacts with the thromboplastinogen (using Quick's term) to form active thromboplastin, which in turn reacts with the "prothrombin complex." This complex is, for the moment, shown as a combination of calcium, calcium co-factor, prothrombin components A and B, and factor 5. The exact relationship of these components to each other and to thrombin production is not clear at present, and a more specific designation is unjustified. The presence of all of them, however, is required for adequate production of thrombin under the influence of thromboplastin. Once formed, thrombin reacts with fibrinogen, possibly by the sulphhydryl mechanism already discussed. It is possible that some intermediate fibrinogen product is formed, though whether this is "pro-fibrin" or "fibrinogen B" or whether these two are identical is not yet established; the supposition that there are two different thrombins does not seem sufficiently supported to justify their inclusion. The so-called "autocatalytic reaction," illustrated in the diagram as the activation of

thromboplastinogen by some effect of thrombin formation, is largely speculative and is merely one explanation of an observed phenomenon. Owren considers that an actual factor, "factor 6," is produced which activates prothrombin directly. The arguments for and against this contention are too involved to pursue here, but it can be said that since the activity ascribed to factor 6 might well be due to one of the other known factors involved, or even to a physical effect, and since the assumption of its existence complicates, rather than simplifies, the concept of thromboplastic action, the principle of Ockam's razor, never very popular with the constructors of coagulation theories, should reluctantly be applied.

The diagram illustrates also the probable inhibitors of coagulation, and it should be realized that only when the rate of production of the clotting factors exceeds the rate at which they are neutralized by inhibitors can coagulation occur. The fibrinolytic system is included, partly for the sake of completeness, since the disposal of blood clots is a physiological necessity, and partly as a reminder that it may play a much more important part in the coagulation mechanism than is now apparent.

It may seem curious that blood coagulation, which is after all a subsidiary and non-essential fragment of total bodily activity, should involve the multiplicity of factors illustrated, and a complexity which grows rather than resolves with increasing knowledge. Perhaps it is that in this particular subject the rather intensive investigation it has received has merely revealed more clearly the almost limitless complexity that underlies even the simplest of biological processes. It may be, however, that the conception of separate interacting factors is wrong in principle, being based on the artificial reactions of portions of plasma removed by force. Future workers may possibly agree with Pickering, who wrote twenty years ago that "instead of postulating a large number of bodies reacting in unknown ways, the plasma is seen as a unit capable of easily reacting to foreign substances by its unsatisfied side-chains, but returning to a normal condition when the foreign material has been eliminated."

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REFERENCES

- Addis, T. (1911). *J. Path. Bact.*, **15**, 427.
- Aggeler, P. M., Howard, J., Lucia, S. P., Clark, W., and Astaff, A. (1946). *Blood*, **1**, 224.
- Aggeler, P. M., and Lucia, S. P. (1938). *Proc. Soc. exp. Biol. N.Y.*, **38**, 11.
- Allen, E. V. (1947). *J. Amer. med. Ass.*, **134**, 323.
- Allen, E. V., Barker, N. W., and Waugh, J. M. (1942). *J. Amer. med. Ass.*, **120**, 1009.
- Allen, J. G., Julian, O. C., and Dragstedt, L. R. (1940). *Arch. Surg.*, **41**, 873.
- Allen, J. G., and Jacobson, L. O. (1947). *Science*, **105**, 388.
- Almquist, H. J. (1941). *Physiol. Rev.*, **21**, 194.
- Almquist, H. J., and Klose, A. A. (1939a). *Biochem. J.*, **33**, 1055.
- Almquist, H. J., and Klose, A. A. (1939b). *J. Amer. chem. Soc.*, **61**, 1923.
- Almquist, H. J., Pentler, C. F., and Mecchi, E. (1938). *Proc. Soc. exp. Biol. N.Y.*, **38**, 336.
- Almquist, H. J., and Stokstad, E. L. R. (1935). *J. biol. Chem.*, **111**, 105.
- Ansbacher, S., and Fernholz, E. (1939). *J. Amer. chem. Soc.*, **61**, 1924.
- Apitz, K. (1937). *Z. ges. exp. Med.*, **101**, 552.
- Apitz, K. (1939). *Z. ges. exp. Med.*, **105**, 88.
- Arthus, M., and Pagès, C. (1890). *Arch. Physiol. Nom. Path.*, **2**, 739.
- Astrup, T. (1944). *Acta physiol. scand.*, **7**, Suppl. 21.
- Astrup, T., and Darling, S. (1942). *Acta physiol. scand.*, **3**, 168.
- Austin, V. T., and Quastler, H. (1945). *Amer. J. med. Sci.*, **210**, 491.
- Barnett, B., and Macfarlane, R. G. (1934). *Proc. zool. Soc.*, 977.
- Barratt, J. O. W. (1920). *Biochem. J.*, **14**, 189.
