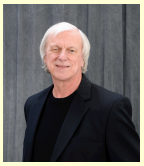
Cell-based Coagulation

Coagulation System Overview And Cell-Based Coagulation



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Your Interactive Hemostasis Resource
george@fritsmafactor.com

AGNESIAN HEALTHCARE

1

Coagulation Summary

- Platelets
- Plasma-based cascade
- Coagulation controls
- Fibrinolysis
- Cell-based coagulation
- Virtues and limitations

3/22/2015 Cell-based Coagulation 2

3 YO Boy: Chronic Joint Pain

A 3 year-old boy experienced painful joints. His hematologist ordered a prothrombin time (PT), activated partial thromboplastin time (PTT), and platelet count.

Results:

	Result	RI
Platelet count	324,000/uL	150–400,000/uL
PT	12.9 sec	12.6–14.6 sec
PTT	67 sec	25–35 sec

3

What are the Possibilities?

- Liver disease, vitamin K deficiency, renal disease?
  - No symptoms, normal diet, PT normal
  - Liver enzymes normal
- Lupus anticoagulant?
  - Unlikely in child
- Inherited single factor deficiency

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PT and PTT Test Results in Inherited Coagulopathies

PT	PTT	Congenital Single Factor Deficiency (Hemophilia)
Long	Normal	VII
Long	Long	X, V, II, and fibrinogen[1]
Normal	Long	VIII, IX, XI
		Contact factors: XII, prekallikrein, high MW kininogen[2]

1. PT and PTT are prolonged only when fibrinogen is < 100 mg/dL
2. Contact factor deficiencies affect PTT results, but do not cause bleeding

A 3 Year-old Boy: Easy Bruising Single Factor Assay Results

Factor	Result
VIII	12%
IX	95%
XI	108%
RI	50–150%

- Implications of factor VIII deficiency: hemophilia A
- The mild form accounts for late onset and mild symptoms
- Therapy: RICE, DDAVP, tranexamic acid, FVIII concentrate

6

Cell-based Coagulation

Plasma-Based Coagulation Model 1964–2004

- Davie EW, Ratnoff OD. Waterfall sequence for intrinsic blood clotting. Science 1964; 145: 1310–12.
- Macfarlane RG. An enzyme cascade in the blood clotting mechanism and its function as a biological amplifier. Nature 1964; 202: 498–9.
- Nemerson Y. The tissue factor pathway of blood coagulation. Semin Hematol 1992;29:170–6.

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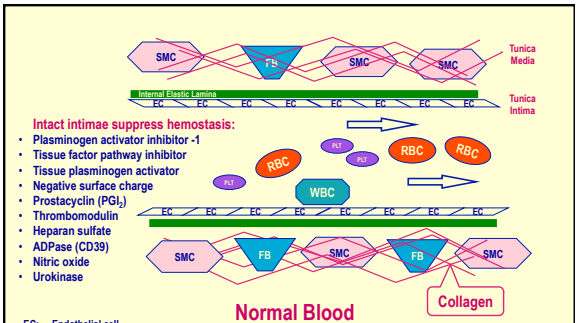

Fritsma GA. Platelet production, structure, and function. In Keohane EM, Smith LJ, Walenga JM. Rodak's Hematology Clinical Principles and Applications. 2015, Elsevier

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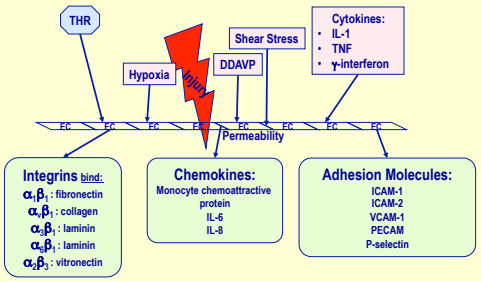
Injured Endothelial Cells Become Hemostatic



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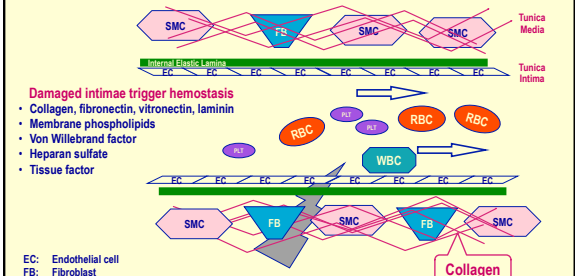

