Rheological and biochemical analyses on blood coagulation
~ Discovery of a new pathway under stagnant flow conditions ~

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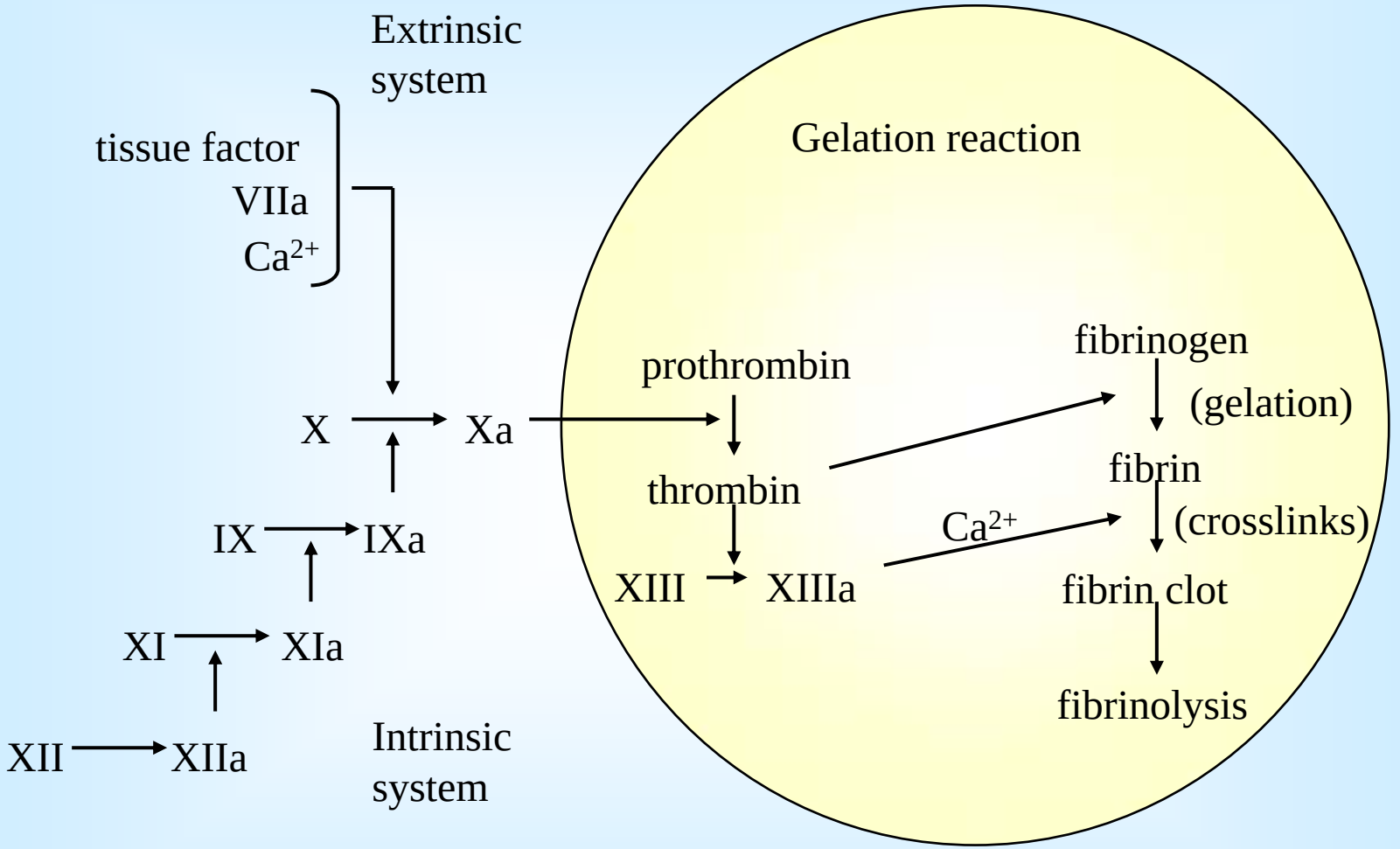
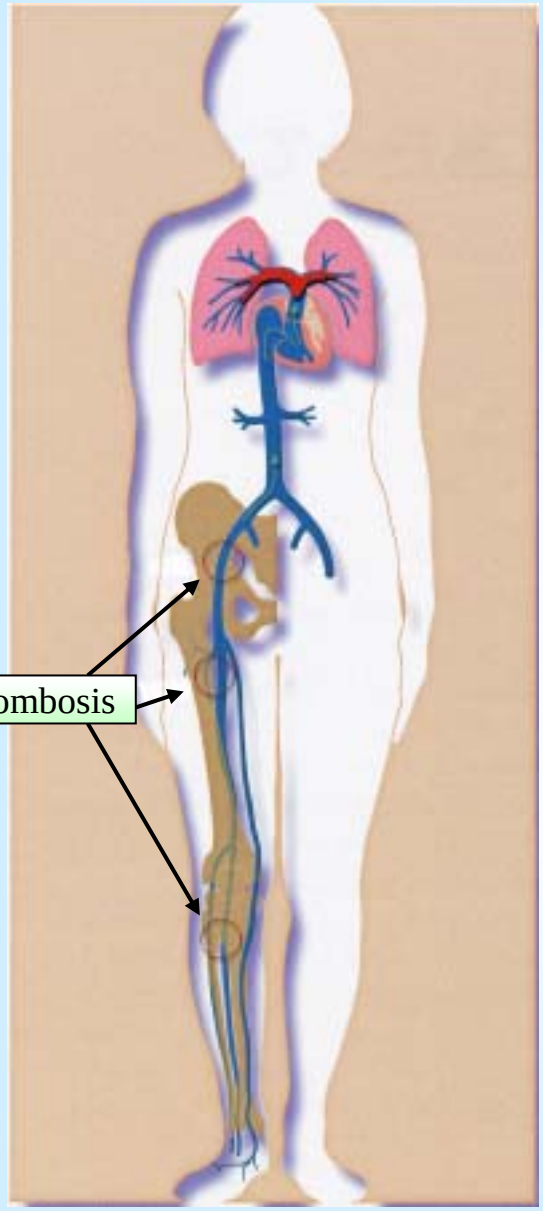
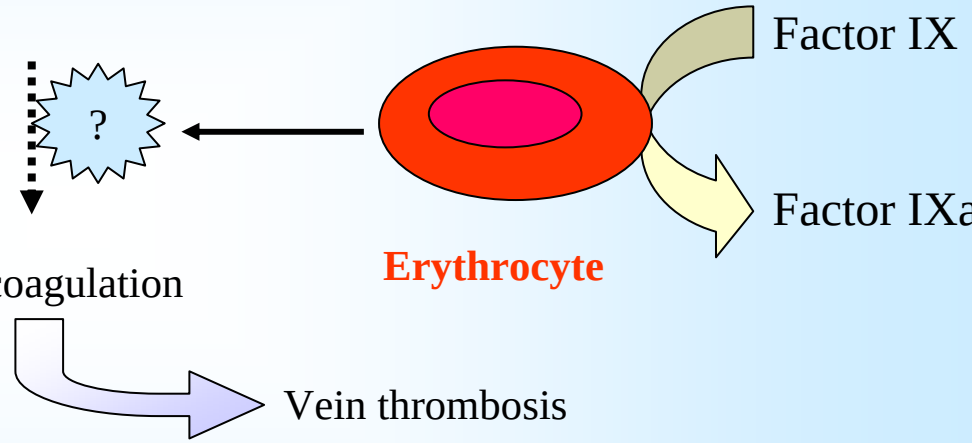