- Baumbarger, J. P. (1941). *Amer. J. Physiol.*, **133**, 206.
- Bellis, C. J., and Scott, F. H. (1932). *Proc. Soc. exp. Biol. N.Y.*, **30**, 1373.
- Bendien, W. M., and Creveld, S. van (1937). *Amer. J. Dis. Child.*, **54**, 713.
- Bendien, W. M., and Creveld, S. van (1939). *Acta med. scand.*, **99**, 12.
- Bergmann, M., and Niemann, C. (1937). *J. biol. Chem.*, **118**, 301.
- Bergström, S. (1936). *Z. Physiol. Chem.*, **238**, 163.
- Bering, E. A. (1944). *J. clin. Invest.*, **23**, 586.
- Bertrand, L., and Quivy, D. (1946). *Rev. d'Hematol.*, **1**, 278.
- Best, C. H. (1940). *The Harvey Lectures*, **36**, 66.
- Best, C. H., Cowan, C., and Maclean, D. L. (1938). *J. Physiol.*, **92**, 20.
- Birch, C. L. (1932). *J. Amer. med. Ass.*, **99**, 1566.
- Bingham, J. B., Meyer, O. O., Pohle, F. J. (1941). *Amer. J. med. Sci.*, **202**, 563.
- Bizzozero, J. (1882). *Virchows Arch. Path. Anat.*, **90**, 261.
- de Blainville (1834). *Gaz. Med.*, **521**.
- Bordet, J. (1920). *Ann. Inst. Pasteur*, **34**, 561.
- Bordet, J., and Delange, L. (1912). *Ann. Inst. Pasteur*, **26**, 737.
- Bordet, J., and Delange, L. (1914). *Ann. Bull. Soc. roy. de Sci., Bruxelles*, **72**, 87.
- Brinkhous, K. M. (1939). *Amer. J. med. Sci.*, **198**, 509.
- Brinkhous, K. M. (1940). *Medicine*, **19**, 329.
- Brinkhous, K. M. (1947). *Proc. Soc. exp. Biol. N.Y.*, **66**, 117.
- Brinkhous, K. M., Smith, H. P., and Warner, E. D. (1937). *Amer. J. med. Sci.*, **193**, 475.
- Brinkhous, K. M., Smith, H. P., Warner, E. D., and Seegers, W. H. (1939). *Amer. J. Physiol.*, **125**, 683.
- Buchanan, A. (1835). *Lond. med. Gaz.*, **18**, 50.
- Bürker, K. (1907). *Pflüg. Arch. ges. Physiol.*, **118**, 452.
- Butt, H. R., Allen, E. V., and Bollman, J. L. (1941). *Proc. Mayo Clin.*, **16**, 388.
- Butt, H. R., Snell, A. M., and Osterberg, A. E. (1938). *Proc. Mayo Clin.*, **13**, 74.
- Campbell, H. A., and Link, K. P. (1941). *J. biol. Chem.*, **138**, 21.
- Campbell, H. A., Smith, W. K., Roberts, W. L., and Link, K. P. (1941). *J. biol. Chem.*, **138**, 1.
- Chak, I. M., and Giri, K. V. (1948). *Nature*, **161**, 354.
- Chaptal, H. A. (1797). *Ann. Chimie*, **21**, 284.
- Chargaff, E., Bancroft, F. W., and Stanley-Brown, M. (1936). *J. biol. Chem.*, **116**, 237.
- Chargaff, E., Bendich, A., Cohen, S. S. (1944). *J. biol. Chem.*, **156**, 161.
- Chargaff, E., and Ziff, M. (1941). *J. biol. Chem.*, **138**, 787.
- Chargaff, E., Ziff, M., and Cohen, S. S. (1940). *J. biol. Chem.*, **135**, 351.
- Charles, A. F., Fisher, A. M., and Scott, D. A. (1934). *Trans. roy. Soc. Canada*, **28**, 49.
- Charles, A. F., and Scott, D. A. (1933). *J. biol. Chem.*, **102**, 425, 431, 437.
- Clowes, G. H. A. (1917). *Amer. J. Physiol.*, **42**, 610.
- Cohen, S. S., and Chargaff, E. (1940). *J. biol. Chem.*, **136**, 243.
- Cohn, E. J. (1946). *Blood*, **1**, 3.
- Collingwood, B. J., and MacMahon, M. T. (1912). *J. Physiol.*, **45**, 119.
- Collins, E. N., and Hoffmann, A. D. (1943). *Cleveland Clin. Quart.*, **10**, 105.
- Conley, C. L., and Morse, W. I. (1948). *Amer. J. med. Sci.*, **215**, 158.
- Crafoord, C. (1939). *Acta chir. scand.*, **82**, 319.
- Crafoord, C., and Jörpes, E. (1941). *J. Amer. med. Ass.*, **116**, 2831.
- Cramer, W., and Pringle, H. (1912). *J. Physiol. (Proc.)*, **45**, 11.
