Recent Advances in Thrombosis

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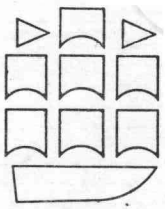

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Preface

The considerable popularity and success of *Recent Advances in Blood Coagulation* suggested the need for a companion volume dedicated specifically to thrombosis. Advances in this field in the last five to ten years have been considerable and have resulted from a multidisciplinary approach. This is exemplified by the differing backgrounds of the various distinguished contributors to the present volume.

The vast amount of research can be gauged from the fact that eleven simultaneous sessions were required to accommodate the offered papers at the 1972 Congress of the International Society on Thrombosis and Haemostasis. Proceedings of such meetings appear annually and several thousand scientific papers related to thrombosis are published in the medical press each year. The purpose of a recent advances volume is to put these original contributions into their correct perspective.

The selection of topics at any one moment must necessarily be limited to those fields in which investigation has been most active. The contributions made by morbid anatomists, epidemiologists, clinicians, biochemists, coagulationists, platelet workers, thrombolytic investigators and immunologists have been to the forefront in the last decade and all are represented in this edition. The participation of contributors from many different centres has helped to ensure a balanced overall view of developments.

Particular gratitude is due to the contributors for the great care they have all taken with their manuscripts, which has resulted in the minimum delay in publication. Thanks are also due to the publishers, Churchill Livingstone, for the efficiency with which they dealt with the manuscript.

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P. A. Owren

BASIC MECHANISMS OF THROMBOSIS

The following phrase, which is borrowed from a radio address by Sir Winston Churchill in 1939, might appropriately characterise the problem complex of thrombosis: ". . . it is a riddle wrapped in a mystery inside an enigma". The ingenuity of researchers in divining its answer is characterised by two periods of foresighted conjecture, with a long drowsy interval of about 40 years. In the eighteenth century period, the masters of gross pathology were surprisingly successful. The morphological phenomena of the thrombotic process were described in detail and distinct ideas, which are still valid, concerning etiology and pathogenesis were formulated (Virchow, 1856). Many of these brilliant discoveries however, sank into oblivion and had to be rediscovered in the recent epoch of research.

Carl von Rokitansky in 1842 developed the concept of deposition of material from the blood stream on the intima of arteries (mural thrombi of today) as an important factor in atherosclerosis, and the presence of fibrin in atheromatous plaques was described 60 years ago (Mallory, 1912). These findings were disregarded until Duguid (1946) reported his important studies on the role of thrombosis in the development of atheromatosis of the coronary arteries.

The first event in thrombosis, the local accumulation of platelets, was familiar to many pathologists at the turn of the century and William Welch gave an outstanding description in 1899. The appraisal of this important discovery had to wait for nearly 50 years. This neglect of platelets is rather astonishing in view of the excellent studies by Bizzozero (1882) who definitely recognised their essentiality. The term 'viscous metamorphosis', which is still used, was introduced by Eberth and Schimmelbusch in 1886 and 'irreversible aggregation' was described in detail by Wright and Minot in 1917. This description is one of the very few lights during the long dark period of thrombosis research of more than 30 years from the beginning of our century.

Coagulation research has had a similar development. After the intensive research period, which culminated in the classical theory of

Morawitz in 1905, coagulation research also went into hibernation for nearly 40 years. It was roused to active life when Quick introduced his one-stage prothrombin time method and particularly when the first new clotting factor, factor V, was discovered in 1943 (Owren, 1944, 1947). A new epoch then started with discoveries of several new clotting factors followed by intensive studies on the biochemistry of blood coagulation. After about 15 years, this period lost some of its vigour, when no more new factors could be visualised and because the function of blood platelets eventually came into the limelight. The intense studies on the biochemistry of platelets and their function in haemostasis and thrombosis, which have now persisted for about 10 years, was initiated by Hellem's discovery (1958) of a platelet aggregating factor in red blood cells and its subsequent identification as adenosine-5-diphosphate (ADP) by Gaarder et al. in 1961.