Extrinsic system
tissue factor
VIIa
Ca2+
X
Xa
IX
IXa
XI
XIa
XII
XIIa
Intrinsic system
Gelation reaction
prothrombin
thrombin
XIII
XIIIa
Ca2+
fibrinogen
(gelation)
fibrin
(crosslinks)
fibrin clot
fibrinolysis



Stasis
Erythrocyte aggregation
High hematocrit



Activation of coagulation factor IX
on erythrocyte membrane

Time of onset of coagulation of blood sample

Blood sample	Cell number (cells/ μl)	Coagulation time (min)
Whole blood		31.2±5.5
PFP	Platelets < 100	not coagulated
PRP	Platelets < 1-40 x 10^4 (Leukocytes 1-6.4 x 10^3)	54.3±14.3
PFP + Granulocytes	Granulocytes 0.2 - 3.5 x 10^3	58.3±6.3
PFP + Erythrocytes	Erythrocytes < 5 x 10^5	not coagulated
	2 x 10^6	64.6±28.8
	> 4 x 10^6	30.0±2.9

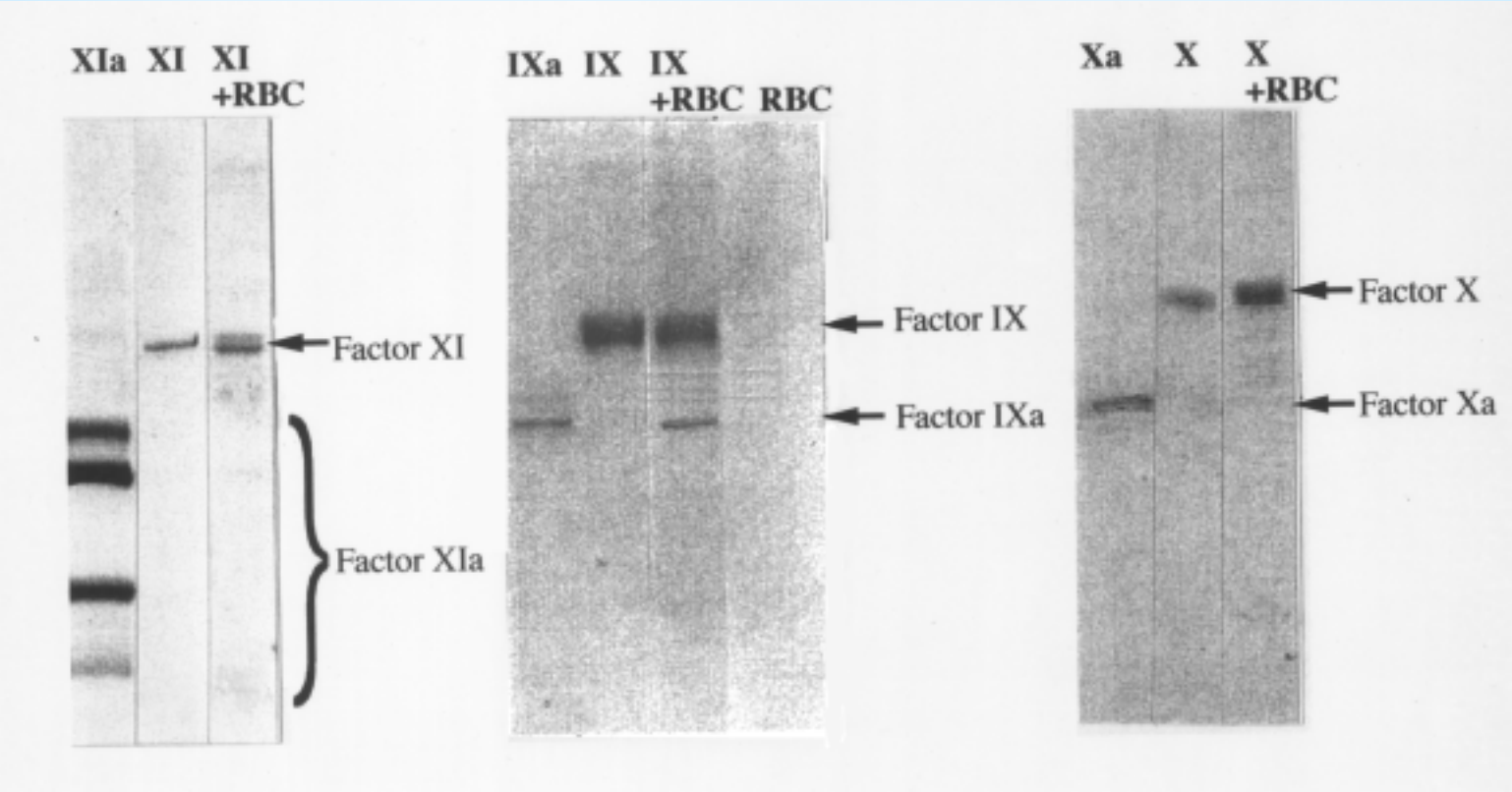
S.Kawakami, et al.: Biorheology, 32(5): 521-36, 1995

Coagulation of coagulation factor-deficient PFP supplemented with erythrocytes

PFP sample	Coagulation time (min)
PFP (normal)	29.9±2.4
Factor VII-, XI or XII-deficient	28.1±3.8
Factor IX-, VIII- or X-deficient	not coagulated