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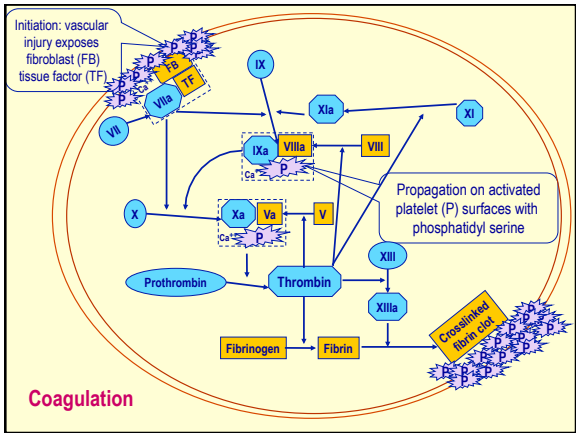
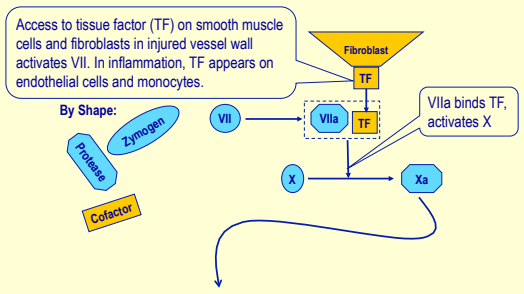


Tissue Factor "*Extrinsic*" Pathway



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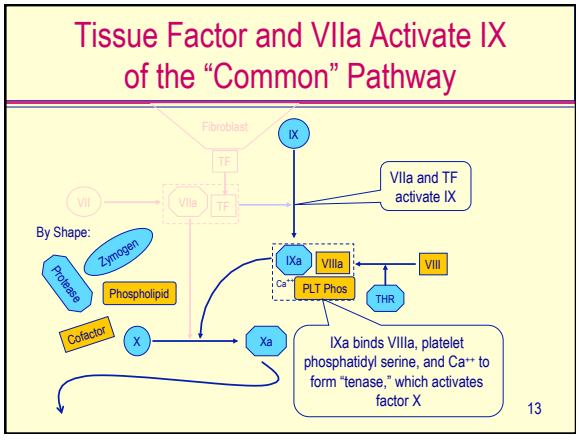
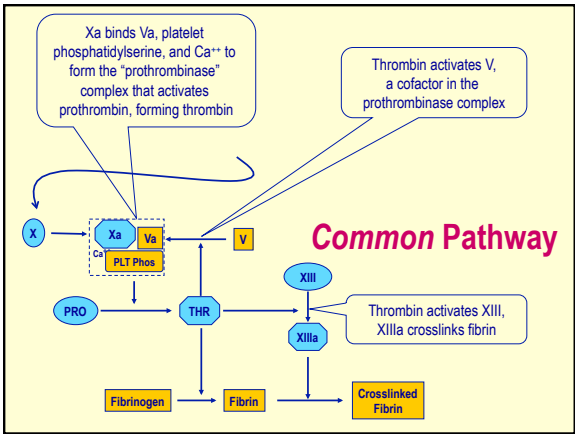
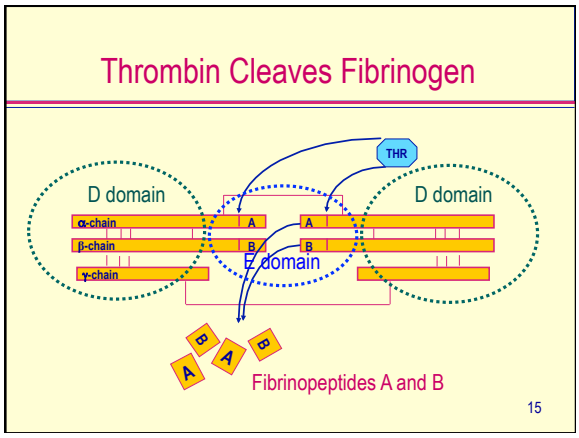
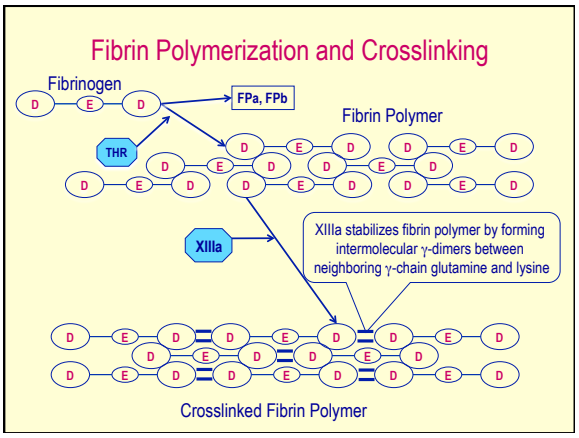
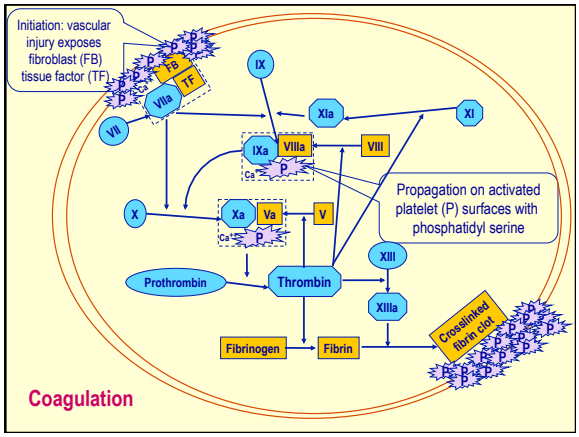
Cell-based Coagulation