The development of research in fibrinolysis and thrombolysis, which are intimately related to thrombosis, started only about 15 years ago. It was then realised that these processes are regulated by a specific enzyme system, the plasminogen-plasmin system. It has since become evident that the deposition of fibrin and its subsequent resolution operate in a dynamic equilibrium as a fundamental biologic mechanism. Research on fibrinolysis therefore has become an integral part of thrombosis research. The progress in this field has been very impressive as evidenced by the very large number of publications covering its many aspects of biochemistry, physiology, methodology and pathology (recent reviews by Kontinen, 1968; Fearnley, 1969; Astrup, 1969; Sherry, 1969). The development of the fibrinolytic therapy has a major current interest.

The most elusive component of Virchow's pathogenetic concept of thrombus formation, the vessel wall, and particularly the function of the endothelial cells, has only lately attracted the research interest it deserves.

Virchow also realised the pathogenetic role of retarded blood flow or stasis, and clinicians have since ancient times been well aware of the increased incidence of thrombosis by immobilisation, pregnancy, congestive heart failure and other pathological states associated with a retardation of the venous circulation. Retarded blood flow alone, however, does not easily produce thrombosis or coagulation in normal veins as demonstrated already by Hewson's classical experiment in 1771 with the trapping of venous blood between two ligatures. Wessler (1952, 1955) however, by using a similar *in vivo* model, confirmed the contribution of stasis to intravascular coagulation and thrombosis and he successfully applied this model for analysing the thrombogenic effect of serum and activated clotting factors (Wessler et al., 1967).

Recent research on the rheology of human blood in relation to thrombosis has yielded further valuable contributions to a better understanding of the effects of stasis, the mechanism of viscosity changes and their importance for the conversion of a static column of blood into a venous

thrombus. Analysis of changes from the normal laminar to a non-laminar or turbulent blood flow in arteries, by various intimal lesions, has contributed to a better comprehension of arterial thrombus formation.

The modern multidisciplinary approach to the solving of the many different and puzzling problems of thrombosis includes both the basic sciences (biochemistry, enzymology, molecular biology, cellular metabolism and electron microscopy of the 'microcosmos') and the whole spectrum of clinical sciences.

Improvements of phlebography and the introduction of new diagnostic techniques, such as the ^{125}I-fibrinogen scanning, have revealed that venous thromboembolism is of far greater magnitude and scope than recognised from clinical diagnosis and necropsy data. It has been realised that the thrombotic process is often clinically silent, and if not, the diversity of symptoms and signs are easily misinterpreted. Whether a thrombotic process attains clinical significance probably depends on the inhibitory systems, the activity of the fibrinolytic system and the normal clearing mechanisms of the reticuloendothelial system, removing 'activated' products.

Some of these many aspects of thrombosis are discussed by the other contributors. I shall restrict my comments to a few recent developments in the study of basic mechanisms, which might be useful in the search for a better quality of service to our patients.

THE CONCEPT OF PATHOGENESIS

Until the rediscovery of the important function of platelets in thrombosis about 15 years ago, the consensus of opinions was largely that thrombosis is a variant of coagulation, and anticoagulation was supposed to be the only logical therapeutic answer. When research on platelets in thrombosis progressed, it fascinated also the coagulationists and it still dominates the scene. The prospective therapeutic answer in vogue today is the antiplatelet agents. In spite of the ruling passion having shifted from coagulation to platelet function, it is fully realised by everybody that both processes are indispensable for thrombus formation and both are consequently targets for antithrombotic therapy. Which approach, single or combined, will be most successful, remains to be seen.

From the comprehensive studies on the morphology and biochemistry of both hemostasis and thrombosis in recent years has evolved the following pathogenetic concept concerning the main sequences of reactions: (1) Endothelial damage results in the exposure of subendothelial tissue to the blood stream. (2) Platelets adhere to collagen, to the basement membrane and probably to some fine fibrils of unknown nature (T'Sao