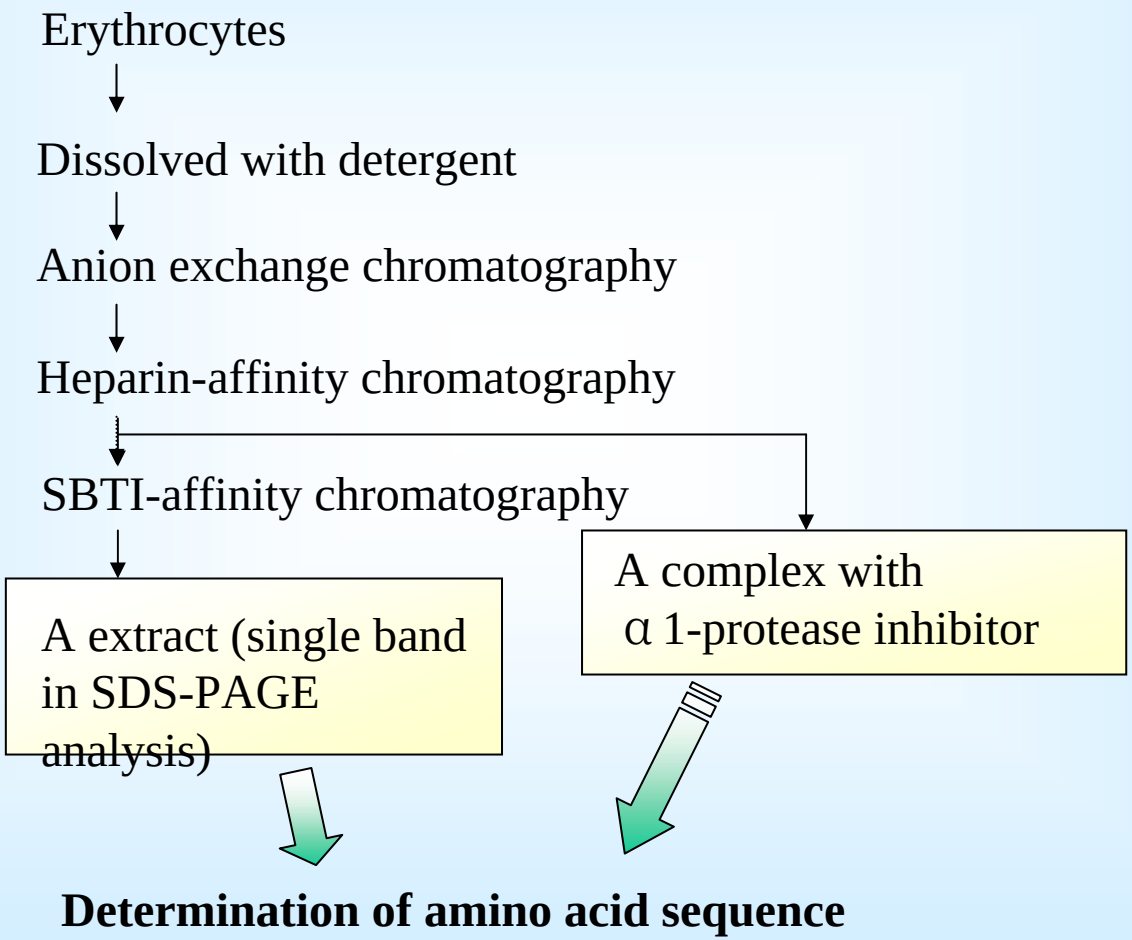
S.Kawakami, et al.: Biorheology, 32(5): 521-36, 1995

Factor XI Factor IX Factor X

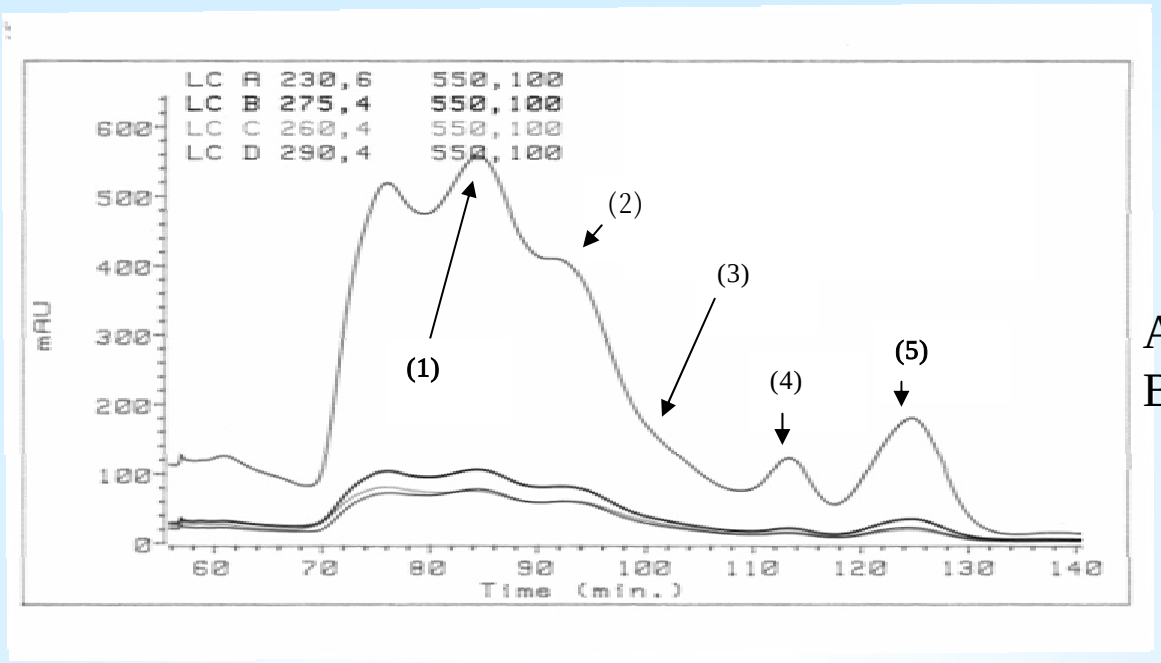


SDS-PAGE analysis of activation of coagulation factors by erythrocyte membrane

Purification Procedure



Affinity chromatography on heparin sepharose column

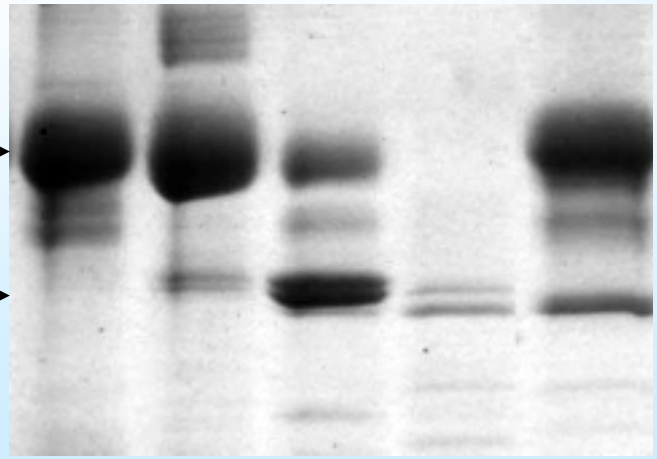


A: 50mM TrisHCl
B: A + 0.8M NaCl

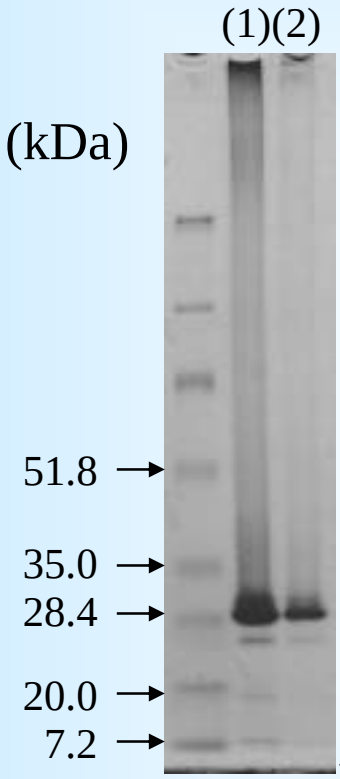
(1) (2) (3) (4) (5)