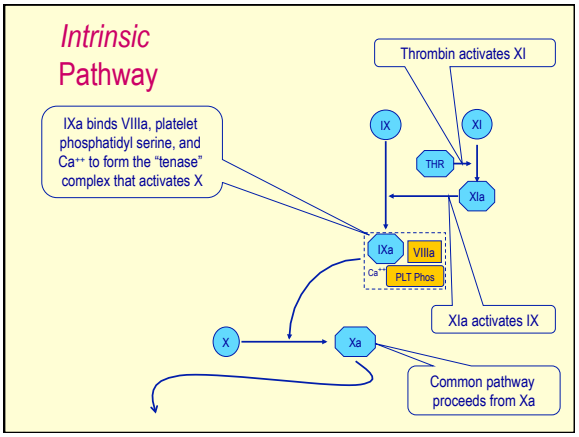






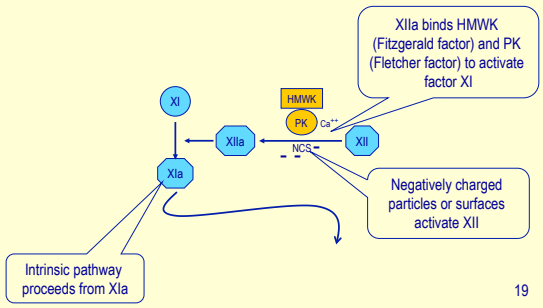






Cell-based Coagulation

In Vitro Contact Activation



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Vitamin K

- Necessary for normal activity of prothrombin (II) and factors VII, IX, and X
- Also necessary for normal activity of control proteins C, S, and Z
- Mediates γ-carboxylation of glutamic acid
- Necessary for Ca^{++} fixation to phospholipid
- Affected by oral anticoagulants

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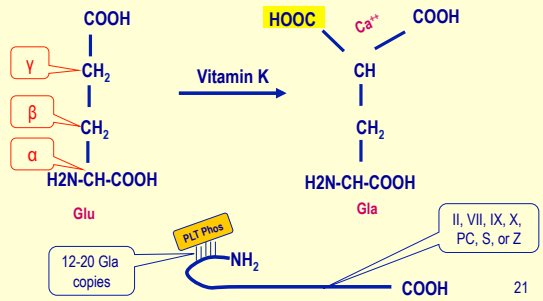
γ-carboxylation of Glutamic Acid