- Crawford, T., and Nassim, J. R. (1944). *Lancet*, **2**, 404.
- Dale, D. J., and Jaques, L. B. (1942). *Canad. med. Ass. J.*, **46**, 546.
- Dale, H. H., and Laidlaw, P. P. (1911). *J. Path. Bact.*, **16**, 351.
- Dale, H. H., and Walpole, G. St. (1916). *Biochem. J.*, **10**, 331.
- Dam, H. (1935). *Biochem. J.*, **29**, 1273.
- Dam, H., and Glavind, J. (1938). *Biochem. J.*, **32**, 485.
- Dam, H., and Schönheyder, F. (1934). *Biochem. J.*, **28**, 1355.
- Dam, H., Schönheyder, F., and Tage-Hansen, E. (1936). *Biochem. J.*, **30**, 1075.
- Davidson, C. S., Freed, J. H., and MacDonald, H. (1945). *Amer. J. med. Sci.*, **210**, 634.
- Davidson, C. S., and MacDonald, H. (1943). *Amer. J. med. Sci.*, **205**, 24.
- Davis, D. (1911). *Amer. J. Physiol.*, **29**, 160.
- Denis, P. S. (1859). "Mémoire sur le Sang." Paris. Ballière.
- Douglas, S. R., and Colebrook, L. (1916). *Lancet*, **2**, 180.
- Dungern, M. von (1937). *Z. Biol.*, **98**, 136.
- Eagle, H. (1935a). *J. gen. Physiol.*, **18**, 531, 547.
- Eagle, H. (1935b). *J. gen. Physiol.*, **18**, 813.
- Eagle, H. (1937). *J. exp. Med.*, **65**, 613.
- Eagle, H., and Harris, T. N. (1937). *J. gen. Physiol.*, **20**, 543.
- Estrev, S., Médal, L. S., and Dameshek, W. (1946). *Blood*, **1**, 504.
- Evans, J. A., and Boller, R. J. (1946). *J. Amer. med. Ass.*, **131**, 879.
- Fantl, P., and Nance, M. H. (1946a). *Nature*, **158**, 708.
- Fantl, P., and Nance, M. H. (1946b). *Med. J. Aust.*, **2**, 125.
- Fantl, P., and Nance, M. H. (1947). *Med. J. Aust.*, **2**, 133.
- Feissly, R. (1942). *Schweiz. med. Wschr.*, **72**, 516.
- Feissly, R. (1944). *Helv. med. acta*, **11**, 177.
- Feissly, R. (1945a). *Helv. med. acta*, **12**, 467.
- Feissly, R. (1945b). *Helv. med. acta*, **12**, 215.
- Feissly, R. (1945c). *Schweiz. med. Wschr.*, **75**, 696.
- Ferguson, J. H. (1937). *Amer. J. Physiol.*, **119**, 755.
- Ferguson, J. H. (1942). *Proc. Soc. exp. Biol. N.Y.*, **51**, 373.
- Ferguson, J. H. (1943). *Science*, **97**, 319.
- Ferguson, J. H., and Erickson, B. N. (1939). *Amer. J. Physiol.*, **126**, 661.
- Ferguson, J. H., Travis, B. L., and Gerheime, E. B. (1947). *Proc. Soc. exp. Biol. N.Y.*, **64**, 285.
- Ferry, J. D., and Morrison, P. R. (1944). *J. clin. Invest.*, **23**, 566.
- Fieser, L. F., Campbell, W. P., Fry, E. M., and Gates, M. D. (1939). *J. Amer. chem. Soc.*, **61**, 2559.
- Fischer, A. (1935a). *Biochem. Z.*, **278**, 320.
- Fischer, A. (1935b). *Biochem. Z.*, **279**, 108.
- Fisher, B. (1947). *Amer. J. clin. Path.*, **17**, 471.
- Fleming, A., and Fish, E. W. (1947). *Brit. med. J.*, **2**, 242.
- Fonio, A. (1914). *Mitt. a. d. Grenzgeb. Med. Chir.*, **27**, 642.
- Foster, D. P., and Whipple, G. H. (1921). *Amer. J. Physiol.*, **58**, 365, 379, 393, 407.
- Freund, E. (1888). *Wien. Med. Jahrb.*, **3**, 259. Quoted by Jaques and others, 1946.
- Fuchs, H. J. (1929). *Z. Immunforsch.*, **62**, 107.
- Fullerton, H. W. (1940). *Lancet*, **2**, 195.
- Gamgee, A. (1879). *J. Physiol.*, **2**, 145.
- Gasser, H. S. (1917). *Amer. J. Physiol.*, **42**, 378.
- Glazko, A. J., and Ferguson, J. H. (1940). *J. gen. Physiol.*, **24**, 169.
- Gratia, A., and Levene, P. A. (1922). *J. biol. Chem.*, **50**, 455.