Factor IX →

Factor IXa →

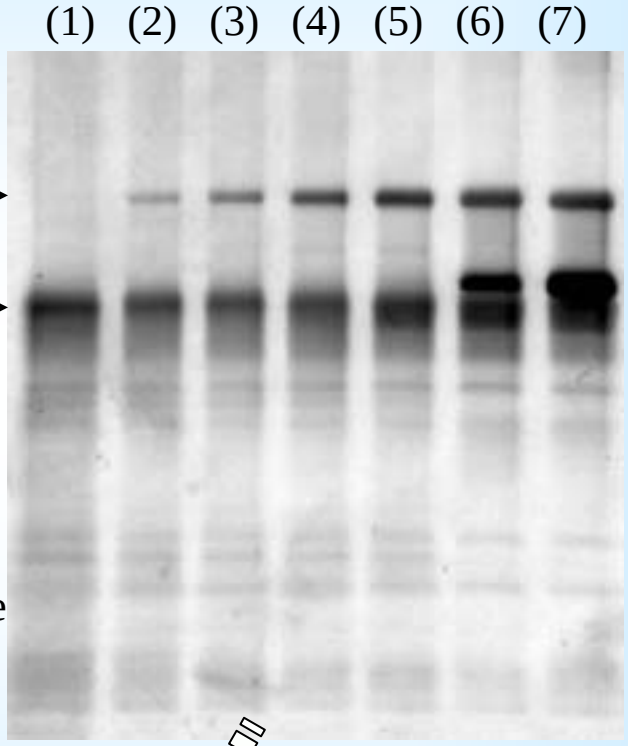


Purified by SBTI-affinity chromatography



(1) Neutrophil elastase
(2) Purified enzyme

Complex formation with α 1-protease inhibitor



Analysis of N-terminal sequence

N-Terminal amino acid sequence of factor IX-activating enzyme on erythrocyte membrane