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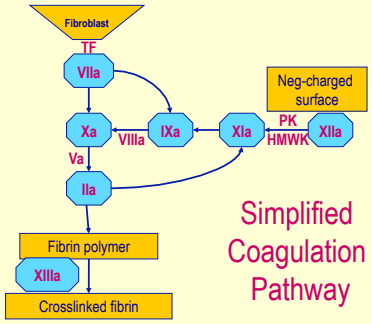
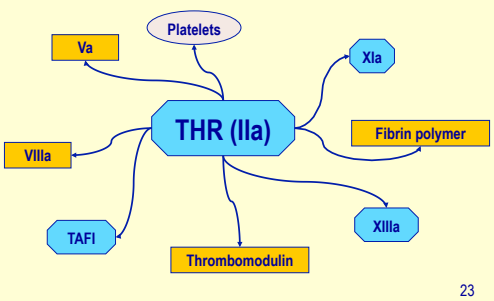
Simplified Coagulation Pathway



Thrombin Properties



Mann KG. Thrombin formation. Chest 2003; 124:4S-10S

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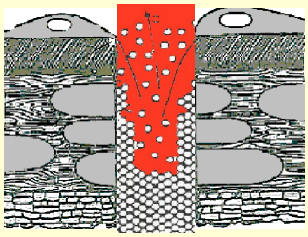
Procoagulant Concentrations and Their Plasma Half-lives

Factor	Category	Half-life	Plasma Level	Hemostatic Level
Fibrinogen (I)	Substrate	4 days	280 mg/dL	50 mg/dL
Prothrombin (II)	Protease	60 hours	1300 μg/mL	20%
V	Cofactor	16 hours	680 μg/mL	25%
VII	Protease	6 hours	120 μg/mL	20%
VIII	Cofactor	12 hours	0.24 μg/mL	30%
IX	Protease	24 hours	5 μg/mL	30%
X	Protease	30 hours	10 μg/mL	25%
XI	Protease	2-3 days	6 μg/mL	25%
XIII	Transglutaminase	7-10 days	290 μg/mL	2-3%
VWF	Cofactor	30 hours	6 μg/mL	50%

Cell-based Coagulation

The Platelet Clot or "White" Clot

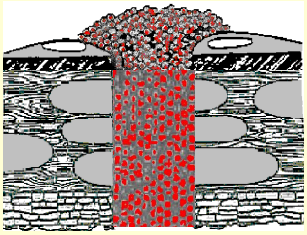
- Composed of platelets and von Willebrand factor
- The endpoint of "primary" hemostasis
- Complete hemostasis in invertebrates and lower vertebrates



Courtesy of Kathy Jacobs, Chronolog, Inc

The Fibrin Clot or "Red" Clot

- Composed of platelets, fibrin, and RBCs
- The endpoint of "secondary" hemostasis
- Complete hemostasis in higher vertebrates



Courtesy of Kathy Jacobs, Chronolog, Inc

Platelet Adhesion Properties

- Platelets bind vessel wall via VWF and fibrin
  - Platelet receptors GP Ia/IIa, IV, and VI bind intimal collagen
  - Platelet receptors GP Ib/V/IX and GP IIb/IIIa adhere to VWF and fibrin
  - GP IIb/IIIa supports platelet aggregation
- Platelets bind other adhesive proteins: thrombospondin, fibronectin
- Fibrin binds platelet interior actin: clot retraction

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A 7 Year-old Girl: Elective Surgery

A healthy 7 year-old girl was scheduled for elective outpatient surgery. The surgeon ordered a screening platelet count, PT, and PTT. She had experienced no bleeding.

Results:

	Result	RI
Platelet count	237,000/uL	150–400,000/uL
PT	13.5 sec	12.6–14.6 sec
PTT (APTT)	47 sec	25–35 sec

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A 7 Year-old Girl: Elective Surgery

Mixing studies were performed to determine the cause for the prolonged PTT. Results:

Test	Result
PTT patient	47 sec
PTT control	29 sec
PTT 1:1 patient/control	32 sec
2h incubated control	34 sec
2h incubated PTT 1:1	36.5 sec
PTT RI	25–35 sec

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What are the Possibilities?