- Graville, G. D., and Lehman, H. (1944). *J. Physiol.*, **103**, 175.
- Grünig, W. (1943). *Naturwiss.*, **31**, 399.
- Hammarsten, O. (1877). *Pflüg. Arch. ges. Physiol.*, **14**, 211.
- Hawn, C. van Z., and Porter, K. R. (1947). *J. exp. Med.*, **86**, 285.
- Heard, W. N. (1917). *J. Physiol.*, **51**, 294.
- Henderson, J. L., Donaldson, G. M. M., and Scarborough, H. (1945). *Quart. J. Med.*, **38**, 101.
- Herbert, F. K. (1940). *Biochem. J.*, **34**, 1554.
- Herman, J. v., and Worschitz, F. (1935). *Fortschr. Röntgenstr.*, **52**, 80.
- Hewson, W. (1770). "Works Sydenham Soc. 1846." p. 1.
- Hines, L. E., and Kessler, D. L. (1945). *J. Amer. Med. Ass.*, **128**, 794.
- Hirschboeck, J. S., and Coffey, W. R., Jr. (1943). *Amer. J. med. Sci.*, **205**, 727.
- Hölmgren, H., and Wilander, O. (1937). *Z. Mikrosk. anat. Forsch.*, **42**, 242.
- Howell, W. H. (1910). *Amer. J. Physiol.*, **26**, 453.
- Howell, W. H. (1911). *Amer. J. Physiol.*, **29**, 187.
- Howell, W. H. (1912). *Amer. J. Physiol.*, **31**, 1.
- Howell, W. H. (1925). *Amer. J. Physiol.*, **71**, 553.
- Howell, W. H. (1935). *Physiol. Rev.*, **15**, 435.
- Howell, W. H., and Cekada, E. B. (1926). *Amer. J. Physiol.*, **78**, 500.
- Howell, W. H., and Holt, E. (1918). *Amer. J. Physiol.*, **47**, 328.
- Huggatt, A. St. G. (1934). *Quart. J. Pharm. Pharmacol.*, **7**, 372.
- Huggatt, A. St. G., and Rowe, F. M. (1933). *J. Physiol.*, **80**, 82.
- Hunter, J. (1794). "Treatise on the Blood." London. 1812, **1**, 43.
- Jaques, L. B. (1938). *Biochem. J.*, **32**, 1181.
- Jaques, L. B., and Dunlop, A. P. (1945a). *Amer. J. Physiol.*, **143**, 355.
- Jaques, L. B., and Dunlop, A. P. (1945b). *Amer. J. Physiol.*, **145**, 67.
- Jaques, L. B., Fidler, E., Feldsted, F. T., and Macdonald, A. G. (1946). *Canad. med. Ass. J.*, **55**, 26.
- Jaques, L. B., and Waters, E. T. (1941). *J. Physiol.*, **99**, 454.
- Jaques, L. B., Waters, E. T., and Charles, A. F. (1942). *J. biol. Chem.*, **144**, 229.
- Jones, T. B., and Smith, H. E. (1930). *Amer. J. Physiol.*, **94**, 144.
- Jörpes, E., and Bergström, S. (1937). *J. biol. Chem.*, **118**, 447.
- Joules, H., and Macfarlane, R. G. (1938). *Lancet*, **1**, 715.
- Kark, R., Souter, A. W., and Hayward, J. C. (1940). *Quart. J. Med.*, **33**, 247.

- Kato, K. (1940). *Amer. J. clin. Path.*, **10**, 147.
- Kunitz, M. (1945). *Science*, **101**, 668.
- Kunitz, M. (1946). *J. gen. Physiol.*, **29**, 149.
- Kunitz, M., and Northrop, J. H. (1936). *J. gen. Physiol.*, **19**, 991.
- Laki, K. (1943). "Studies from Institute Medical Chemistry University of Szeged." **3**, 5.
- Laki, K., and Mommaerts, W. F. H. M. (1945). *Nature*, **156**, 664.
- Lamb, G. (1903). "Scientific Memoirs by Officers of Medical and Sanitary Depts. of the Govt. of India." New Series. Nos. 3, 4, 5.
- Lampert, H. (1930). *Münch. med. Wschr.*, **77**, 586.
- Lancet Correspondence. (1947). *Lancet*, **2**, 669, 772.
- Leathes, J. B., and Mellanby, J. (1939). *J. Physiol.*, **96**, 38p.
- Lee, R. I., and White, P. D. (1913). *Amer. J. med. Sci.*, **145**, 495.
- Lehmann, J. (1944). *Lancet*, **1**, 493.
- Lempert, H. (1948). *Brit. med. J.*, **1**, 125.
- Lenggenhager, K. (1935). *Helv. med. acta*, **1**, 527.
- Lenggenhager, K. (1936). *Klin. Wschr.*, **15**, 1835.
- Lenggenhager, K. (1946). *Schweiz. med. Wschr.*, **76**, 410.