Amino acid sequence of human leukocyte elastase

```
  1 ivggrrarph awpfmvslql rgghfcgatl iapnfvmsaa hcvanvnvra vrvvlgahnl
 61 srreptrqvf avqrifengy dpvnllndiv ilqlngsati nanvqvaqlp aqgrrlgngv
121 qclamgwgll grnrgiasvl qelnvtvvts lcrrsnvctl vrgrqagvcf gdsgsplvcn
181 glihgiasfv rggcasglyp dafapvaqfv nwidsiix
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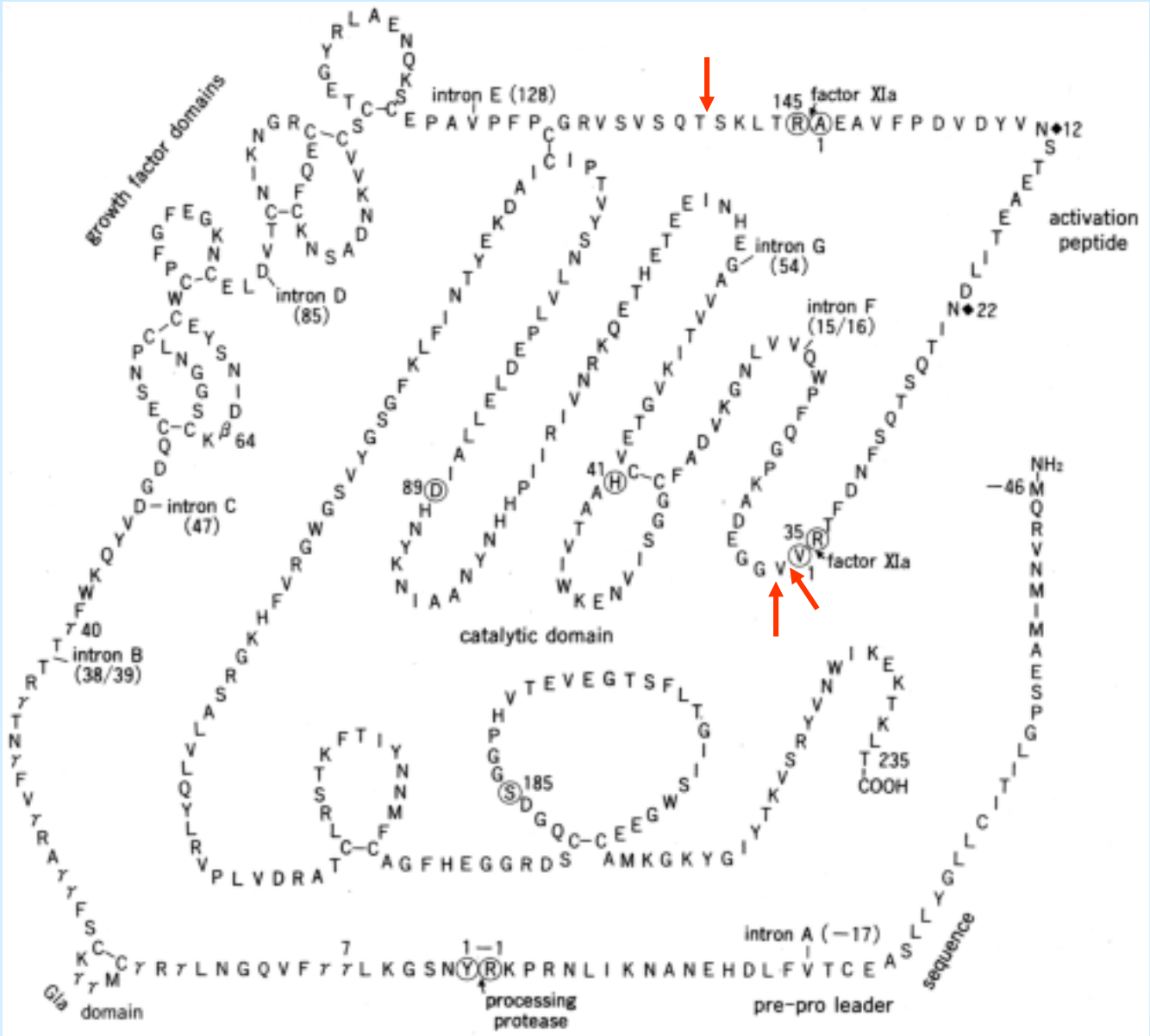
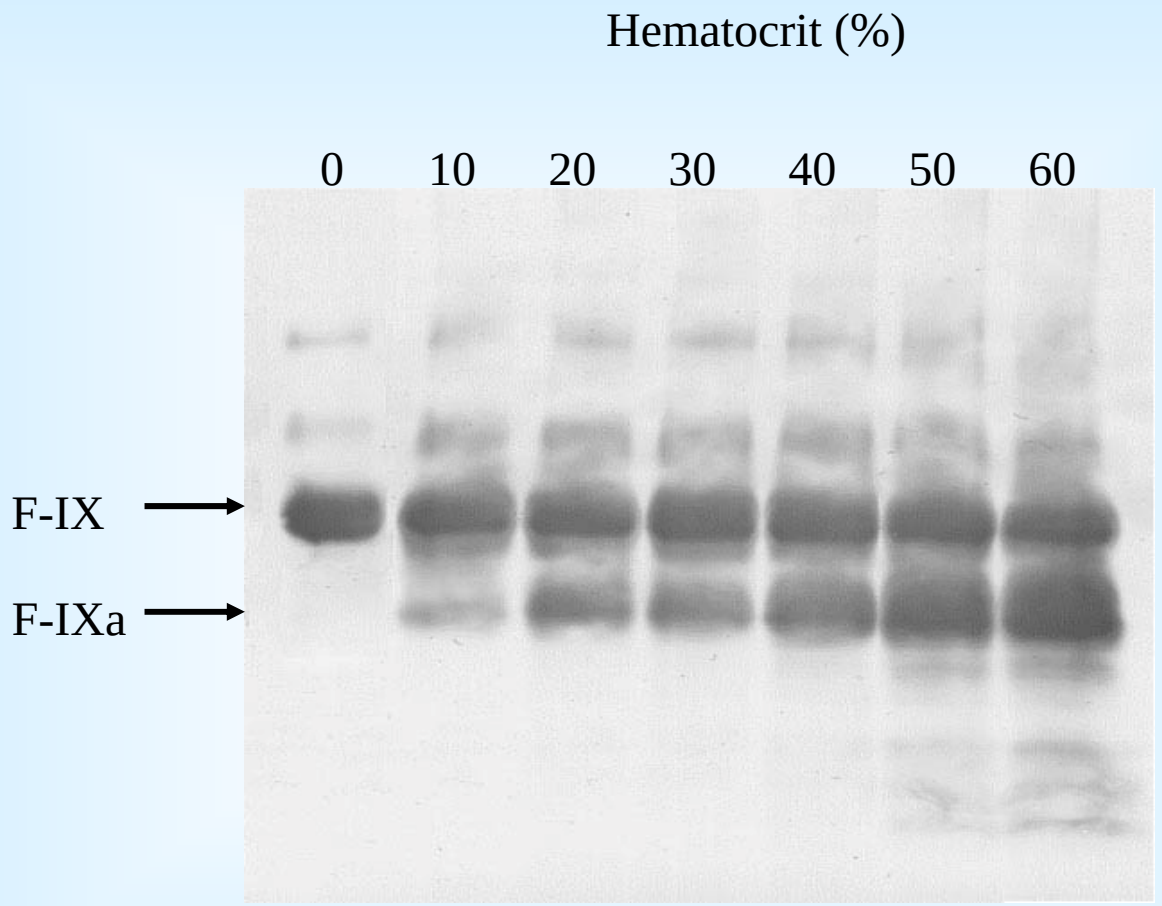
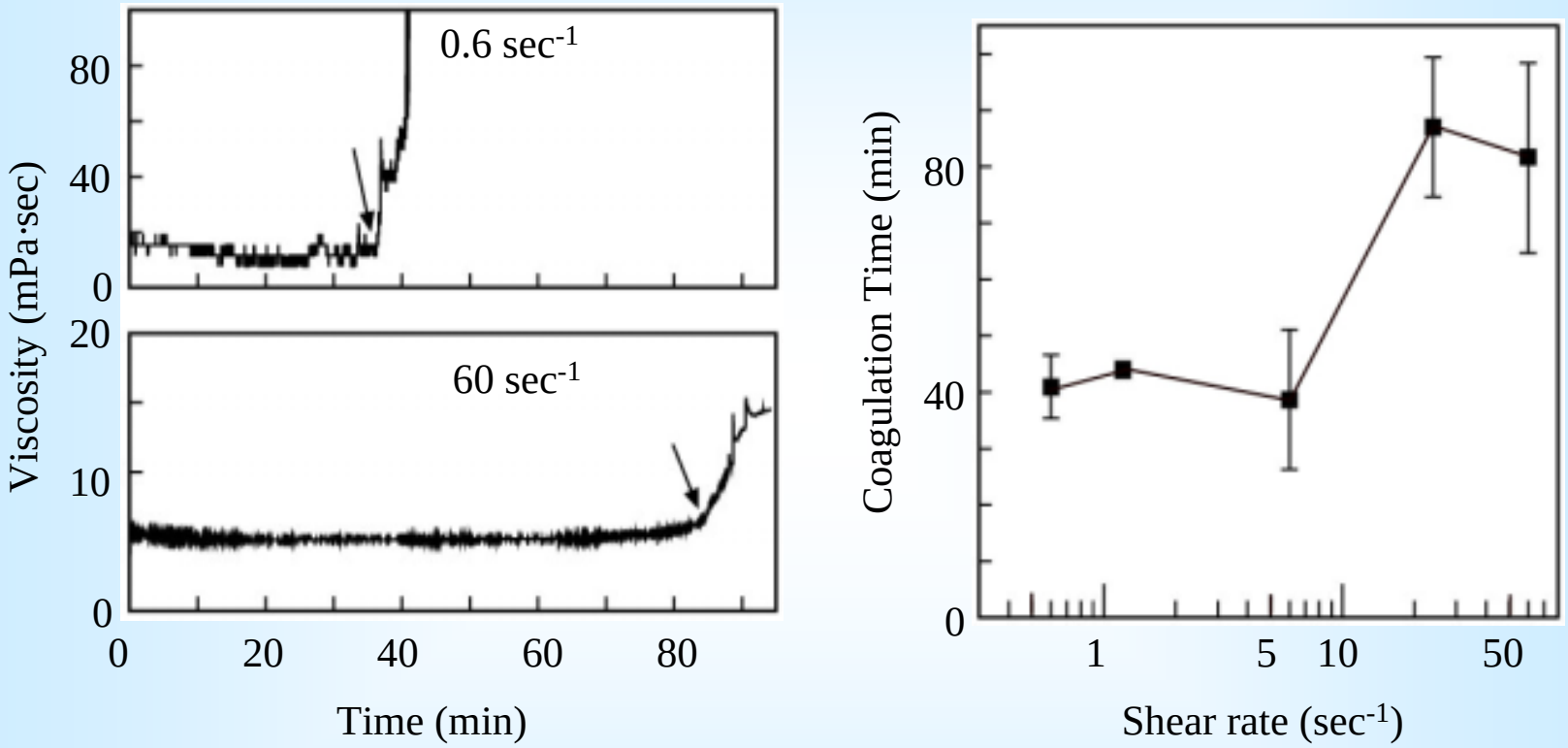