and Glagov, 1970). (3) Collagen is a very effective inducer of the so called 'platelet release reaction' with discharge of several platelet substances contained in the platelet granules: adenosine diphosphate (ADP), platelet factor 3 (PF3), platelet factor 4 (PF4), 5-hydroxytryptamine, catecholamines and a number of platelet enzymes. (4) ADP, in the presence of co-factors (fibrinogen, Hageman-factor?, anti-Willebrand factor?, gammaglobulins?) and assisted by platelet factor 4 and serotonin, produces progressive cohesion and aggregation of new platelets from the blood stream, forming loose platelet aggregates, which are often detached and carried away with the blood stream. (5) The next stage requires thrombin which produces consolidation of the platelet aggregates, associated with a complete release of the platelet factors mentioned, structural platelet changes (viscous metamorphosis), irreversibility of the aggregates and the appearance of polymerised fibrin at the periphery of the platelet thrombus as a definite sign of the presence of thrombin. (6) Blood coagulation and formation of a 'red thrombus', whenever the rheologic conditions are favourable. (7) Fibrinolysis, thrombolysis.

The pertinent question is whether this concept, which is mainly based on experimental studies on haemostasis and thrombosis, also applies to spontaneous clinical thrombosis. There is general agreement on the two principal reactions: the platelet release reaction, initiating progressive aggregation and the formation of thrombin, producing consolidation and fibrin. The concept implies, however, that the platelet release reaction is triggered by collagen (or other components of connective tissue). Further, it has been suggested by many researchers that the thrombin formation is triggered by tissue thromboplastin.

This hypothesis fits rather well with *arterial thrombosis*. Recent investigations, using step-serial sections with a detailed histologic examination of thrombi in human coronary and cerebral arteries, have demonstrated, first, that most myocardial infarcts are caused by thrombotic occlusions, and second, that most instances of occlusive thrombi are caused by rupture of an atheromatous plaque (Chapman, 1965; Friedman et al., 1966; Constantinides, 1966, 1969; Harland, 1969). The great differences, which have been reported previously, in the incidence of coronary thrombosis in hearts with infarcts, presumably have resulted from differences in the selection of material and particularly from differences in the thoroughness of examination. Constantinides (1966, 1969), who found that all thrombi were associated with breaks in the atherosclerotic or fibrosed arterial wall, points out that most of the cracks were narrow, short and difficult to identify without connective tissue stains. The subthrombic breaks presumably preceded and caused the thrombi, because they were overlaid and filled with a platelet mass and blood had often penetrated through the fracture into the wall. By rupture of the intima, triggers for both the release reaction and coagulation, collagen and tissue thromboplastin, are made available. It seems

doubtful, however, that tissue thromboplastin would be the only trigger of coagulation. Polymerised fibrin is absent at just the sites where a rapid extrinsic coagulation would be anticipated, i.e. within the platelet mass and at the contact area between the platelet thrombus and the vessel wall. Fibrin appears only at the surface of the platelet thrombus, facing the blood stream (Hovig, 1962, 1969). It is difficult to explain how tissue thromboplastin from the damaged intima should have its main effect at this more distant location. It seems more likely, therefore, that the platelet aggregates, as such, can activate coagulation. Research on venous thrombosis has strengthened this assumption.

THE TRIGGER MECHANISM OF COAGULATION IN VENOUS THROMBOSIS

Roskam in 1922 suggested that "l'atmosphère plasmatique des plaquettes" may play a role in haemostasis. In 1955 Hjort et al. demonstrated that factor V from plasma is strongly adsorbed on the platelet surface, displaying the activity which was previously called platelet factor I. It has since been demonstrated that all the plasma clotting factors are represented on the platelet surface. The clinical significance of the platelet surface clotting system for haemostasis was demonstrated several years ago (Owren, 1960; Borchgrevink and Owren, 1961), when it was found that transfusions of normal platelets temporarily restored the prolonged secondary bleeding time in haemophilia A and B and factor V deficiency, and also the prolonged primary bleeding time in patients who had been overtreated with anticoagulants. These effects occurred without any change in the plasma clotting systems, and therefore had to be ascribed to an effect of the transfused platelets, i.e. thrombin for haemostasis was provided by the platelet surface clotting factors of the transfused platelets. An 'intrinsic' trigger for platelet surface coagulation was postulated, but its origin and nature has remained hypothetical until Walsh (1972) demonstrated that platelets can initiate intrinsic coagulation by enhancing the formation of the 'contact activation product' from factors XII and XI. This platelet activity, termed 'contact product forming activity' (CPFA), was specifically and rapidly stimulated by physiological concentrations of ADP, also in the absence of calcium and platelet aggregation. The activity was independent of PF3. Walsh showed that the activating effect of platelet CPFA was completely dependent upon the presence of factor XII and partially dependent upon the presence of factor XI. It presumably has a similar effect as glass and activates factor XII on the platelet surface with subsequent formation of the activator complex. It might be assumed therefore, that the release reaction results in the appearance of CPFA with formation of the activation product and activation of intrinsic