- Lester, D. (1944). *J. biol. Chem.*, **154**, 305.
- Lewis, J. H., Soulier, J. P., and Taylor, F. H. L. (1946b). *J. clin. Invest.*, **25**, 876.
- Lewis, J. H., Tagnon, H. J., Davidson, C. S., Minot, G. R., and Taylor, F. H. L. (1946a). *Blood*, **1**, 166.
- Light, R. U., and Prentice, H. R. (1945). *Arch. Surg.*, **51**, 69.
- Link, K. P. (1943). *Harvey Lect.*, **39**, 162.
- Link, K. P., Overman, R. S., Sullivan, W. R., Hueber, C. F., and Scheel, L. D. (1943). *J. biol. Chem.*, **147**, 463.
- Lister, J. (1863). Croonian Lecture, June, 1863. *Proc. roy. Soc.*, **12**, 580.
- Loewe, L., and Rosenblatt, P. (1944). *Amer. J. med. Sci.*, **208**, 54.
- Loomis, E. C., and Seegers, W. H. (1947). *Amer. J. Physiol.*, **148**, 563.
- Lozner, E. L., Jolliffe, L. S., and Taylor, F. H. L. (1940). *Amer. J. med. Sci.*, **199**, 318.
- Lozner, E. L., Taylor, F. H. L., and MacDonald, H. (1942). *J. clin. Invest.*, **21**, 241.
- Lyons, R. N. (1945a). *Aust. J. exp. Biol.*, **23**, 131.
- Lyons, R. N. (1945b). *Nature*, **155**, 633.
- Macfarlane, R. G. (1935). *St. Bart's Hosp. Rep.*, **68**, 229.
- Macfarlane, R. G. (1937a). *Lancet*, **1**, 10.
- Macfarlane, R. G. (1937b). *Med. Annual*, 415.
- Macfarlane, R. G. (1938a). M.D. Thesis, Univ. London.
- Macfarlane, R. G. (1938b). *Lancet*, **1**, 309.
- Macfarlane, R. G. (1939). *Lancet*, **1**, 1199.
- Macfarlane, R. G. (1941). *Quart. J. Med.*, **33**, 1.
- Macfarlane, R. G. (1942). *Proc. roy. Soc. Med.*, **35**, 410.
- Macfarlane, R. G. (1943). *Brit. med. J.*, **2**, 541.
- Macfarlane, R. G. (1945). *Proc. roy. Soc. Med.*, **38**, 399.
- Macfarlane, R. G. (1947). *J. Physiol.*, **106**, 104.
- Macfarlane, R. G., and Barnett, B. (1934). *Lancet*, **2**, 985.
- Macfarlane, R. G., and Biggs, R. (1946). *Lancet*, **2**, 862.
- Macfarlane, R. G., and Biggs, R. (1948). *Blood*. (In the press.)
- Macfarlane, R. G., and Pilling, J. (1946a). *Lancet*, **2**, 562.
- Macfarlane, R. G., and Pilling, J. (1946b). *Lancet*, **1**, 888.
- Macfarlane, R. G., Trevan, J. W., and Attwood, A. M. P. (1941). *J. Physiol.*, **99**, 7p.
- Maddison, F. W., and Quick, A. J. (1945). *Amer. J. med. Sci.*, **209**, 443.
- Malpighi, M. (1666). "De Polypo Cordis." Opera Omnia Ludg. Batav., 1687. Pt. 2, p. 311.
- Maltaner, F., and Johnston, E. (1921). *J. Immunol.*, **6**, 349.
- Martin, C. J. (1894). *J. Physiol.*, **15**, 380.
- Mayer, M., and Zeiss, H. (1920). *Arch. Schiffs Tropen. Hyg.*, **24**, 257.
- McLean, J. (1916). *Amer. J. Physiol.*, **41**, 250.
- Meard, W. N. (1917). *J. Physiol.*, **51**, 294.
- Mellanby, J. (1909). *J. Physiol.*, **38**, 28, 441.
- Mellanby, J. (1930). *Proc. roy. Soc. Lond. B.*, **107**, 271.
- Mellanby, J. (1933). *Proc. roy. Soc. Lond. B.*, **113**, 93.
- Mellanby, J. (1934). *Proc. roy. Soc. Lond. B.*, **116**, 1.
- Mellanby, J. (1935). *Proc. roy. Soc. Lond. B.*, **117**, 352.
- Mellanby, J., and Pratt, C. L. G. (1938). *Proc. roy. Soc. Lond. B.*, **125**, 204.
- Mertz, E. T., Seegers, W. H., and Smith, H. P. (1939). *Proc. Soc. exp. Biol. N.Y.*, **42**, 604.
- Meyer, O. O., Bingham, J. B., and Axelrod, V. H. (1942). *Amer. J. med. Sci.*, **204**, 11.