Underlined: the observed amino acid sequence



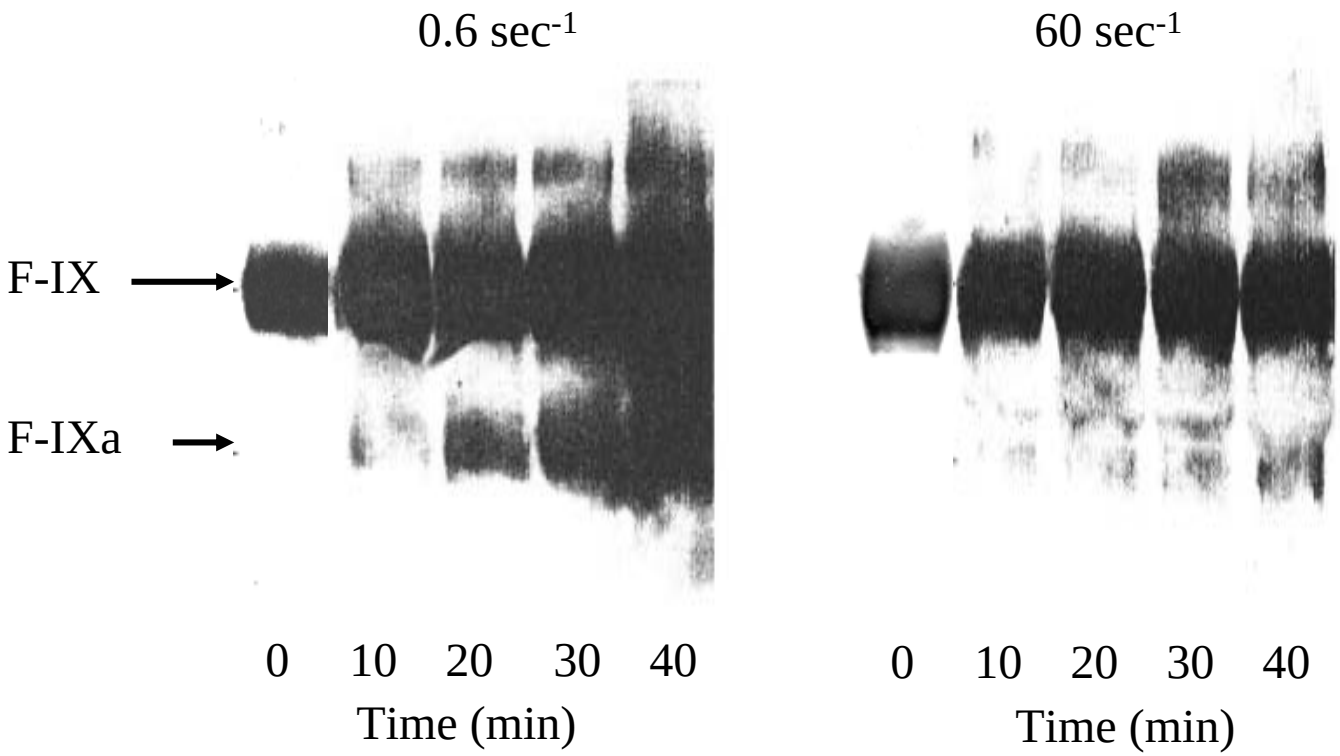
Sites of Factor IX cleaved by the extract



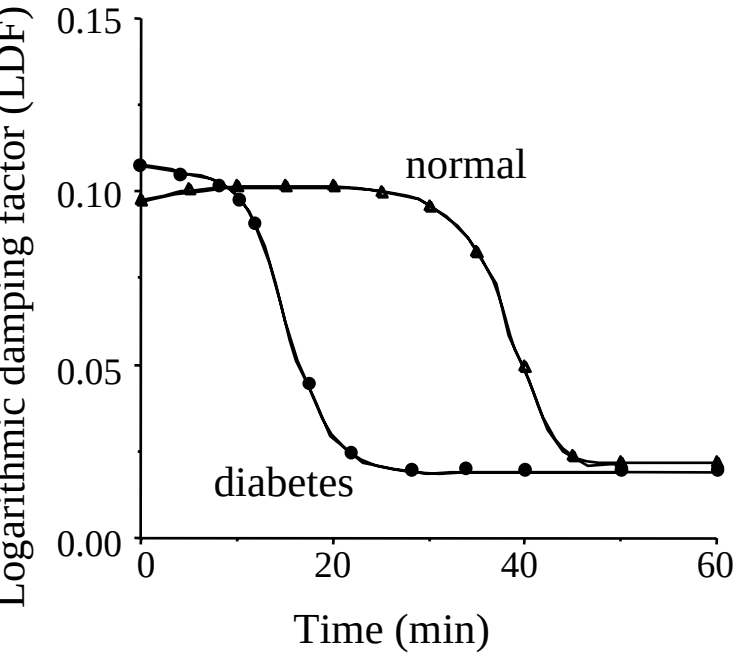
Effect of hematocrit on activation of factor IX

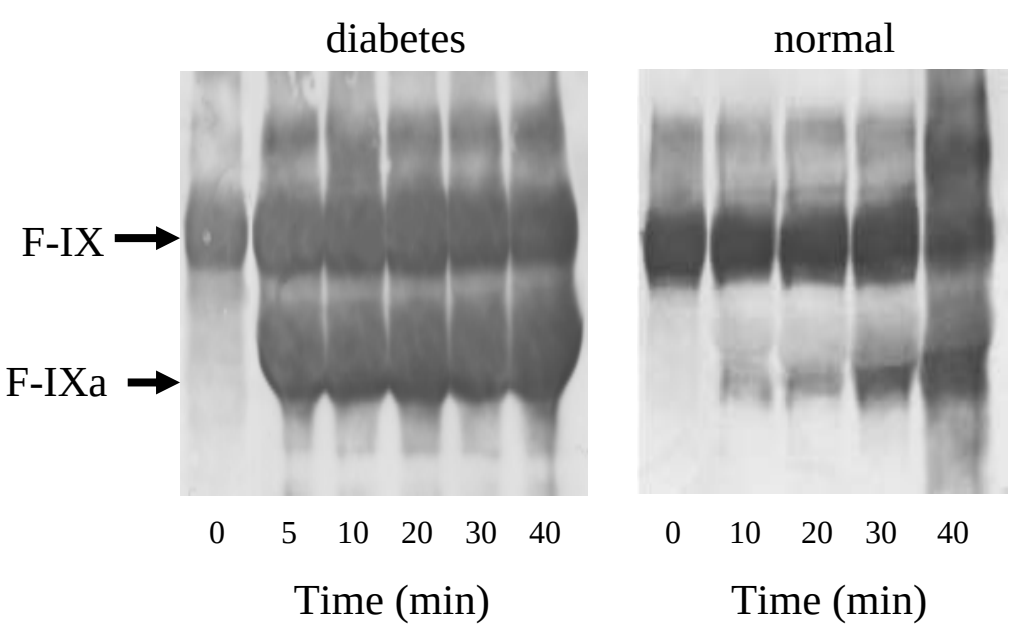


Effect of flow shear rate on coagulation time



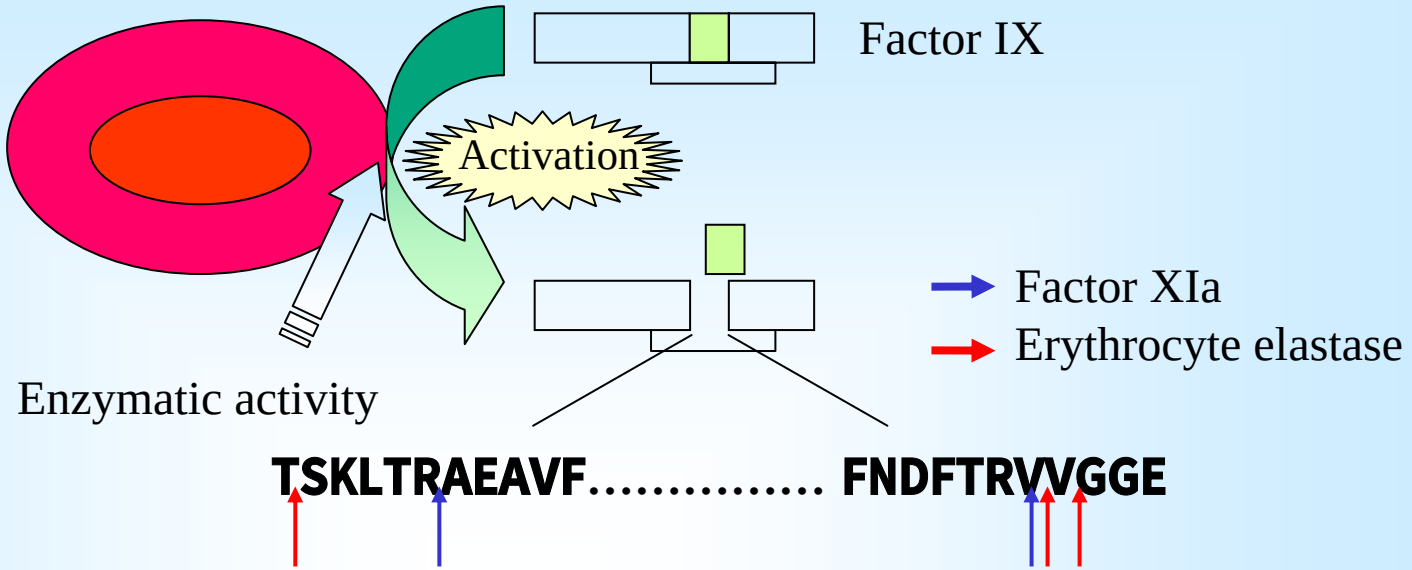
Effect of flow shear rate on activation of factor IX



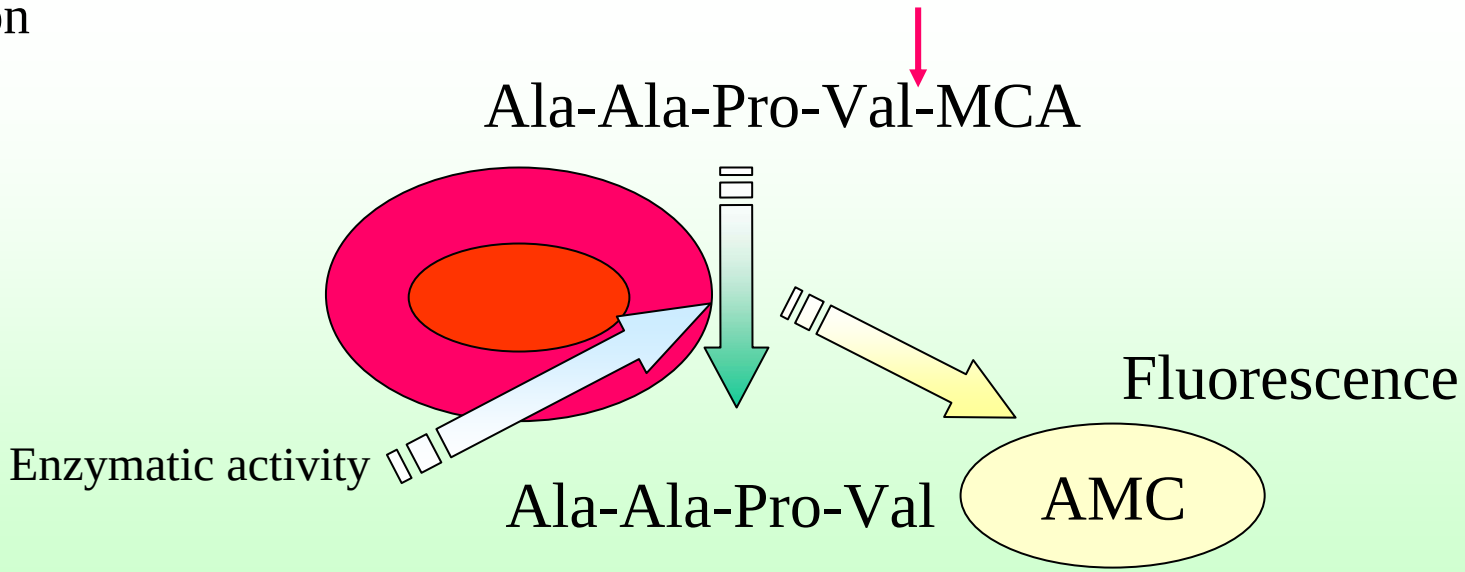


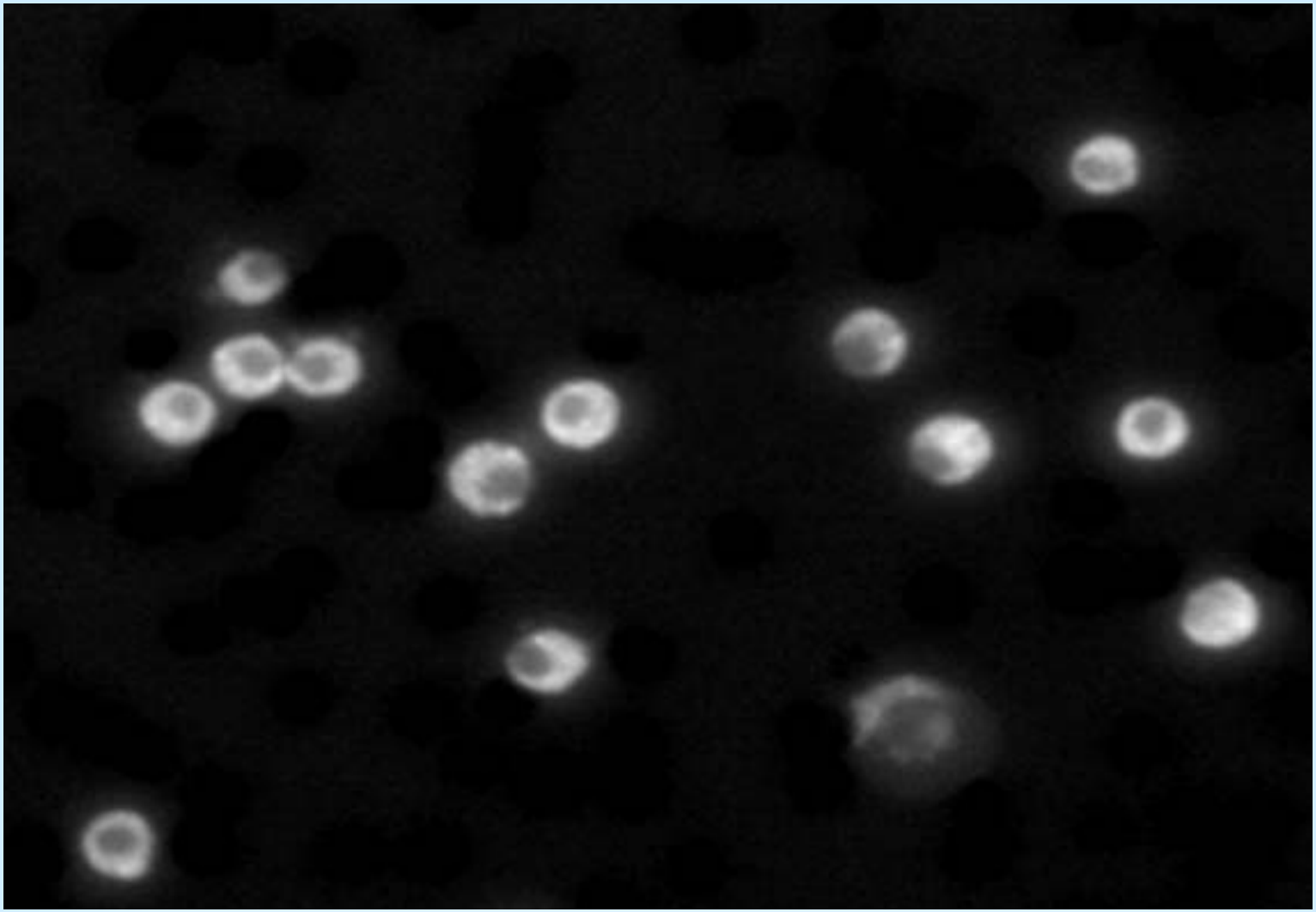