- Lupus anticoagulant inhibitor?
  - No: immediate correction within 10% of control
- Specific inhibitor?
  - No: 2h correction to within 10% of 2h control
- Liver disease, vitamin K deficiency, renal?
  - No symptoms, PT normal, liver enzymes normal
- Inherited single factor deficiency?

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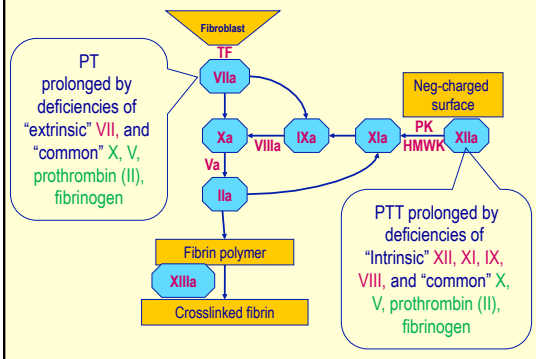
Cell-based Coagulation

PT and PTT Test Results in Inherited Coagulopathies

PT	PTT	Congenital Single Factor Deficiency (Hemophilia)
Long	Normal	VII
Long	Long	X, V, II, and fibrinogen[1]
Normal	Long	VIII, IX, XI
		Contact factors: XII, prekallikrein, high MW kininogen[2]

1. PT and PTT prolonged when fibrinogen is < 100 mg/dL
2. Contact factor deficiencies affect PTT results, but do not cause bleeding

31



A 7 Year-old Girl: Elective Surgery Factor Assay Results

Intrinsic Pathway Factor	Result
Factor XI	123%
Factor XII	37%
PK	97%
HMWK	89%
RI	50–150%

- Bleeding implications of factor XII deficiency: none
- Incidence: 3%

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True or False

1. Coagulation is triggered by exposure to tissue factor.
2. Coagulation is also triggered by the in vivo activation of factor XII.
3. Factor VIII deficiency causes bleeding.
4. Coagulation can occur without platelet phosphatidylserine.

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Antithrombin

- Plasma antithrombin activity is upregulated by endothelial cell heparan sulfate, a long-chain glycosaminoglycan
- Therapeutic heparin upregulates plasma antithrombin 1000-2000×
- Antithrombin is a serine protease inhibitor (SERPIN) that neutralizes thrombin and Xa

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Glycosaminoglycan

- Linear heteropolysaccharide
  - Disaccharide repeating unit
  - Chondroitin sulfate
  - Dermatan sulfate
  - Keratan sulfate
  - Heparan sulfate
  - Hyaluronic acid
  - Heparin

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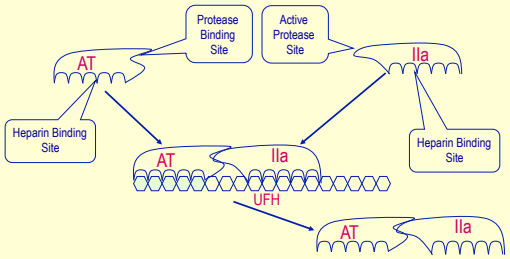
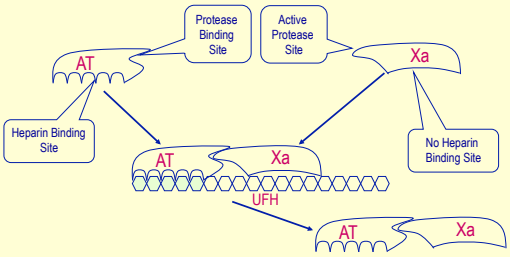
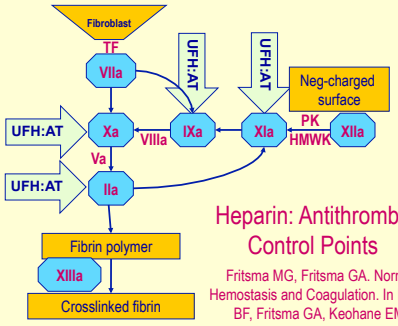
Cell-based Coagulation

Unfractionated Heparin Binds Antithrombin to Thrombin



Unfractionated Heparin Binds Antithrombin to Xa





Heparin: Antithrombin Control Points

Fritsma MG, Fritsma GA. Normal Hemostasis and Coagulation. In Rodak BF, Fritsma GA, Keohane EM. Hematology Clinical Principles and Applications, 4th Edition. 2012, Elsevier