- Meyer, O. O., and Howard, B. (1943). *Proc. Soc. exp. Biol. N.Y.*, **53**, 234.
- Mills, C. A. (1921). *J. biol. Chem.*, **46**, 135, 167.
- Minot, G. R., Davidson, C. S., Lewis, J. H., Tagnon, H. J., and Taylor, F. H. L. (1945). *J. clin. Invest.*, **24**, 704.
- Minot, G. R., and Lee, R. I. (1916). *Arch. internat. Med.*, **18**, 474.
- Morawitz, P. (1905). *Ergebn. Physiol.*, **4**, 307.
- Morawitz, P. (1924). "Abderhalden's Handbuch der Biol. Arbeitsmethoden," **4**, 3, 249.
- Morawitz, P., and Lossen, J. (1908). *Dtsch. Arch. klin. Med.*, **94**, 110.
- Moses, C. (1945). *Proc. Soc. exp. Biol. N.Y.*, **59**, 25.
- Munro, F. L., Hart, E. R., Munro, M. P., and Walkling, A. A. (1945). *Amer. J. Physiol.*, **145**, 206.
- Munro, F. L., and Jones, H. W. (1943). *Amer. J. med. Sci.*, **206**, 710.
- Murray, B. G. (1940). *Arch. Surg.*, **40**, 307.
- Murray, D. W., Jaques, L. D., Perrett, T. S., and Best, C. H. (1937). *Surgery*, **2**, 163.
- Nanninga, L. (1946). *Arch. Néerlandaise de Physiol.*, **28**, 241.
- Neuhof, H., and Hirschfeld, S. (1922). *Ann. Surg.*, **76**, 1.
- Nitshe, G. A., Gerards, H. W., and Deutsch, H. F. (1947). *J. Lab. clin. Med.*, **32**, 410.
- Nolf, P. (1905). *Arch. internat. Physiol.*, **3**, 1.
- Nolf, P. (1908). *Arch. internat. Physiol.*, **6**, 1.
- Nolf, P. (1938). *Medicine*, **17**, 381.
- Nolf, P. (1945). *Arch. internat. de Pharmacodynam. et de therap.*, **71**, 1, and **70**, 5, 323.
- Nolf, P. (1947). XVII. Int. Physiol. Cong. Abs. 204.
- Nordbø, R. (1927). *Biochem. Ztschr.*, **190**, 150.
- Nygaard, K. K. (1941). "Hemorrhagic Diseases." St. Louis.
- Oliver, J., Bloom, F., and Mangieri, C. (1947). *J. exp. Med.*, **86**, 107.
- Oneal, W. J., and Lam, C. R. (1945). *Amer. J. med. Sci.*, **210**, 181.
- Owen, C. H., Hoffman, G. R., Ziffren, S. E., and Smith, H. P. (1939). *Proc. Soc. exp. Biol. N.Y.*, **41**, 181.
- Owren, P. A. (1947a). *Acta med. scand. Suppl.* No. 194.
- Owren, P. A. (1947b). *Lancet*, **1**, 446.
- Page, R. C., and Russell, H. K. (1941). *J. lab. clin. Med.*, **26**, 1366.
- Parfentjev, I. A. (1941). *Amer. J. med. Sci.*, **202**, 578.
- Patek, A. J., and Stetson, R. P. (1936). *J. clin. Invest.*, **15**, 531.
- Patek, A. J., and Taylor, F. H. L. (1936). *Science*, **84**, 271.
- Patek, A. J., and Taylor, F. H. L. (1937). *J. clin. Invest.*, **16**, 113.
- Perkins, W. (1946). *Blood*, **1**, 497.
- Petit (1735). *Hist. de l'Acad. Roy. des Sciences*, p. 14.
- Pickering, J. W. (1928). "The Blood Plasma in Health and Disease." Heinemann. London.
- Pinniger, J. L., and Prunty, F. T. G. (1946). *Brit. J. exp. Path.*, **27**, 200.
- Quick, A. J. (1935a). *J. Immunol.*, **29**, 87.
- Quick, A. J. (1935b). *J. biol. Chem. (Proc.)*, **109**, 73.
- Quick, A. J. (1936a). *Amer. J. Physiol.*, **114**, 382.
- Quick, A. J. (1936b). *Amer. J. Physiol.*, **115**, 317.
- Quick, A. J. (1937). *Amer. J. Physiol.*, **118**, 260.
- Quick, A. J. (1938a). *J. Amer. med. Ass.*, **110**, 1658.
- Quick, A. J. (1938b). *Amer. J. Physiol.*, **123**, 712.
- Quick, A. J. (1939). *Pennsylvania Med. J.*, **43**, 125.
- Quick, A. J. (1940). *Amer. J. Physiol.*, **131**, 455.
- Quick, A. J. (1941a). *Amer. J. med. Sci.*, **201**, 469.
- Quick, A. J. (1941b). *Amer. J. Physiol.*, **132**, 1, 239.
