THE PATHOGENESIS OF THROMBOSIS

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The pathogenesis of thrombosis has excited the curiosity and interest of clinicians and pathologists for a great many years. In modern times the ever-increasing incidence of thrombosis and embolism in Western countries has resulted in a vast amount of research into the problem. This has been directed along many channels, being based on clinical, laboratory, sociological and so-called 'epidemiological' studies. Thrombosis and the factors giving rise to it may be classified in a variety of ways. One of the most valuable classifications was provided as long ago as 1924 by Aschoff.³ The mechanisms leading to thrombus formation were classified under four headings on theoretical grounds. The first was changes in the coagulability of the blood, the second changes in the formed elements of the blood, the third changes in the circulatory blood flow and the last changes in the vessel walls.

The first problem which arises therefore is whether there is a demonstrable increase in coagulability in cases of recent thrombosis. Attempts have been made to show this by the use of blood coagulation time techniques. Finger-prick capillary clotting times, clotting times by venous method of Lee and White and modified methods employing non-wettable surfaces of lusteroid or silicone have been used in patients with thrombosis and parallel controls. In each case claims have been made that the method showed shortened clotting times in the thrombotic patients but in each and every instance later detailed studies were unable to substantiate the claim.

The use of the clotting time methods to demonstrate increased coagulability was based on two basic assumptions. The first was that a local predisposition to thrombus formation in a coronary or peripheral vessel would be associated with a general increase in coagulability of the entire blood registered at a point distant from the clot, and the second was that the methods in current use would be sensitive enough to measure these

changes. The failure of the blood clotting times to register increased coagulability was regarded by some investigators not as evidence of its absence but of lack of sensitivity of these methods to the changes in recent thrombosis. A further technique was evolved, therefore, which depended upon the addition of an inhibitor of coagulation to clotting times to prolong the tests in the hope of detecting differences which might not have been apparent before. The only method to be used on any large scale has been the addition of heparin to tests of clotting times.

The heparin tolerance test was introduced by the Canadians Waugh and Ruddick in 1944.²⁹ It was, in fact, a venous blood coagulation time method performed in nine separate tubes, increasing amounts of heparin being added to them. It was thus possible to draw an *in vitro* heparin tolerance curve for an individual. Claims were made that shortened clotting times (i.e. increased heparin tolerance) were present in cases of recent thrombosis.¹⁹ Later work, including a survey published from Oxford in 1953,¹⁴ failed to substantiate this claim.

The trouble with the Waugh-Ruddick test was that it was elaborate, was difficult to perform and took one to two hours to complete. Thus spontaneous changes in coagulability which occur when blood is stored in glass tubes and which were insufficiently appreciated at the time were a source of error. The heparin plasma clotting time test²² is a single tube test based on the heparin tolerance principle. Plasma was preferred to whole blood to improve the end-point and a standard preparation of heparin was used which has been found to be stable for several months. Using this method under standardized conditions reproducible results were obtained. A normal range was divided from 100 healthy adults. A series of hospitalized patients without evidence of thrombosis was then observed as a control group. The results are given in Fig. 1. The mean coagulation time was shorter than that of the normal group. In Fig. 2 are given the results in 64 cases of recent thrombosis tested within 48 hours of onset. Here there was significant

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FIG. 1.—Heparin plasma clotting times in 71 cases with no evidence of thrombosis.

FIG. 2.—Results of heparin plasma clotting times in 64 cases of recent thrombosis.

shortening of the clotting time compared with the normal group and the group of patients in hospital without evidence of thrombosis. The distribution is somewhat uneven but this was not a homogeneous group. In fact the 17 cases of deep vein thrombosis included had a significantly shorter

FIG. 3.—The effect of adding increasing volumes of stored normal serum (B), and serum products obtained by alumina adsorption and elution (C). Line (A) is the simple effect of dilution with normal saline, calcium being present in greater amounts than the optimum in the heparin plasma clotting mixture.

clotting time than the 34 cases of coronary thrombosis included in the series. This finding will be referred to later.