The Protein C Control Pathway

- Protein C activated by thrombomodulin-bound thrombin
- Becomes serine protease specific for Va and VIIIa
- Forms cell-surface complex with protein S, digests Va, VIIIa

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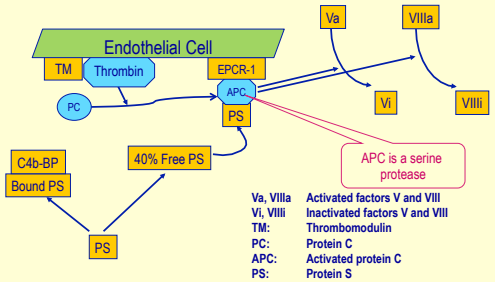
Protein C Control Pathway



Va, VIIIa	Activated factors V and VIII
Vi, VIIIi	Inactivated factors V and VIII
TM:	Thrombomodulin
PC:	Protein C
APC:	Activated protein C
PS:	Protein S
C4b-BP	C4b binding protein
EPCR-1	Endothelial protein C receptor

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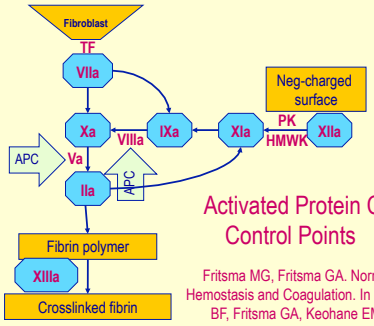
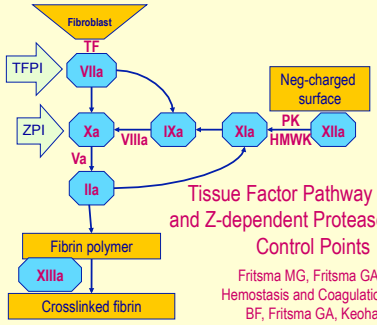


Activated Protein C Control Points

Fritsma MG, Fritsma GA. Normal Hemostasis and Coagulation. In Rodak BF, Fritsma GA, Keohane EM. Hematology Clinical Principles and Applications, 4th Edition. 2012, Elsevier

Cell-based Coagulation



Tissue Factor Pathway Inhibitor and Z-dependent Protease Inhibitor Control Points

Fritsma MG, Fritsma GA. Normal Hemostasis and Coagulation. In Rodak BF, Fritsma GA, Keohane EM. Hematology Clinical Principles and Applications, 4th Edition. 2012, Elsevier

True or False

1. Antithrombin deficiency confers a risk of thrombosis.
2. Protein S excess confers a risk of hemorrhage.
3. Deep vein thrombosis is common in 30-year-olds.
4. Though thrombin is procoagulant, it activates protein C on endothelial cells.

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Virtues of the Plasma Coagulation System Model

- It models coagulation as a series of amplifying proteolytic reactions
  - Each protease cleaves and activates the subsequent substrate zymogen in the series
- It recognizes the participation of platelet anionic phospholipids, mainly phosphatidylserine
  - Inert although essential assembly site
- It models the screening tests PT and PTT as corresponding to the "extrinsic" and "intrinsic" systems

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Limitations of the Plasma Coagulation System

- If there is a separate tissue factor pathway, why doesn't VIIa/TF activate enough X to compensate for a lack of factor VIII or IX in hemophilia?
- If VIII or IX deficiency both cause severe bleeding, why is XI deficiency bleeding mild and variable?
- Why is no fibrin generated when the platelet count is less than 10,000/uL?
- Why does aspirin reduce thrombin formation?

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The Answer

- There is no "extrinsic," "common," or "intrinsic" pathway
- There is no plasma coagulation
- Coagulation occurs only under the control of cells

Hoffman M, Cichon LJH: Practical coagulation for the blood banker. Transfusion 53:1594-1602, 2013.