Coagulation time and enzymatic activity of erythrocyte from different donors

oagulation reaction

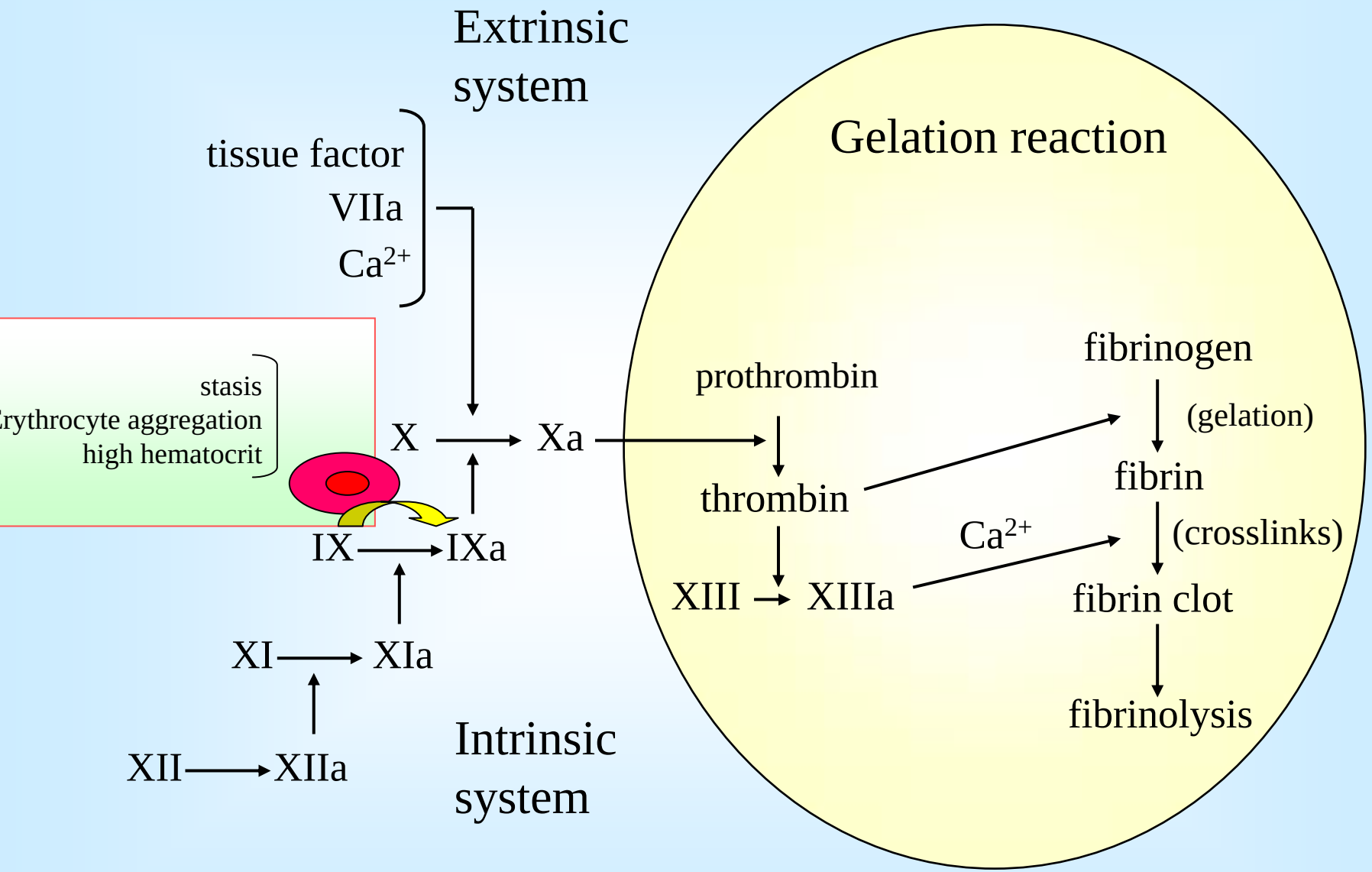


hemical reaction





Observation of erythrocytes labeled with elastase-specific fluorogenic substrate


Extrinsic system
tissue factor
VIIa
Ca2+
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Erythrocyte aggregation
high hematocrit
X
Xa
IX
IXa
XI
XIa
XII
XIIa
Intrinsic system
Gelation reaction
prothrombin
thrombin
XIII
XIIIa
Ca2+
fibrinogen
(gelation)
fibrin
(crosslinks)
fibrin clot
fibrinolysis

Conclusion

1. Factor IX is activated by enzyme on erythrocyte membrane. The enzymes are homologous to leukocyte elastase, The enzyme activates Factor IX by cleaving several bonds which sites are close to the bonds cleaved by Factor XIa.

2. Identification of Factor IX activating enzyme on erythrocyte membrane could provide a frame work for defining roles in cardiovascular coagulation and for refining strategies in pharmaceutical development.