Does the demonstration of shortened heparin clotting times in cases of recent thrombosis mean that the presence of increased coagulability has been established? This has not been finally settled. It has been shown²⁵ that normal stored serum reduces the anticoagulant effect of heparin on plasma clotting (Fig. 3). This activity is present in factor VII solutions prepared by the alumina-adsorption of serum but does not appear to depend on the presence of factor VII or of Christmas factor, or on the anti-heparin activity of platelets described by Van Creveld *et al.*⁵ An agent with similar physical properties has been found by Wessler and co-workers³⁰ in the United States to be responsible for the production of thrombi in experimental animals. It thus appears possible that the shortened heparin clotting times may in fact be secondary to a formed thrombus, resulting from the release of serum products into the circulation.²⁵ The fact that patients with large vessel occlusion had shorter clotting times than the patients with coronary thrombosis lends some support to this view.

With the lack of clear demonstration of increased coagulability using methods for the coagulation time of the blood, can more specific

FIG. 4.—Simplified scheme for 'intrinsic' blood coagulation.

methods demonstrate changes producing a hyper-coagulable state? With increasing knowledge of the blood coagulation pattern obtained in recent years there has been a multiplication in the techniques available to us. Contact with a wettable surface activates the thromboplastin complex consisting of Hageman factor, P.T.A., Christmas factor, antihæmophilic globulin and Prower Stuart factor. These in the presence of prothrombin, factor V, and calcium and perhaps accelerated by factor VII, produce thrombin. Thrombin is the enzyme responsible for the conversion of fibrinogen to the fibrin clot. A simple outline of this scheme is given in Fig. 4.

Prothrombin times were introduced by Quick in 1935,²⁷ since the administration of the anti-coagulant drugs of the coumarin type became general medical practice in the late 1940s there has been an opportunity to observe large groups of patients with this technique. Although claims were made that the method showed shortened clotting times in thrombosis it is now accepted that this is not the case.

Prothrombin activity as measured by the Quick method is a complex of prothrombin, factor V, factor VII and Prower Stuart factors. Specific methods exist for these clotting factors. Interesting results were obtained with one of these methods, the specific assay of factor VII, in patients with thrombosis.²⁴ It was found that the plasma of patients with recent thrombosis restored the clotting defect of factor VII deficient plasma more than controls. The results of the mean of 20 cases and 20 controls are given in Figs. 5 and 6. Some confirmation of these results has been given by Alexander,¹ who found increased factor VII activity in some but not all cases of recent thrombosis.

The significance of raised factor VII activity in recent thrombosis is by no means clear. Factor VII is an accelerator of prothrombin conversion and it might be assumed that excess would be associated with increased coagulability. Indeed the demonstration of increased factor VII levels during pregnancy by several groups of investiga-

FIG. 5.—The effect of control plasma and thrombotic plasma on restoring the coagulation defect of factor VII deficient plasma.

FIG. 6.—Factor VII levels. Distribution of readings of 50% dilutions in thrombosis and controls

tors has been taken as evidence of the hyper-coagulable state associated with pregnancy. Factor VII is not consumed during clotting but is activated, excess being present in serum. It is possible, therefore, in this instance also that excess is due to the release of serum products into the circulation.

The thromboplastin generation test was introduced by Biggs and Douglas.⁴ This test was used by Poller in investigations comparing thrombotic patients with controls. It was noted that thromboplastin generation appeared to be increased in the cases of recent thrombosis.²⁴ In the same year McDonald and Edgill¹⁷ found thromboplastin generation to be increased in ischaemic heart disease, and later McDonald¹⁸ showed also that thromboplastin generation was increased in cases of recent coronary thrombosis. The association of increased factor VII activity together with increased thromboplastin generation and shortened heparin clotting times is of interest as it is now well established that factor VII is not

FIG. 7.—Heparin plasma clotting times after operation.

involved in blood thromboplastin generation. There is evidence, therefore, of a combined coagulation anomaly in recent thrombosis.