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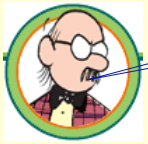
The Cell-Based Coagulation System 2001-Present



Hoffman H, Monroe DM. Coagulation 2006: a modern view of hemostasis. *Hematol Oncol Clin North Am* 2007;21:1-11

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Cell-based Coagulation

Overlapping Coagulation Phases

Dahlbäck B. Blood coagulation. Lancet 2000; 355: 1627-32.

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Initiation: Tissue Factor Bearing Cell

- TF constitutive on fibroblast, smooth muscle cell, or induced on monocytes or endothelial cells
- Exposure of TF cleaves VII → VIIa and binds TF/VIIa
- TF/VIIa cleaves X → Xa; Xa cleaves and binds V → Va
- Xa/Va cleaves prothrombin (Pro, II) → thrombin (Thr, IIa)
- TF/VIIa also cleaves IX → IXa
  - With no VIII around, IXa cannot function
- Free initiation proteases are bound by TFPI, ZPI or AT
- Occurs away from injury site and outside of vessels

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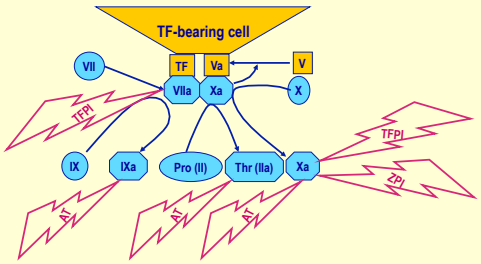
Initiation: TF-bearing Cell



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Amplification: COAT Platelets

- Amplification begins at injury
- Platelets adhere to injury site collagen and VWF
- Platelets contact thrombin-producing tissue factor-bearing cells and become partially activated
- Called "collagen and thrombin-stimulated" (COAT) platelets

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Amplification: COAT Platelet

- Thrombin (IIa) in direct "hand-off" from TF-bearing cells escapes AT and binds nearby COAT platelets
  - Triggers release of VIII/VWF and V from α-granules
  - Cleaves and activates VIII → VIIIa and V → Va
  - Cleaves VIII from VIII/VWF
  - Cleaves and activates XI from plasma and PLT α-granules → XIa
- IXa from TF-bearing cells binds VIIIa on COAT platelets
- Xa from TF-bearing cells binds Va on COAT platelets
- XIa binds COAT platelet membranes
  - Cleaves and activates IX from plasma/platelets → IXa

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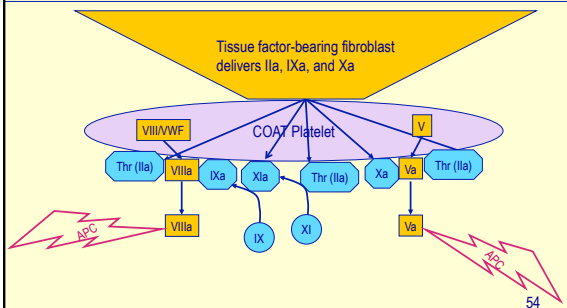
Amplification: COAT Platelet



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Cell-based Coagulation

Propagation: COAT Platelet

- COAT platelet continues to be activated by thrombin, collagen and VWF
- Tenase and prothrombinase complexes now assemble on platelet surface
  - TF/VIIa and Xa/Va are the extrinsic mechanism
  - XIa and IXa/VIIIa are intrinsic
- The pathways now act at the platelet surface to produce high-volume thrombin
- Fibrin polymerization and stabilization

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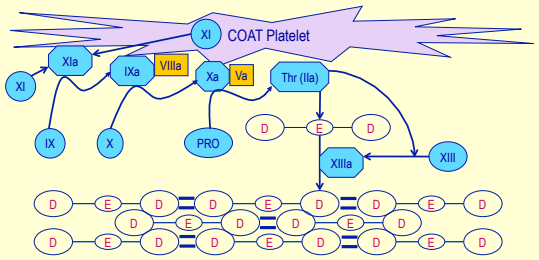
Propagation: COAT Platelet



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Cell Localization

- Tissue factor has to be on a cell that supports prothrombinase activity (Va/Xa)
  - Fibroblasts, smooth muscle cells, also macrophages, monocytes, and endothelial cells when induced
- Malignant cells that make TF but don't support prothrombinase activity are not as effective

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Cell Localization: Platelets