- Quick, A. J. (1942). "The Hemorrhagic Diseases and the Physiology of Hemostasis." Charles C. Thomas. Illinois.
- Quick, A. J. (1943). *Amer. J. Physiol.*, **140**, 212.
- Quick, A. J. (1945a). *J. biol. Chem.*, **161**, 33.
- Quick, A. J. (1945b). *Amer. J. clin. Path.*, **15**, 560.
- Quick, A. J. (1947a). *Amer. J. Physiol.*, **148**, 211.
- Quick, A. J. (1947b). *Amer. J. med. Sci.*, **214**, 272.
- Quick, A. J. (1947c). *Amer. J. Physiol.*, **151**, 63.
- Quick, A. J., and Grossman, A. M. (1939). *Proc. Soc. exp. Biol. N.Y.*, **40**, 647.
- Quick, A. J., Stanley-Brown, M., and Bancroft, F. W. (1935). *Amer. J. med. Sci.*, **190**, 501.
- Rapoport, S. (1947). *Proc. Soc. exp. Biol. N.Y.*, **64**, 478.
- Rapoport, S., Wing, M., and Guest, G. M. (1943). *Proc. Soc. exp. Biol. N.Y.*, **53**, 40.
- Reimann, F. (1941). *Acta med. scand.*, **107**, 95.
- Rhoads, J. E., and FitzHugh, T. (1941). *Amer. J. med. Sci.*, **202**, 662.
- Richardson, B. W. (1858). "The Cause of the Coagulation of the Blood." London. Churchill.
- Robb-Smith, A. H. T. (1945). *Lancet*, **2**, 362.
- Roderick, L. M. (1929). *J. Amer. Vet. med. Ass.*, **74**, 314.
- Roderick, L. M. (1931). *Amer. J. Physiol.*, **96**, 413.
- Rosenfeld, S., and Lenke, S. E. (1935). *Amer. J. med. Sci.*, **190**, 779.
- Rous, P., Gilding, H. P., and Smith, F. (1930). *J. exp. Med.*, **51**, 807.
- Sábrázes, J. (1904). *Folia Haemat.*, **1**, 394.
- Sahli, H. (1905). *Z. klin. Med.*, **56**, 264.
- Schmidt, A. (1892). "Zur Blutlehre." Leipzig. Vogel.
- Schmidt, A. (1895). "Weitere Beiträge zur Blutlehre." Wiesbaden. Bergmann.
- Schmitz, A. (1933). *Z. Physiol. Chem.*, **222**, 155.
- Scott, F. H., and Chamberlain, C. (1934). *Proc. Soc. exp. Biol. N.Y.*, **31**, 1054.
- Seegers, W. H. (1940). *J. biol. Chem.*, **136**, 103.
- Seegers, W. H., Brinkhous, K. M., Smith, H. P., and Warner, E. D. (1938). *J. biol. Chem.*, **126**, 91.
- Seegers, W. H., and Doubt, L. (1944). *Proc. Soc. exp. Biol. N.Y.*, **56**, 72.
- Shapiro, S., Redish, M. H., and Campbell, H. A. (1943a). *Proc. Soc. exp. Biol. N.Y.*, **52**, 12.
- Shapiro, S., Redish, M. H., and Campbell, H. A. (1943b). *Proc. Soc. exp. Biol. N.Y.*, **53**, 251.
- Shapiro, S., Sherwin, B., Redish, M. H., and Campbell, H. A. (1942). *Proc. Soc. exp. Biol. N.Y.*, **50**, 85.
- Shlevin, E. L., and Lederer, M. (1944). *Ann. intern. Med.*, **21**, 332.
- Smith, H. P., Warner, E. D., and Brinkhous, K. M. (1937). *J. exp. Med.*, **66**, 801.
- Smith, H. P., Ziffren, S. E., Owen, C. A., and Hoffman, G. R. (1939). *J. Amer. med. Ass.*, **113**, 380.
- Smith, W., and Hale, J. H. (1944). *Brit. J. exp. Path.*, **25**, 101.
- Solandt, D. Y., and Best, C. H. (1938). *Lancet*, **2**, 130.
- Spooner, M., and Meyer, O. O. (1944). *Amer. J. Physiol.*, **142**, 279.

- Stahmann, M. A., Huebner, C. F., and Link, K. P. (1941). *J. biol. Chem.*, **138**, 513.
- Sternberger, L. A. (1947). *Brit. J. exp. Path.*, **28**, 168.
- Tagnon, H. J., Davidson, C. S., and Taylor, F. H. L. (1942). *J. clin. Invest.*, **22**, 127.
- Tait, J., and Green, F. (1926). *Quart. J. exp. Biol. N.Y.*, **16**, 141.
- Taylor, F. H. L., Lozner, E. L., and Adams, M. A. (1941). *Amer. J. med. Sci.*, **202**, 585.