We cannot leave the subject of coagulability and thrombosis without reference to post-operative coagulability. There is a well-established relationship between surgical operations and a thrombotic tendency. The risk varies in the surveys reported, but it is higher in major abdominal and pelvic operations. Coagulability studies were performed with the heparin plasma clotting time, in a group of 40 patients undergoing a variety of common operations. The maximum fall of clotting time during the 10 days after operation is seen (Fig. 7) to occur on the fifth day after operation. This is somewhat surprising, as it is often stated that the maximum incidence of thrombus formation occurs several days later. Two other surveys by Allen *et al.*² and Gardikas *et al.*⁹ have confirmed that the shortest clotting times occur on the fifth day after operation. Payling Wright²¹ has reviewed the problem and has noted that a maximum increase in fibrinogen, platelet 'stickiness' and slowing of the foot-groin venous flow time occur together at about the tenth day after operation, which is the time of the greatest incidence of thrombosis. It is thus possible that the coagulability changes may not be of prime importance in the causation of post-operative thrombosis, although this view is opposed by Gardikas *et al.*⁹

Clinical thrombosis tends to be episodic in character, the most notorious form being 'thrombosis migrans'. Other forms of the disease may show this tendency to a lesser extent. There is some evidence from coagulability studies which helps to explain this phenomenon. The heparin plasma clotting test was introduced in 1952 as a method for the better control of anticoagulant therapy, as it was felt that control by Quick prothrombin times was not sufficiently safe, thrombosis and haemorrhage on occasion being present at 'safe' levels. It was thought necessary to include two control groups. The first was a group of hospital patients without evidence of thrombosis

FIG. 8.—Spontaneous changes in clotting times in three cases of coronary thrombosis.

FIG. 9.—Spontaneous changes in clotting times and factor VII in coronary thrombosis.

on whom clotting times were performed, at similar periods to the patients on treatment. The second control group consisted of 20 patients with recent thrombosis who did not receive anticoagulant drugs. As was hoped, the controls without thrombosis were stable from day to day, without much variation in any case. The results in the thrombotic controls were completely different, however, shortened clotting times being present initially but followed by a period of reduced coagulability after two to three days which in some cases appeared to amount to a definite coagulation defect. There was then a tendency to return to normal over a period of several days and in several cases the return of shortened clotting times was associated with a further thrombotic episode. This fluctuating pattern was present in varying degree in all the thrombotic group. The patterns of three typical patients suffering from coronary thrombosis are illustrated (Fig. 8).

Is there any more evidence to support the existence of these fluctuations of coagulability?

FIG. 10.—Spontaneous changes in coagulability in a case of myocardial infarction.

- A. Quick's prothrombin time.
- B. Owren's P. and P. method.
- C. Thrombotest.
- D. Thromboplastin generation test.

It is well known that some patients are more sensitive in the early days of anticoagulant treatment than later on and unexplained fluctuations occur. In Fig. 9 are the heparin clotting times and factor VII levels of a case of coronary thrombosis who had two further episodes of myocardial infarction. A fluctuating pattern is present in both and the further episodes came when both factor VII levels were increased and heparin clotting times fell below the lower limit of normal.

During observations on thromboplastin generation in patients on phenindione treatment it was noted that a fair proportion of subjects had patterns of thromboplastin generation which did not appear to have a close relationship to either the dosage of the drug or the level of prothrombin activity.¹⁵ The patterns bore a striking resemblance to the coagulability patterns with the heparin clotting time in untreated thrombosis. This pattern of thromboplastin generation was not present in patients without thrombosis. In Fig. 10 there is a striking picture. This case of myocardial infarction developed a marked coagulation defect two to three days after the clinical onset. This observed defect was present in four different coagulation techniques, the Quick prothrombin time, Owren's P. and P. method, the thromboplastin generation test and the 'thrombotest'. The thrombotest is a new technique devised by Owren²⁰ for the better control of anticoagulant treatment. It has been under trial at Manchester Royal Infirmary and the indications from the investigation are that it is a useful index of coagulability.¹⁵ All four methods show in varying degree the fluctuating coagulation pattern, which is similar to that previously observed with the heparin plasma times. If these changes are commonly present in untreated thrombosis they

appear of importance not only in relation to the evolution of the disease but in connection with the laboratory control and dosage of anticoagulant treatment.

