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Symposium on thrombolytic therapy

The symposium, arranged by Kabi Pharmaceuticals Ltd, London, was held at the Nuffield Department of Surgery and the Radcliffe Infirmary, Oxford, on 24-25 October 1968, with Mr N. L. Browse as chairman. Abstracts from some of the papers follow.

PLASMINOGEN ACTIVATION AND THE COAGULATION PROCESS

C. R. M. PRENTICE, G. P. MCNICOL, AND A. S. DOUGLAS (*University Department of Medicine, Glasgow Royal Infirmary*) In the defibrination syndrome intravascular coagulation and the fibrinolytic process are often associated so that it is difficult to determine the primary cause of the process. The present studies were designed to examine some of the mechanisms by which intravascular coagulation is able to initiate, or is stimulated by, the fibrinolytic process.

Several aspects of the problem have been well explored. Sherry, Fletcher, and Alkjaersig (1959) have suggested that a thrombus may selectively encourage local plasminogen activation as the inhibitors are excluded in the surrounding plasma. Kwaan and McFadzean (1957) have shown that the release of plasminogen activator is augmented by anoxia of venous endothelium. A thrombus, by causing local endothelial ischaemia, could provide the stimulus for activator release. Niewiarowski and Prou-Wartelle (1959) and Iatridis, Wilson, Ferguson, and Rierson (1960) demonstrated that activation of the contact factors accelerates plasminogen activation.

In the present studies, four situations in which coagulation and plasminogen activation were associated are described. Particular emphasis has been given to the linking mechanisms between these processes.

The first studies were with urokinase. Using a proprietary preparation of urokinase a satisfactory fibrinolytic response was found in patients, but a transient coagulative effect was sometimes also seen. There was shortening of the recalcification time associated with a marked rise in factor VIII activity. Plasma obtained at this time formed a thrombin-clottable cryoprecipitate at 4°C. To determine if this effect was due to contamination of urokinase with thromboplastic substances or whether it was the direct result of plasminogen activation, volunteers were given two infusions on separate occasions. The infusions consisted of first, urokinase, and second, urokinase preceded by aminocaproic acid to inhibit plasminogen activation. The coagulative effect in the recipients' plasma was, on the whole, greater when urokinase was administered alone than when it was given with aminocaproic acid. It is suggested that this coagulative effect was the result of plasminogen activation by urokinase. Possibly the patients' fibrinogen may have been converted into a cold-precipitable form with consequent activation or release of factor VIII activity.

The second studies were made following exertion. In volunteers, hard exertion produced the expected increase in plasma fibrinolytic activity and in factor VIII levels. Subjects who exercised following the administration of aminocaproic acid also showed a normal rise in factor VIII levels. It appears that the factor VIII increase is not dependent on plasminogen activation. Two splenectomized patients showed a normal rise of factor VIII activity following exertion. It is unlikely that factor VIII released by the spleen is responsible for the elevation of this factor seen after exercise. Platelet aggregation was increased and disaggregation impaired following exertion.

The third series of studies were of three children from East Africa who developed thrombotic gangrene of the fingers, but they showed defective fibrinolysis associated with increased levels of fibrinolytic inhibitors. It is possible that this abnormality was, in part at least, responsible for their disease (Turpie, Forbes, and McNicol, 1967).

The fourth series were of studies made during Caesarian section. Here, blood samples were taken simultaneously from peripheral and uterine veins. A progressive shortening of the whole blood clotting time, increase in factor VIII activity, and increased fibrinolysis were noted after placental separation. The changes were all greater in the uterine venous blood than in forearm vein blood. In some subjects raised levels of fibrinogen degradation products were found in the uterine venous blood, but not in the peripheral blood.

It is apparent that both the coagulation and fibrinolytic processes are called into action following uterine incision and placental separation. Of practical importance is the observation that the uterine venous blood shows marked changes which are diminished by the time it reaches the periphery. This confirms the observation that the body has a great capacity to inhibit the active enzymes concerned with haemostasis. It also indicates that the peripheral venous blood may not give an accurate reflection of changes that occur locally in body tissues.

These four situations, which reflect the close association between coagulation and fibrinolysis, illustrate that there are definite, but ill-defined, linking mechanisms between them. Further study in these areas is needed to increase our understanding of the physiology of haemostasis.