coagulation. The release reaction also liberates phospholipid micelles, PF3, which is necessary for the formation of the complex of activated factor IX, factor VIII and calcium, which activates factor X, and further for the formation of the prothrombinase-complex of activated factor X, factor V and calcium, which activates prothrombin to thrombin. Under physiological conditions therefore, the release reaction will presumably result in the activation of the intrinsic clotting system and thrombin formation on the platelet surface. Because there is no evidence of endothelial damage which possibly could release thromboplastin from the subendothelial tissue, and because platelets and other blood cells contain minimal if any tissue thromboplastin, it is unlikely that the extrinsic clotting system plays any important role in venous thrombosis. The major importance of the intrinsic clotting system for venous thrombosis, as compared with the extrinsic, is also evident from the fact that venous thrombo-embolism has never been observed in haemophilia A or B, but several cases have been seen in factor VII-deficiency, in which the extrinsic system is out of function. Because the effect of CPFA is dependent upon factor XII, an important question still remains to be answered. What triggered the formation of thrombin in Mr Hageman's fatal venous thrombo-embolism?

A second question in venous thrombosis is: What is the physiological trigger of the release reaction in the absence of collagen?

THE INDUCER OF THE PLATELET RELEASE REACTION IN VENOUS THROMBOSIS

There are few studies reported on the detailed structure of early venous thrombi and of the vein wall at the site of attachment. Thrombi in valve pockets have usually propagated and are organised when seen, and the initial changes cannot be observed. The part of the thrombus near the apex of the valve pocket, which is likely to be the site of the nidus, has shown a varying picture. Paterson (1969) found the majority of the white thrombi consisting of platelet masses. Sevitt (1970) found predominantly fibrin-red cell masses with only tiny foci of aggregated platelets. Which was the very first process, platelet aggregation or coagulation, could not be decided. There was no difference in the histology of the endothelium of valve pockets with and without thrombi (Sevitt, 1970), and there was definitely no exposure of collagen to induce the release reaction. It has been speculated that there might exist alterations of the endothelial cell membrane, leading to molecular derangements involving a change in charge distribution, causing platelet adhesion, or that platelets adhere to the basement membrane in gaps between the endothelial cells. Such gaps have been demonstrated by electron microscopy. Experimental evidence suggests, however, that

such adhesion would not induce the release reaction and progressive aggregation.

Among the very large number of platelet release inducers which have recently been recognised, several might play a role physiologically or in pathological conditions. In the group of low molecular substances, like ADP, the most important are adrenalin, noradrenalin and serotonin. In the collagen group we have antigen antibody complexes, aggregated gamma globulins and fibrin precursors (Solum, 1966). In the group of proteases of the trypsin-like character, there might exist other physiological proteases in addition to thrombin, but this is not known. Some of these agents might possibly act as inducers under certain circumstances, but none of them has been actually identified as the first-rate cause of the platelet release reaction in venous thrombosis.

