Section of Pathology

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Papers

Hæmorrhage and Fibrinolysis

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Bleeding and increased fibrinolysis are often found together. The association has been reported following operations on the lungs and prostate, gastro-intestinal hæmorrhage, obstetric accidents, diffuse malignant conditions, extracorporeal shunts, hepatic cirrhosis, and in the terminal stages of many different diseases (reviewed by Sherry *et al.* 1959). How often the fibrinolysis has contributed to the cause of the bleeding in these states is not yet known.

The intravascular injection of coagulants into animals causes a secondary stimulation of fibrinolysis (Stefanini 1958) and in many patients the increase in fibrinolysis may be a protective response to intravascular coagulation. If the increase is excessive it can, on the other hand, be the cause of massive tissue hæmorrhage, particularly at sites of trauma. The use of fibrinolytic enzymes for the treatment of thrombo-embolic disease has clarified our understanding of the mechanism by which this bleeding is produced and it is now possible to discuss how further information can be obtained by the application of the existing methods for the measurement of fibrinolysis.

The Physiological Basis of Fibrinolysis

A series of enzymes are concerned with the digestion of fibrin in the body, and these are in dynamic equilibrium with inhibitors in the blood. Different enzymes can be found in blood, tissues and urine which act as plasminogen activators. They convert plasminogen, a plasma globulin which has no proteolytic action, into the enzyme plasmin. This enzyme, in turn, splits the insoluble fibrin molecule into soluble polypeptides.

Plasmin is an enzyme of the trypsin class and can attack arginine and lysine bonds in many different proteins. In blood any plasmin which is not adsorbed to fibrin is neutralized by inhibitors present in the α-globulin fraction. Free plasmin can only be demonstrated if these antiplasmins are completely absent, a rare occurrence, or if the plasminogen activator level rises to such an extent that the rate of plasmin formation exceeds the rate at which it can combine with the antiplasmins. It is only when free plasmin appears that proteins other than fibrin are degraded.

The Mechanism Causing Bleeding

Free plasmin can destroy the clotting factors fibrinogen, factor V, and factor VIII. The breakdown products of fibrinogen, which produce a fibrin polymerization defect, have been shown to be even more important as a cause of bleeding than the actual loss of essential clotting factors (Fletcher *et al.* 1962).

During normal blood coagulation thrombin splits off a small portion of the fibrinogen molecule; the fibrin monomers which are formed then polymerize to form a fibrin clot which undergoes gelation (Scheraga 1958). When fibrinolysis is excessive free plasmin also splits the fibrinogen molecule but at a site different from that attacked by thrombin. Among the products of this action are fragments of high molecular weight which become caught up with the polymerization of fibrin monomers but, because they lack the necessary molecular structure to complete the process, block the final polymerization of fibrin. The clot formed in the presence of these breakdown products is very weak and friable (Fig 1) and their concentration in the blood has been shown to parallel the severity of bleeding due to fibrinolysis (Alkjaersig *et al.* 1962).

Fibrinogen breakdown products are only formed when the concentration of a plasminogen activator, from whatever source it is derived, increases some twenty or thirty times above the physiological level of the blood activator and causes the appearance of free plasmin. The release

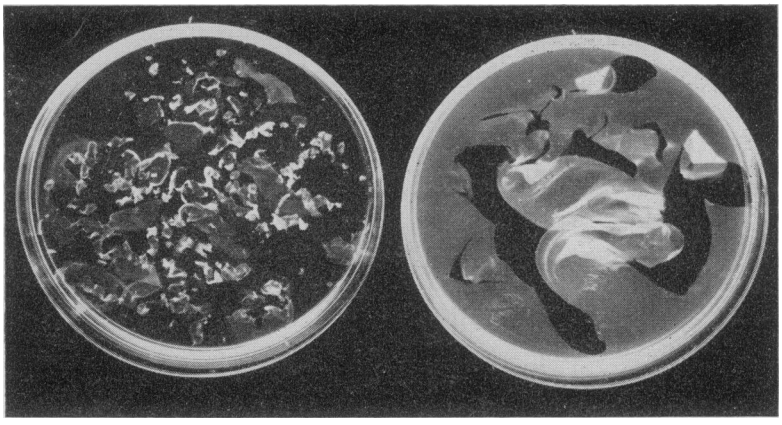

Fig 1 *Two plasma clots broken by stirring with a rod; showing the friable nature of the clot formed in the presence of fibrinogen breakdown products (left) in contrast with the normal clot (right)*

of plasmin may be general, with high activator levels throughout the circulation. These can be demonstrated directly by their action on a fibrin substrate. Alternatively very high concentrations of activator may be released in one part of the circulation causing the local appearance of plasmin. Elsewhere dilution of activator in the circulating blood may reduce the concentration below that needed to produce free plasmin and direct estimation in blood collected from an antecubital vein may show fibrinolytic activity within the normal range (Tsapogas *et al.* 1962). This local release of plasmin can only be recognized indirectly by the effect on the blood concentrations of fibrinogen and plasminogen.

