

Thursday, July 16, 1981

Poster Presentations

Fibrinogen - VII

Synthesis, Structure, Polymerization

11:00-12:30 h

Grand Ballroom Lobby Boards 248-256

0750

FIBRINOGEN AND HEMATOCRIT IN VISCOSITY MEASUREMENTS. J. Grotta, R. Bigelow, J. Olson, D. Haynie. Department of Neurology, University of Texas Medical School, Houston, Tex.

We have studied the relative contribution of fibrinogen and hematocrit to viscosity preparatory to therapeutic reduction of viscosity in a rat model of cerebral ischemia.

Assuming Newtonian flow characteristics, we first devised a model of the interaction of these variables. Estimating the relative volume of RBCs, plasma, and visco-active proteins suspended in the plasma, assuming random collision of these components, and assuming that viscosity is dependent only on RBCs and vaso-active proteins, we have derived the following relationship: $\text{viscosity} \propto K_1(\text{Hct}+1)^{-K_2}$. We then performed a linear model analysis of measured viscosity (range 1.5-12.3 cp) vs measured fibrinogen (range 0-270 mg%) and hematocrit (range 0-.45) in rat blood which was composed of either normal whole blood or mixtures of washed RBCs, defibrinogenated plasma, and known quantities of fibrinogen. Viscosity was measured in a Wells-Brookfield cone-plate microviscometer at shear rates ranging from 22.5 sec⁻¹ to -450 sec⁻¹.

Hematocrit had a significant effect on viscosity at all shear rates measured, though this effect was greater at lower shear rates. Fibrinogen had no effect on viscosity at shear rates above 22.5 sec⁻¹.

These relationships are consistent with previously published data demonstrating an effect of normal concentrations of fibrinogen on viscosity only at shear rates below those accurately measured by our viscometer (i.e., less than 11 sec⁻¹). We did not study fibrinogen concentrations over 300 mg%. Furthermore, there did not appear to be an interaction between fibrinogen and Hct, indicating that the absence of fibrinogen at shear rates measured in this study did not affect the properties of RBCs as they pertain to viscosity.

The accuracy of our model enables us to correct for varying Hct from animal to animal as long as measurements are performed in the Newtonian range. Presently we are testing a model of Hct and fibrinogen vs viscosity applicable to shear rates at which blood becomes non-Newtonian.

John Grotta

0751

ACUTE PHASE STIMULATION OF FIBRINOGEN SYNTHESIS. EVIDENCE AGAINST A MAJOR MEDIATOR ROLE FOR GRANULOCYTES.

L.M. Kernoff, J. Colman and E. Rawlings. Department of Haematology, Groote Schuur Hospital and the MRC Liver Research Group, University of Cape Town Medical School, Cape Town, South Africa.

This study evaluates the neutrophil granulocyte as a possible mediator of the acute phase protein response. 300g male rats received 1 ml mineral turpentine IM to create a reproducible inflammatory lesion. Five, 16 and 40 hr later i) "in vivo" plasma fibrinogen, transferrin and albumin concentrations were determined, and ii) livers were removed perfused with rat blood and the synthesis rates (SR) of these proteins measured directly by the ¹⁴C carbonate method of McFarlane and Reeve. The results in Table I demonstrate i) that both fibrinogen and transferrin are positive reactants but that the latter's response is by comparison slower, ii) that the "in vivo" fall in albumin concentration results from increased catabolism/redistribution since the SR remains normal. In further experiments rats were rendered neutropenic (0.3mg/kg N. mustard) prior to turpentine injection and the 40 hr measurements repeated. Neutropenia, 0.26 x 10⁹/l (cont. = 2.17) failed to prevent the previously observed changes in protein concentration and SR (Table II). Rats receiving N. mustard only (neutropenic controls) did not differ from normal.

	Fibrinogen		Transferrin		Albumin	
	conc. (g/l)	SR (mg/hr)	conc	SR	conc	SR
5 hr	4.3*	0.77*	4.3	0.22	20.6*	-
16	7.3*	2.10*	4.8	1.93*	17.4*	2.92
40	9.4*	2.31*	5.5*	2.84*	12.5*	3.52
Control	3.2	0.16	4.2	0.13	26.6	3.80

* = significantly different from control (p<.01 or <.001)

	N. Mustard/Turpentine		N. Mustard
	conc.	SR	conc.
Fibrinogen	10.2g/l	2.25 mg/l	3.7
Transferrin	5.6	4.12	3.5
Albumin	13.6	2.59	25.5

0752

ISOLATION, CHARACTERIZATION, AND STEROID HORMONE REGULATION OF SYNTHESIS OF FIBRINOGEN FROM *XENOPUS* LAEVIS. L.J. Wanhg, L.J. Holland, and J.W. Weisel. Department of Biology, Brandeis University, Waltham, MA 02254.

Fibrinogen was prepared from *Xenopus* plasma by a modified mammalian procedure. The purified protein was 95% clottable upon addition of bovine thrombin and SDS-PAGE showed four polypeptide bands which comigrated with polypeptides found in unfractionated plasma. The four polypeptides have molecular weights of 60K, 55K, 52K and 50K Daltons. Upon thrombin cleavage the 60K polypeptide yields a 53K product while the 55K and 52K polypeptides yield a product of 51K. Comparison of the cleavage products of human, bovine, and frog fibrinogens with the chain specific proteolytic venoms, copperhead Venzyme and botroxobin, demonstrates that the 60K polypeptide of *Xenopus* fibrinogen is the B β subunit while the 55K and 52K polypeptides are both A α chains. The 50K polypeptide is the γ chain. In contrast to other species, the frog B β and β chains are larger than the A α and α chain. Primary cultures of purified *Xenopus* liver parenchymal cells maintained for several weeks in a defined culture medium secrete fibrinogen. ³⁵S-methionine labeling of the secreted proteins showed radioactive polypeptides which comigrate with the 60K, 55K and 50K polypeptides of fibrinogen. The liver-synthesized polypeptides are selectively precipitated by a rabbit antiserum prepared against frog fibrinogen. The radioactive A α and B β chains are cleaved by thrombin. We conclude that the A α polypeptide is secreted as a 55K product but undergoes modification in circulation to give the 52K polypeptide. Synthesis and secretion of fibrinogen as well as several other proteins in liver cultures is dependent upon the continuous presence of a glucocorticoid. Wheat germ *in vitro* translation products of mRNA from livers of dexamethasone-treated frogs include three polypeptides of molecular weights similar to those of liver secreted fibrinogen. Thrombin selectively cleaved the two larger translation products identifying them as the frog B β and A α chains. Thus, the polypeptides of frog fibrinogens are translated from separate mRNAs.