It has been shown that mechanical trauma can cause release of ADP from both platelets and red cells, followed by platelet aggregation. This mechanism is presumably involved in the formation of platelet thrombi in the cage of an artificial heart valve and in arterial grafts. High velocity gradients favour platelet aggregation, and mechanical release of ADP probably plays a role for the localisation of platelet aggregates and thrombi at sites of arterial stenosis, branching and curvatures. It is less likely, however, that similar mechanical damage of erythrocytes or platelets would occur during stasis in the venous circulation. Neither is there any evidence of a local accumulation of any of the known platelet release inducers. Experimental evidence indicates that retardation of the venous blood flow usually requires additional co-operative factors in order to produce venous thrombosis and these assisting factors might originate at distance and be carried to the place of venous stasis. Wessler et al. (1967) demonstrated the thrombogenic effect of activated clotting factors. They suggested that the state of activation plays a greater role than the actual concentration of the non-activated factors. Others have characterised the 'hypercoagulable state' by an increase in clotting factors (fibrinogen, factor VIII) an increase in accelerators of prothrombinase generation, by a reduction of the normal inhibitors (antithrombin 3), by a reduced fibrinolytic activity or other mechanisms. None of these changes, as such, are known to induce the platelet release reaction and aggregation, except if coagulation is initiated with the formation of small amounts of thrombin. If intermediate coagulation products, activated factors or complexes, are trapped in the stagnant pool of a valve pocket, conditions seem extremely favourable for local thrombin formation, inducing both platelet aggregation and fibrin-red cell thrombosis of the type observed in clinical venous thrombosis. If the process is started, it progresses by its own inherent mechanisms as already mentioned. There is no doubt that coagulation products enter the circulation in many clinical conditions associated with tissue damage (surgery, injury, burns, parturition, etc.), and if these are not quickly

cleared by the RES or inactivated, they might settle at local areas of stasis. This hypothesis would explain also Mr Hageman's thrombosis. Wessler et al. (1967) have demonstrated that very minute amounts of activated factor X are thrombogenic in the stasis model in animals.

Much interest has recently also been focused on the small platelet aggregates which can arise in flowing blood and be trapped as microembolism or adhere to the endothelium at sites of eddy currents or stasis and act as a nidus for thrombus formation. Platelet aggregates in flowing blood have been produced experimentally by injection of ADP, collagen, thrombin, viruses, bacteria, bacterial endotoxin, lipids, fatty acids, mobilisation of fatty acids by ACTH, and by antigen in sensitised animals. The same phenomenon has been observed in patients after major vascular surgery, after massive blood transfusion, injuries and burns and in acute haemolytic anaemia. Studies on platelet aggregates in the arterial system has indicated that intraluminal platelet aggregation might be the initial event in thrombosis, and the adherence to the vessel wall a subsequent stage (Jørgensen, 1969). The occurrence of coagulation products and platelet aggregates in the circulating blood is closely related to the syndrome of disseminated intravascular coagulation (see recent monographs: McKay, 1965; Hardaway, 1966).

THERAPEUTIC CONSIDERATIONS

In the normal physiologic state there are several protective defences against thrombosis. The triggers of both platelet aggregation and coagulation are separated from blood plasma by being concealed either in the subendothelial tissue or within the blood cells. If the trigger of platelet aggregation is released, the aggregates tend to break up and disperse, because of subsequent degradation of ADP by plasma. The platelets then acquire a temporary refractoriness to reaggregation (Holmsen and Rozenberg, 1971). If triggers of coagulation are accidentally liberated into the blood stream, the plasma is provided with protective inhibitors which neutralise activated clotting factors at all stages of the clotting process. If fibrin nevertheless is formed, the fibrinolytic system is automatically activated by the release of tissue activators from the vascular endothelium. Finally and just as important, all products of coagulation are very effectively removed by the RES. Theoretically, there are numerous possibilities of influencing these many mechanisms therapeutically by reducing thrombosis-promoting factors or by increasing inhibiting factors.

The main research in recent years has centred on the modifying of general mechanisms such as platelet function and coagulation. The importance of local factors, however, should be duly recognised. Prevention of stasis by ambulation, as a measure against venous thrombosis

is generally applied whenever possible. The local factor in arterial thrombosis, however, has only recently attracted the interest it deserves. The local factors which trigger the formation of secondary thrombosis seem to be related to the structure of the atheroma. There is a striking difference in histological picture and histochemical composition between the hard fibrous plaques seen in elderly men and premenopausal women and the soft atheroma which is encountered in young and middle-aged men and in elderly women. There is a similar marked difference in the tendency to thrombosis. The soft plaques with abundant lipids and which are scarce in mast cells (heparinocytes) tend to develop necrotic masses and subendothelial haemorrhages, ruptures of 'atheromatous abscesses', ulcerations of intima and exposition of tissue fibres, processes which act as a potent trigger mechanism for secondary occluding thrombi. This type is regularly associated with disturbance of lipid metabolism. Mural thrombi may also be seen on hard plaques, but they are seldom occlusive. However, they tend to become incorporated into the fibroplastic area and to produce narrowings. These plaques have little or no lipids but numerous mast cells in the intima in contrast to the soft plaques, and there is less evidence for disturbed lipid metabolism in these patients. Further studies are needed on the histology and histochemistry in order to find out why the intima cracks and ruptures, and why plaques change from fibrous to soft.