- Thayer, S. A., Cheney, L. C., Binkley, S. B., MacCorquodale, D. W., and Doisey, E. A. (1939). *J. Amer. chem. Soc.*, **61**, 1932.
- Thurer, G. R., and Angevine, D. M. (1947). *Amer. J. Path.*, **23**, 876.
- Timperley, W. A., Naish, A. E., and Clark, G. A. (1936). *Lancet*, **2**, 1142.
- Tocantins, L. M. (1938). *Medicine*, **17**, 155.
- Tocantins, L. M. (1943a). *Fed. Proc.*, **2**, 48.
- Tocantins, L. M. (1943b). *Proc. Soc. exp. Biol. N.Y.*, **54**, 94.
- Tocantins, L. M. (1944a). *Proc. Soc. exp. Biol. N.Y.*, **55**, 291.
- Tocantins, L. M. (1944b). *Proc. Soc. exp. Biol. N.Y.*, **57**, 211.
- Tocantins, L. M. (1945). *Amer. J. Physiol.*, **143**, 67.
- Tocantins, L. M. (1946). *Blood*, **1**, 156.
- Trevan, J. W., and Macfarlane, R. G. (1936). *Med. Res. Council Ann. Rep.*, p. 143.
- Vines, J. W. C. (1921). *J. Physiol.*, **55**, 86, 287.
- Warner, E. D. (1938). *J. exp. Med.*, **68**, 831.
- Warner, E. D., Brinkhous, K. M., and Smith, H. P. (1936). *Amer. J. Physiol.*, **114**, 667.
- Warner, E. D., Brinkhous, K. M., and Smith, H. P. (1937). *Proc. Soc. exp. Biol. N.Y.*, **37**, 628.
- Warner, E. D., Brinkhous, K. M., and Smith, H. P. (1939a). *Proc. Soc. exp. Biol. N.Y.*, **40**, 197.
- Warner, E. D., Brinkhous, K. M., and Smith, H. P. (1939b). *Amer. J. Physiol.*, **125**, 296.
- Warner, E. D., DeGowin, E. L., and Seegers, W. H. (1940). *Proc. Soc. exp. Biol. N.Y.*, **43**, 251.
- Wasserman, L. R., and Stats, D. (1943). *Amer. J. med. Sci.*, **206**, 466.
- Waugh, T. R., and Ruddick, D. W. (1944a). *Canad. med. Ass. J.*, **50**, 547.
- Waugh, T. R., and Ruddick, D. W. (1944b). *Canad. med. Ass. J.*, **51**, 11.
- Wertheimer, E., Shapiro, B., and Fodor-Salmonowicz, I. (1944). *Brit. J. exp. Path.*, **25**, 121.
- Weymouth, F. W. (1913). *Amer. J. Physiol.*, **32**, 266.
- Wilson, S. J. (1944). *Amer. J. clin. Path.*, **14**, 307.
- Witts, L. J. (1940). *Brit. med. J.*, **1**, 484.
- Witts, L. J. (1942). *J. Path. Bact.*, **54**, 516.
- Witts, L. J., and Hobson, F. C. G. (1942). *Brit. med. J.*, **1**, 575.
- Wöhlich, E. (1929). *Ergebn. Physiol.*, **28**, 443.
- Wöhlich, E. (1940). *Ergebn. Physiol.*, **43**, 174.
- Wöhlich, E., and Clamann, H. G. (1932). *Z. Biol.*, **92**, 462.
- Wöhlich, E., and Kiesgen, A. (1936). *Biochem. Z.*, **285**, 200.
- Wöhlich, E., and Köhler, V. (1942). *Biochem. Z.*, **311**, 408.
- Wolpers, C., and Ruska, H. (1939). *Clin. Wschr.*, **2**, 1077.
- Woodhall, B. (1944). *J. Amer. med. Ass.*, **126**, 469.
- Woodlridge, L. C. (1886). *Arch. Annat. Physiol. Suppl.*, 397.
- Woodlridge, L. C. (1889). *J. Physiol.*, **10**, 329.
- Wright, A. E. (1893). *Brit. med. J.*, **2**, 223.
- Wright, H. P. (1941). *J. Path. Bact.*, **53**, 255.
- Wright, H. P. (1942). *J. Path. Bact.*, **54**, 461.
- Wright, H. P. (1944). *J. Path. Bact.*, **56**, 151.
- Wright, H. P. (1946). *Lancet*, **1**, 306.
- Yeager, L. B., Rhoads, P. S., and Freeman, S. (1947). *J. Lab. clin. Med.*, **32**, 502.
- Zak, E. (1912). *Arch. exp. Path. Pharm.*, **70**, 27.
- Zilliacus, H. (1946). *Acta med. scand. Suppl.*, 171.
- Zondek, Bernhard, and Finkelstein, M. (1945). *Proc. Soc. exp. Biol. N.Y.*, **60**, 374.