The Direct Measurement of Fibrinolytic Activity

A fibrin clot is formed in a Petri dish by adding thrombin to a solution of fibrinogen known to contain plasminogen. Three 30 microlitre drops of citrated plasma from the patient to be investigated are placed on the surface of the fibrin and the plate is incubated at 37°C for twenty hours. Fibrinolytic activity in the plasma is proportional to the product of two perpendicular diameters of each area of lysis when plotted on a double logarithmic scale (Astrup & Mullertz 1952).

The normal result depends upon the fibrinogen used to make the plates, and should be decided separately for each laboratory. Normal plasma never shows any activity on plates made from bovine fibrinogen (Armour fraction 1) 300 mg/100 ml of borate buffer pH 8 containing 0·001 molar calcium chloride. Any lysis of these plates is therefore pathological and can be appreciated within one hour of the plasma being placed on the plate, even though a final reading cannot be taken until the next day. Normal blood contains low levels of plasminogen activator which can be detected on the plate only by separating the euglobulin fraction, which contains very little of the blood antiplasmins (Flute 1963).

Further investigations can show the nature of any activity found. Plasmin retains the ability to dissolve fibrin incorporating 10^{-3} molar ε-aminocaproic acid, a concentration which will inhibit plasminogen activators. The normal blood activator gives greater areas of lysis on human than on bovine fibrin plates and is easily destroyed by heating the plasma to 56°C. Tissue activators are resistant to heat and dissolve human and bovine fibrin equally.

A useful screening test is the dilute blood clot lysis time (Fearnley *et al.* 1957). In this 0·2 ml of freshly collected blood is added to a tube containing 1·8 ml of 0·07 molar phosphate buffer, pH 7·4, with 2 NIH (National Institutes of Health) units of thrombin. The clot, which forms within two minutes, retracts within an hour of incubation at 37°C but does not dissolve within one and a half hours unless there is excessive fibrinolytic activity. A clot does not form at all in this test if the fibrinogen level in the blood is less than 150 mg/100 ml or if large amounts of fibrinogen breakdown products are present. The test can then be set up again with the addition of 0·2 ml of bovine fibrinogen 400 mg/100 ml as well as the 0·2 ml of blood.

The Detection of Fibrinogen Breakdown Products

There are three ways for detecting these breakdown products: (1) The friable nature of the clots can be appreciated. (2) Thrombin clotting time, one-stage prothrombin time, and partial thromboplastin time results are prolonged and cannot

Table 1
Illustrative Cases

Test	*Secondary carcinoma of prostate*	*Gastric erosions*	*Amniotic fluid embolism*	*Concealed accidental hæmorrhage*	*Normal values*
Dilute clot lysis time:					
(*a*) Blood	No clot	4 hours	No clot	No clot	2–7 hours
(*b*) Blood and fibrinogen	10 min	6 hours	6 hours	6 hours	2–7 hours
Bovine fibrin plate lysis	480 sq.mm	No lysis	No lysis	No lysis	No lysis
Plasminogen (Sherry casein units per ml)	1·3	1·8	1·0	3·6	3·0–5·0
Fibrinogen (clot weight mg/100 ml)	50	570	300	60	240–500
Thromboplastin generation (Hicks & Pitney)	10%	100%	100%	20%	80–100%
Platelets per c.mm	175,000	303,000	250,000	70,000	200,000 to 500,000

be fully corrected by the addition of normal plasma. (3) Estimates of fibrinogen obtained after clotting the plasma are lower than those obtained by precipitation or immunological methods. These changes are not, however, specific for excessive fibrinolysis; similar effects are seen in these tests if clotting is delayed by circulating anticoagulants, such as heparin.

It is desirable to use different methods of estimating fibrinogen in sequence. To 2 ml of citrated plasma are added 2 ml of 0·025 molar calcium chloride containing 20 NIH units of thrombin and 10^{-2} molar ε–aminocaproic acid, the latter being added to prevent digestion of fibrin during the test. After fifteen minutes at 37°C the clot which forms is removed, dried and weighed (Ingram 1952). The serum from this test is then 25% saturated with ammonium sulphate. No precipitate is formed unless fibrinogen or its breakdown products are still present.

The Measurement of Plasminogen

A significant release of plasmin in the blood must always be accompanied by a decrease in the concentration of its precursor plasminogen. The release of plasmin may be transient or even confined to a particular part of the circulation but fibrinogen breakdown products have a 50% plasma clearance time of 9·5 hours (Fletcher *et al.* 1962) and may still be the cause of bleeding even if fibrinolytic activity is not demonstrably increased in the circulating blood. The measurement of plasminogen is therefore the key to the recognition of excessive fibrinolysis.

Plasminogen is a very stable protein and can be measured reliably by a caseinolytic method (Alkjaersig *et al.* 1959*a*). Normal plasma concentration is between 3·0 and 5·0 Sherry units per ml. A concentration less than 2·0 units per ml is strong evidence that plasmin release has occurred, particularly if the concentration rises to normal levels between twenty-four and forty-eight hours after the original stimulus to fibrinolysis has ceased.

