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LEECH PROSTAGLANDINS AND THE ENZYME DESTABILASE AS THROMBOLYTIC AGENTS OF THE PREPARATIONS FROM THE MEDICINAL LEECHES.
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The thrombolytic effect of the leeching of thromboflebits has been well known from the ancient time. It is provided by the properties of the salivary gland secretion though leech saliva does not show proteolytic activity and does not activate plasminogen to plasmin. But in leech saliva we have found the enzyme destabilase (isopeptidase-glutaminase) which hydrolyze the cross-linked fibrin. This mechanism can be the basis of a new type of fibrinolysis. We have observed the high affinity of destabilase to fibrin that provides the dissolution of thrombous in circulating citrate blood in experiments in vitro. The high affinity of destabilase to fibrin correlates with its high ability to bound L-lysine, which inhibits isopeptidase and glutaminase activity of destabilase. Using radioimmunoassay for 6-keto-PgF_1 we have found prostaglandins in saliva and other preparations from the medicinal leeches:

3400 pg/ml of leech saliva
322 pg/mg of protein (the whole leeches extract)
351 pg/mg of protein (the head region extract)
63 pg/mg of protein (blood from the intestinal tract)

But the leech prostaglandins in contrast to 6-keto-PgF_1 has strongly inhibited platelets aggregation stimulated by thrombin. The leech prostaglandins stimulate thrombolysis in rats after intravenous injection or oral administration. We have supposed that thrombolytic effect of leech prostaglandins is induced by the release of tissue plasminogen activator from vessel wall.

In the experiments on rats it has been shown that thrombolytic effect of leech saliva and preparations from the medicinal leeches is provided by the summary effect of the leech prostaglandins and the enzyme destabilase. After the extraction of prostaglandins by ethylacetate thrombolytic effect is diminished by 40 per cent.

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THROMBOLYTIC EFFECTS OF A POLYANIONIC COMPOUND (PENTOSAN POLYSULPHATE SP54) IN GERIATRIC PATIENTS.
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In geriatric patients the use of streptokinase or urokinase is often limited by contraindications, particularly hypertension. As it has been demonstrated by different authors, the polyanionic compound pentosan polysulphate SP 54 induced an activation of both intrinsic and extrinsic pathway of fibrinolysis and produced a mild decrease of blood prssure. From thise characteristics pentosan polysulphate SP 54 should be an ideal thrombolytic agent in geriatric patients. To clarify this, an open prospective studie was performed. 24 patients (16 females, 8 males) age 65 - 78 years , suffering from thromboembolic processes (8 deep vein thrombosis (DVT), 16 thrombosis of retina vessels (TRV) were included. Pentosan polysulphate SP 54 (BENE-PHARMA München, F.R.G.) was administred by intravenous infusion 300 mg daily over a period of 10 days. Venography or ophthalmoskopie were repeated at day 11.

Results:

	complete lysis	incomplete l.	no lysis
DVT	4	1	3
TRV	8	5	3

Side effects: Bleeding 0 ; thrombocytopenia 2

It is concluded from these results, that pentosan polysulphate SP 54 is an useful thrombolytic agent in particular in geriatric patients suffering from hypertension.

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PROPERTIES OF THE COMPLEX BETWEEN α_2-MACROGLOBULIN AND THE PROTEINASE BRINASE FROM ASPERGILLUS ORYZAE.
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Brinase, a proteinase from Aspergillus oryzae, has previously been shown to have a significant thrombolytic effect in patients suffering from peripheral arterial disease. Brinase is rapidly bound to α_1-proteinase inhibitor and α_2-macroglobulin (α_2M) in plasma. Since binding of a proteinase to α_2M only results in sterical blocking of the active site and not complete inactivation of the enzyme, it has been suggested that brinase in complex with α_2M may retain ability to digest fibrin. To test this hypothesis, we have characterized the binding of brinase to α_2M and the properties of the complex formed. Analyses by several techniques showed that one molecule of α_2M can maximally bind two molecules of brinase. The bait region and the thioester bonds of α_2M were cleaved in this reaction in a similar manner as in the reaction with trypsin. Moreover, a conformational change highly similar to that caused by trypsin was induced in α_2M by brinase, as shown by changes of fluorescence emission, far-u.v. circular dichroism and u.v. absorption difference spectra. Thus, brinase appears to bind to α_2M in the same manner as other proteinases. The activities of free brinase and two forms of brinase-α_2M complex, produced by reaction of the two components in an equimolar ratio or by saturation of inhibitor with enzyme, were compared by two different assays with high-molecular-weight substrates, i.e. hide powder azure or fibrin. The complex formed with equimolar amounts of brinase and α_2M showed ~15% of the activity of free brinase in both assays, whereas the complex formed at saturation of inhibitor with enzyme showed ~35% of the free brinase activity. Although the brinase-α_2M complex, like other α_2M proteinase complexes, is eliminated rapidly from plasma, the ability of the complex to cleave high-molecular-weight substrates may be an explanation of the thrombolytic effect of brinase seen in patients.