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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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SOME OBSERVATIONS ON THROMBOLYSIS *in vitro*¹

P. M. DALAL, P. M. SHAH, M. J. ALLINGTON, AND A. A. SHARP
 (Department of Haematology, Radcliffe Infirmary, Oxford) The widely accepted intrinsic clot lysis theory (Alkjaersig, Fletcher, and Sherry, 1959) as a primary mechanism of thrombolysis postulates that during formation of thrombi plasma plasminogen adsorbed on to the fibrin/clot surface is activated by circulating plasminogen activators with resultant autodigestion of the thrombus.

Studies in this laboratory have revealed that the plasminogen content of plasma, before and after the formation of artificial thrombi by Poole's modification of the Chandler tube method (Poole, 1959), was not altered, more than 90% of the original plasminogen content of the plasma being recoverable in the defibrinated plasma. Furthermore, the plasminogen content of washed and drained artificial thrombi was uniformly low, and any exogenously added plasminogen was easily washed out of the thrombus.

Similarly, the plasminogen content of human thrombi formed spontaneously over plastic surfaces in contact with a circulation *in vivo*, and that of fresh and unorganized native (*in vivo*) thrombi obtained by operative intervention, without exception, was low.

In an artificial circulation, lysis of Chandler tube thrombi prepared from native blood, or plasma, or from recalcified platelet-rich or platelet-depleted citrated human plasma could not be readily achieved by perfusion with a wide variety of concentrations of plasminogen activators in saline-buffer or plasma. On the other hand, perfusion with human plasmin (grade A, Kabi) in saline led to predictable thrombolysis, the rate of lysis being the function of the enzyme concentration used.

From this study, it has been possible to place recent and unorganized native (*in vivo*) thrombi and emboli into three broad categories: (1) thrombi and emboli, which were deficient in intrinsic plasminogen; (2) thrombi and emboli which, apart from containing only trace amounts of plasminogen, were also rich in an antiplasmin-like activity; and (3) thrombi and emboli which were deficient in plasminogen but rich in a plasminogen activator.

In an artificial circulation, thrombi of the first category behaved similarly to the Chandler tube thrombi, definite lysis only occurring when perfused by human plasmin. Thrombi in the second category failed to lyse with both plasminogen activators and with low concentrations of plasmin; however, when treated by agents known to induce a 'peptone effect' (Astrup, 1968), successful thrombolysis could be achieved with low concentrations of plasmin. Thrombi in the final category lysed slowly but spontaneously, and it was possible to enhance the rate of lysis by perfusion with plasminogen activators.

The physico-chemical properties of the plasminogen activator isolated from saline extracts of thrombi of the latter category were different from those of tissue activators of plasminogen, leukoproteases, urokinase, and streptokinase (Dalal, Shah, Allington, and Sharp, 1969).

Recent unorganized thrombi and emboli of all categories were found to be permeable to various blood components, including plasma plasminogen and antiplasmins, and it was possible to flush red cells out of such thrombi by prolonged gentle perfusion finally leaving a pearly white coralline network of fibrin.

On the basis of these and our other findings, it is suggested that the selective thrombus activator of plasminogen binds with a globulin fraction of plasma and that this complex activates the plasminogen permeating through the thrombus mass to an enzymatically active plasmin-globulin complex. This complex could not be inhibited by circulating antiplasmins. Thus this enzymatically active plasmin-globulin complex could digest the fibrin core of a thrombus in the presence of circulating antiplasmins and it is suggested that this may be the mechanism responsible for thrombolysis *in vivo* (Dalal, P. M., Shah, P. M., Sharp, A. A., and Allington, M. J., 1969).

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THROMBUS DISSOLUTION

A. A. SHARP (Department of Haematology, Radcliffe Infirmary, Oxford) Thrombi have been assumed by many to be standard units made up of fibrin interspersed with platelets, red cells, and white cells and we tend to assume that all thrombi are equal in the eyes of God. Yet it must be obvious to all that there are thrombi and thrombi and that the nature of the fibrin matrix must vary depending on the site of thrombus formation.

Studies in our laboratory have shown that there are considerable differences between fibrin clots formed in a test tube, those formed artificially in a Chandler tube *in vitro*, and those native thrombi formed *in vivo*. Further, there are considerable differences between a thrombus formed on the wall of a vessel, and a thrombus occluding an artery or vein. There are also differences between fresh and old thrombi, age having a variable influence on morphology. When considering thrombolysis there appear to be thrombi which can lyse spontaneously, those which can be lysed by extraneous factors, and those which can never lyse or be lysed (Dalal, Shah, Sharp, and Allington, 1969). Also when certain thrombi are perfused, the perfusate can be shown to pass easily through the interstices of the fibrin, washing out the red cells and trapped plasma. Therefore, it is reasonable to assume that even occluding thrombi may allow the