Clinical Applications

It is of great practical importance to detect bleeding due to excessive fibrinolysis, because this may now be counteracted with the inhibitor ε-aminocaproic acid (Alkjaersig *et al.* 1959*b*). It is too early to assign fibrinolysis to a full list of clinical associations but further knowledge can be acquired by a study of blood plasminogen concentration in the bleeding disorders.

Results of these tests in some typical examples are shown in Table 1. Low plasminogen values were found in 3 patients. The first, a man with secondary carcinoma of the prostate, also had high blood levels of a plasminogen activator of tissue origin. The bleeding was reduced after treatment with ε-aminocaproic acid and multiple transfusions to replace the deficiency of coagulation factors. The other 2 cases were a man with multiple gastric erosions and a woman with amniotic fluid embolism. Neither showed increased blood activator at the time the tests were made, which was while they were actually bleeding; plasmin release may have been transient or confined to one part of the circulation. Both had fibrinogen levels within the normal range. In the woman the concentration of fibrinogen breakdown products was sufficient to prevent clot formation in the dilute blood clot lysis test.

In contrast, the patient with a concealed accidental hæmorrhage, in common with 4 other cases of this type that have been studied, had a very low level of blood fibrinogen but no evidence of excessive fibrinolysis at any time.

Summary

Bleeding may be caused by a defect in the polymerization of fibrin due to excessive fibrino-

lytic activity which causes free plasmin to appear in the blood. This is always associated with a fall in the blood level of plasminogen. High levels of plasminogen activator, or plasmin, and low levels of fibrinogen may also be found but these are not essential to the diagnosis of bleeding due to fibrinolysis.

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Lipoproteins and Fibrinolysis

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During the last eight years there has been considerable interest in the influence of lipoproteins upon fibrinolytic activity. The reason for this is the relationship between serum lipoprotein levels and the incidence of myocardial ischæmia. Racial groups such as the Bantu in whom coronary insufficiency is almost unknown have significantly lower levels of serum lipoprotein than do age-matched Europeans (Walker & Arvidsson 1954, Bronte-Stewart *et al.* 1955). Also within one racial group, living in the same environment, individuals with a high lipoprotein level are more prone to coronary heart disease than those with a lower level (Dawber *et al.* 1962). It has not yet been shown how a high circulating level of lipoprotein can lead to atherosclerosis and thrombosis but one hypothesis put forward is that lipoprotein inhibits spontaneous fibrinolysis (Greig 1956, Kwaan & McFadzean 1957). It is believed that coagulation is going on continuously within the circulation, the fibrinogen molecule being converted to fibrin which is then removed by the fibrinolytic system; thus, if fibrinolytic activity is depressed intravascular fibrin will persist and become incorporated into the arterial wall, leading to stenosis and occlusion (Astrup 1956).

In normal circulating plasma, spontaneous fibrinolysis is thought to result from the interaction of plasminogen, activator, plasmin and antiplasmins. The inactive pro-enzyme plasminogen is converted to plasmin by an activator which is released from endothelium. This activator is always present in circulating plasma and is increased in response to certain stimuli of which exercise, anxiety, adrenaline injection and local ischæmia are the best known. Plasmin, which is the actual fibrinolytic enzyme, does not normally exist free in plasma, the small amounts which may be formed being inactivated by inhibitors called antiplasmins. The removal of intravascular fibrin is thought to occur by the following mechanism: as fibrinogen is converted to fibrin, plasminogen and activator are adsorbed on to the fibrin molecule; activator in plasma surrounding the clot is also adsorbed on to the fibrin surface and some activator diffuses into the thrombus. On the fibrin surface plasminogen and activator react together so that plasmin is formed in intimate contact with the fibrin molecule. In this situation plasmin is at least partially protected from the antiplasmins and, by this induced substrate specificity, the fibrin to which it is adsorbed is lysed (Fearnley 1961).

The *in vivo* lysis of fibrin may in theory be inhibited by lipoprotein in one or more of the following ways: (1) Activator release into the circulation may be depressed. (2) Activation of plasminogen to plasmin may be inhibited. (3) The proteolysis of fibrin by plasmin may be inhibited either by a physical interference with the proteolytic reaction, or lipoprotein may in some way enhance antiplasmin activity. (4) The adsorption to the fibrin molecule of either activator or plasminogen or both may be blocked. (5) Diffusion of activator into the clot may be impeded. There is no single *in vitro* technique which can be used to investigate all these possibilities.

Most of the experimental work has dealt with the effect of alimentary lipæmia, that is to say, the effect of circulating chylomicrons upon fibrinolysis. For the purpose of this paper, serum lipoproteins may be considered in two groups: (1) Chylomicrons that appear in plasma only after a fatty meal. (2) The lipoproteins that are present in plasma all the time and of which the low-density or β-lipoproteins are of importance in relation to myocardial ischæmia. No direct work has been done to compare the fibrinolytic activity of people with differing levels of β-lipoprotein but the results of some inter-racial comparisons are