The changes in the formed elements of the blood is the second of Aschoff's theoretical mechanisms of thrombus production. Of the changes in the red cells both anaemia and polycythaemia are associated with a clotting tendency. The platelets have been intensively studied in view of their known role in haemostasis. Increased platelet 'stickiness' has been found in the thrombotic state by several authors in recent years, e.g. Spooner *et al.*²⁸

Changes in the circulatory dynamics have been insufficiently studied owing to the difficulty of simulating the events in the body in test tube experiments. Circulatory slowing is associated with the post-operative state and occurs after bed rest, both of which are associated with a predisposition to thrombosis. When blood clotting times are performed in silicone tubes, a firm solid clot results after some delay. The result when the silicone tube is put in a suspension mixer instead of a stationary rack is completely different, however. The mixer rotates slowly at 30 revolutions per minute and imparts a continuous to-and-fro motion to the blood. Under these conditions a solid clot never forms, but clotting takes the form of the deposition of a firm web of fibrin on the walls of the tube. If a tube were removed after one hour, a small solid clot formed in addition, but after 24 hours no solid clot formed. Related to the events in an elastic artery, this suggests that the circulatory dynamics may govern the nature of the resulting clot. A solid clot may, therefore, only form when the circulation is slowed or arrested; where there is a good circulatory flow, clotting may take the form of the deposition of fibrin on the walls of a vessel which supports Duguid's thrombogenic theory of atherosclerosis.²⁶

This brings us to the fourth of Aschoff's mechanisms, the changes in blood vessel walls. Damage, disease, and inflammation predispose to intravascular clotting. The anatomical association between atherosclerosis and thrombosis has long been recognized. Duguid⁸ revived Rokitsansky's theory that atherosclerosis resulted from the organization of clots. Histological evidence and experimental evidence for this is derived from the injection of fibrin particles into rabbits.^{11, 13} In Duguid's view, atherosclerosis formation and thrombosis are part of one single pathological process. There are difficulties in the unreserved acceptance of this theory as far as coronary thrombosis is concerned.

There has been a vast amount of research in recent years on the part played by fats in blood

coagulation and thrombosis. This has been based on dietetic surveys, sociological, clinical and laboratory studies. The effects of fats have been studied on clotting *in vitro* and the effects of fatty meals in the human subject by numerous authors. The association between abnormal lipoproteins and a predisposition to thrombosis has been studied.^{6,10} From the vast amount of evidence accumulated it appears that fat and particularly animal fat may play some part in the predisposition to thrombosis as well as to atherosclerosis formation, although the extent of this is not yet defined.

Other considerations in relation to thrombosis should be mentioned for the sake of completeness, although it would be out of place to consider them in detail in this survey devoted primarily to laboratory studies. Thrombosis is associated with certain clinical states, e.g. diabetes, hypertension, xanthomatosis and malignancy. It is also associated with a familial or racial tendency. From surveys it appears that cigarette smoking may predispose to thrombus formation.^{7,12} Insufficient physical exercise may also play a part as suggested by the occupational survey in London Transport workers by Morris.¹⁸

Thus a multiplicity of factors and influences are involved in the pathogenesis of thrombosis. Coagulability studies appear to be of importance, as do changes in the circulatory blood flow from the scanty evidence available. The extent of the role played by fats is not yet finally decided. Further research may result in the discovery of a 'trigger mechanism'. It is much more likely, however, that it will confirm the need to consider

many different causes and influences in the problem of intravascular clotting.

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