Although a local atherosclerotic change in most instances is a prerequisite for arterial thrombosis and a slowing down of the circulation is an important contributing factor for the development of venous thrombosis, there is convincing evidence for assuming the presence of general factors which predispose to thrombotic complications in various clinical conditions. A number of characteristic changes have been postulated, e.g. shortened whole blood clotting time, shortened partial thromboplastin time, increased concentration of fibrinogen or of fibrinogen B, excess of factor VII (characteristically increased in pregnancy), increased activity of factors V and VIII, presence of activated clotting factors, signs of intravascular coagulation (fibrin degradation products), increased platelet number or stickiness, decrease in the concentration of factors which normally antagonise blood coagulation such as the antithrombins and heparin or reduction of the normal fibrinolytic activity. Many tests for assessing these changes have been used for disclosing an increased tendency to thrombosis. Results have been at best equivocal.

The therapeutic goal is to develop reliable predictive methods for quantitating the thrombosing tendency or risk of thrombosis in the individual patient and to have effective prophylactic measures available when needed. The predictive power of available tests at present clearly falls very short of the ideal and we do not have ideal methods for prophylaxis and treatment. The clinical judgment based on empirical risk

factors such as age, obesity, varicosities, history of previous thrombosis, type of disease (malignancy, congestive heart failure) and type of operation has so far had a better predictive reliability than laboratory tests. It must nevertheless be presumed, however, that clinical risk factors are mediated through definite changes in one or more of the fundamental interplaying mechanisms, but these changes might be quite different in different clinical conditions.

There is obviously a large number of factors which might accelerate or inhibit coagulation, fibrinolysis and platelet function and influence the endothelium and the RES and their interactions. It is futile, therefore to search for one single factor or disturbance in all cases. The question is to find which changes are most common. It is well known that lack of antithrombin 3 results in a high incidence of thrombosis, even in children, but this is a rare anomaly. Further, this observation does not allow the conclusion that a moderate reduction of antithrombin 3, such as produced by 'the pill', is the only cause of the increased risk of thrombosis in that situation.

Recent developments in antithrombotic therapy provide different approaches, i.e. antiplatelet therapy, anticoagulant therapy and fibrinolytic-thrombolytic therapy. The possibility of inhibiting the platelet release reaction as a preventive measure against thrombosis has caused an enormous interest. The observation of hereditary platelet disorders with defective-ADP-release (storage-pool deficiency, release deficiency), associated with a bleeding tendency, has strengthened the assumption that therapeutic modification of platelet aggregation might have clinical application. Different types of congenital anomalies have been observed with differences in the adhesion of platelets to collagen and in the collagen and thrombin-induced aggregation (Hardisty and Hutton, 1967; Hirsh et al., 1967; Weiss, 1967; Papayannis et al., 1971). These congenital anomalies might be useful for disclosing the biochemical disturbances involved and they might thereby improve our understanding of the mechanisms involved in platelet adhesion, the release reaction and aggregation. The overt bleeding tendency in these patients is a warning that therapeutic manipulation of platelet function also carries a risk of bleeding complications similar to that of anticoagulant therapy. If new and effective drugs are developed therefore, the treatment presumably might have to be guided with adequate control methods.

The recent development of methods for analysing and quantitating the release reaction under different circumstances has permitted the experimental testing of a large number of antiplatelet agents. Several of these have side-effects and are unlikely to become antithrombotic drugs for clinical use. Others have been in clinical use for other purposes for many years and have recently been tested for antithrombotic effect in various experimental models and are at present subjected to clinical