- No TF on platelets, so they have to adjoin TF bearing cells
- No factor VII or VIIa receptor site on platelets
- Thrombin cleaves protease activated receptor (PAR)
- COAT activation moves phosphatidylserine to outer leaflet to support "tenase" and "prothrombinase"
- GP Ib/V/IX binds VWF, COAT platelets adhere to injury sites
- Glycoprotein IIb/IIIa binds fibrinogen and VWF
- Platelet provides surface receptors for VIIIa, Va, IX, X, XI
- Subsequent non-COAT platelet layer damps the reaction

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Coagulation Control: Endothelial Cells

- Thrombomodulin (TM): binds thrombin and activates protein C → APC
- Endothelial protein C receptor (EPCR-1) binds APC to surface
- APC binds protein S, inactivates Va and VIIIa
- Heparan-like glycosaminoglycans activate AT
- Cell-surface ADPase neutralizes platelet ADP

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Virtues of the Cellular Coagulation System

- If there is a separate tissue factor pathway, why can't the activation of factor X by VIIa/TF compensate for a lack of factor VIII or IX in hemophiliacs?
  - Because the activation of X by VIIa/TF occurs on the wrong cell—the fibroblast
  - Free Xa is inhibited by AT and TFPI—it does not diffuse to a platelet
- If VIII or IX deficiency both cause severe bleeding, why is XI deficiency bleeding variable?
  - Free IXa can transfer to the platelet as AT has less effect
  - Free IXa on platelet bypasses need for factor XI

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Cell-based Coagulation

Thrombosis and Cellular Coagulation

- If thrombosis is hemostasis that occurs on endothelial cells, therapy could target the vulnerable endothelial cells
- Aspirin effect on platelets also slows coagulation
- Thrombocytopenia also slows coagulation

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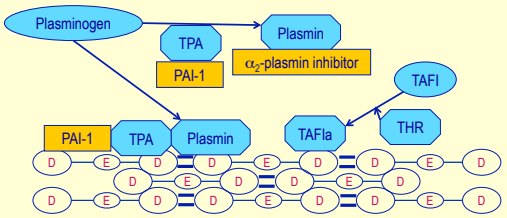
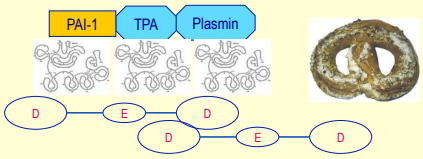
Fibrinolysis: Fibrin Degradation



TPA: Tissue plasminogen activator
PAI-1 Plasminogen activator inhibitor-1
THR Thrombin (factor IIa)
TAFI Thrombin activatable fibrinolysis inhibitor

Kringle Adhesion



TPA: Tissue plasminogen activator
PAI-1 Plasminogen activator inhibitor-1

Fibrinolysis

- PAI-1, TPA, and plasmin bind fibrin polymers through "kringle" structures
- Plasmin cleaves fibrin at selected lysine and arginine residues
- TAFIa cleaves fibrin C-terminal lysines that plasmin requires for their cofactor activity

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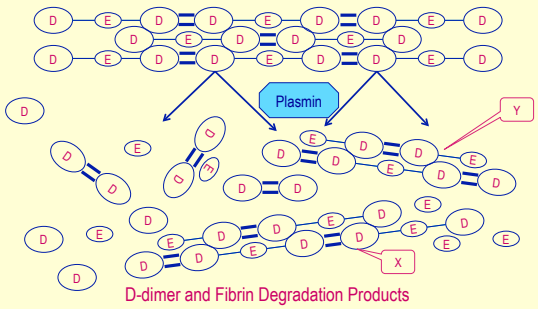
Fibrinolysis: Fibrin Degradation



D-dimer and Fibrin Degradation Products

Wound Healing

- Fibrin attaches to platelet actin, clot retracts
- Neutrophils that first appear are replaced by macrophages
- Macrophages secrete vascular endothelial cell growth factor (VEGF), triggers neovascularization
- Platelets secrete platelet derived growth factor that attracts and stimulates fibroblasts and smooth muscle cells

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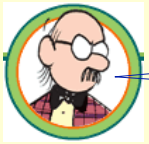
Cell-based Coagulation

Thank You For Listening



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The End

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