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LOCAL FACTORS IN THROMBOLYSIS

A. S. TODD (*Department of Pathology, The University of Dundee*) It has been shown that the activator of plasminogen in tissue is concentrated in the endothelium of blood vessels, especially that of veins and venules (Todd, 1959). Recently, by modifying the time and temperature of incubation (4°C for 24 to 48 hours), the histological technique for identifying plasminogen activator ('fibrinolysis autography') has been improved enabling a greater range of activator concentrations to be detected within a single preparation. This modification has now been used in the study of thrombosis. It is found that the endothelium adjacent to thrombus in veins, pulmonary arteries, cardiac atria, and coronary arteries contains activator. In cases where the thrombus has been loosened, activator can be detected on the fibrin surface, and sometimes lines and foci of activator are found buried within the thrombus, apparently trapped after retraction and rethrombosis. A similar distribution of activator can be demonstrated in pulmonary emboli. Most of the coronary thrombi examined were rich in plasminogen activator. It is, therefore, reasonable to assume that plasminogen activator from endothelium in the venous and coronary circulation plays a major part in the loosening of thrombus and the detachment of emboli.

The endothelium of thrombosed systemic arteries rarely shows fibrinolytic activity, although foci of activator are found deep within mural thrombi from heart chambers and great vessels, apparently related to leucocytes. The platelet-rich lines of Zahn appear to be resistant to plasmin digestion, thus accounting for their prominence in older thrombi.

Coronary and intramyocardial arteries normally show activator in the endothelium, although at lower concentrations than that in veins of comparable size. The degree of fibrinolytic activity seems unrelated to the amount of intimal thickening. Renal arterioles and glomeruli are also active to about the same extent.

Experiments with vessels from limbs subjected to ischaemia during amputation show that the arteries can develop activator concentrations equivalent to those found in veins. Thus, fibrinolytic activity in arteries may be controlled by stimuli of metabolic origin related to the efficiency of their blood supply.

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A further paper on fibrinolysis in animals was read by Dr Chushne Hawkey.

PHARMACOLOGICAL ENHANCEMENT OF FIBRINOLYSIS

G. R. FEARNLEY AND R. CHAKRABARTI (*Gloucestershire Royal Hospital, Gloucester*) The discovery that normal blood has spontaneous fibrinolytic activity (Fearnley and Tweed, 1953) due to a plasminogen activator (Flute, 1960) which is adsorbed to fibrin clot (Fearnley, 1953), led to a concept of how natural fibrinolysis may function as a fibrin-clearing and hence antithrombotic mechanism in arteries: the concept of 'fibrinolysis by adsorption' (Fearnley, 1953, 1961). The situation in veins may differ somewhat from that in arteries since blood fibrinolytic activity appears to derive mainly from the venous side of the circulatory system, and in veins fibrinolysis may be an important function of contiguous vascular endothelium. Evidence has been obtained of an association between defective blood fibrinolytic activity and coronary artery disease and that the former may adversely affect prognosis (Chakrabarti, Fearnley, Hocking, Delitheos, and Clarke, 1966; Chakrabarti, Hocking, Fearnley, Mann, Attwell, and Jackson, 1968).

Over the past 10 years a number of drugs given orally, including the sulphonylureas, the biguanides, and anabolic steroids, have been discovered to increase blood fibrinolytic activity but resistance, in this respect eventually develops (Fearnley, 1964). Latterly phenformin or metformin combined with the anabolic steroid ethyloestrenol have been found to produce a pronounced and sustained increase of blood fibrinolytic activity, together with reduction of plasma fibrinogen levels in a majority of patients with occlusive vascular disease (Fearnley, Chakrabarti, and Hocking, 1967). In addition to these effects, phenformin plus ethyloestrenol decreases platelet stickiness and serum cholesterol levels, whereas metformin plus ethyloestrenol has an adverse influence on both these measurements (Chakrabarti and Fearnley, 1967; Fearnley, Chakrabarti, and Evans, 1968a). Clofibrate (Atromid-S) though effective in reducing serum cholesterol and plasma fibrinogen, has been found to have only a temporary effect on platelet stickiness; and, in contrast to the original Atromid which contained androsterone, to have antifibrinolytic properties, as judged by prolongation of the dilute blood clot lysis times of patients treated with this drug (Fearnley, Chakrabarti, and Evans, 1968b). Recent studies in our laboratory indicate that treatment of arteriopathic patients with phenformin plus ethyloestrenol is associated with a pronounced increase of fibrin degradation products, which provides the first evidence that an increase of blood fibrinolytic activity as measured *in vitro* is accompanied by the breakdown of fibrin/fibrinogen *in vivo*. Hence this combination of drugs appears to produce therapeutic defibrination. Phenformin plus ethyloestrenol, because of its favourable and sustained effects on four factors associated with ischaemic disease, *ie*, fibrinolysis, plasma fibrinogen, platelet stickiness, and serum cholesterol, would seem to be suitable for trial as a prophylactic measure in survivors of vascular occlusions.

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