Objectives

• Understand how hemostasis relates to the body’s response to tissue injury
• Differentiate the newer cell-mediated model from the classic cascade model
• Describe the basic coagulation tests and how they relate to the clotting cascade

we will talk about the coagulation cascade and compare and contrast this with newer cellular models of how coagulation works in the body.
we will also highlight some defects/ things that can go wrong in hemostasis.
when there is an injury, the body must mount a response to halt immediate damage, deal with an infection, and heal the wound and restore tissue function. The first step in this process is coagulation which not only stops bleeding, but produces mediators such as growth factors and cytokines which help condition and direct the rest of this process...
Primary Hemostasis
Platelets Adhere & Activate at Sites of Injury

Platelets are anucleate fragments of cells that circulate in the blood and are normally disc shaped. In this form, they are not responsive and not sticky.

First step in hemostasis involves platelets.

When there is an injury or inflammation, they change shape and bind to the extracellular matrix and to each other. They can stop bleeding by themselves, and they can express lipids on their surface upon activation that provide a good site for the coagulation reactions to take place.
Secondary Hemostasis

Coagulation proteins act on platelet surfaces to form fibrin, which stabilizes the platelet plug.
How can we make sense of hemostasis?

It's complicated, but we will try to highlight key features that will help us make sense of things that happen in our pts.
In 1904 Paul Morawitz proposed a model of coagulation.
More and more factors were discovered and named different things, and it all went down hill from there.....

- Hemostasis was well studied because of hemophilia in royal families
  - fibrinogen
  - prothrombin
  - accelerator (AC-) globulin
  - Antihemophilic Factor
  - Antihemophilic Factor B
  - Antihemophilic Globulin (AHG)
  - Antihemophilic Globulin A
  - Autoprothrombin I
  - Autoprothrombin II
  - Autoprothrombin III
  - Beta cothromboplastin
  - Christmas Factor
  - Contact Factor
  - Cothromboplastin
  - Facteur Antihemophilique A
  - Fibrin Stabilizing Factor
  - Thromboplastic Plasma Component
  - Thromboplastinogen
  - Hageman Factor
  - Hemophilia A factor
  - Hemophilia B Factor

- Hemophilia C factor
- Labile Factor
- Laki-Lorand Factor
- Pavlovsky Factor
- Plasma Thromboplastic Factor
- Plasma Thromboplastic Factor A
- Plasma Thromboplastin Antecedent (PTA)
- Plasma Thromboplastin Component
- Plasmakinin
- Platelet Cofactor
- Proaccelerin
- Proconvertin
- Prothrombokinase
- Protransglutamidase
- Prower Factor
- Robbins Factor
- Serum Factor
- Serum Prothrombin Conversion Accelerator (SPCA)
- Stable Factor
- Stuart Factor
- Stuart-Prower Factor
- Thrombokatalysin
In 1958 the International Society on Thrombosis and Hemostasis convened a conference to standardize the nomenclature. That’s how we got all those roman numerals. At this point though, we still didn’t know how it worked - this is why the roman numerals are not in the right order.
**Coagulation Proteins**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Synonyms</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Fibrinogen</td>
<td>polymer unit</td>
</tr>
<tr>
<td>II</td>
<td>Prothrombin</td>
<td>protease</td>
</tr>
<tr>
<td>III</td>
<td>Tissue thromboplastin, tissue factor</td>
<td>cofactor</td>
</tr>
<tr>
<td>IV</td>
<td>Calcium</td>
<td>we don't call Calcium factor IV either...</td>
</tr>
<tr>
<td>V</td>
<td>Accelerator globulin, proaccelerin, labile factor</td>
<td>cofactor</td>
</tr>
<tr>
<td>VII</td>
<td>Proconvertin, stable factor</td>
<td>protease</td>
</tr>
<tr>
<td>VIII</td>
<td>Antihemophilic factor or globulin</td>
<td>cofactor</td>
</tr>
</tbody>
</table>

**Factor**

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<thead>
<tr>
<th>Factor</th>
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</thead>
<tbody>
<tr>
<td>IX</td>
<td>Christmas factor, plasma thromboplastin component</td>
<td>protease</td>
</tr>
<tr>
<td>X</td>
<td>Stuart factor, Stuart-Prower factor</td>
<td>protease</td>
</tr>
<tr>
<td>XI</td>
<td>Plasma thromboplastin antecedent</td>
<td>protease</td>
</tr>
<tr>
<td>XII</td>
<td>Hageman factor</td>
<td>protease</td>
</tr>
<tr>
<td>XIII</td>
<td>Fibrin stabilizing factor, fibrinoligase Fibrin crosslinker</td>
<td>cofactor</td>
</tr>
<tr>
<td>------</td>
<td>Prekallikrein (Fletcher factor)</td>
<td>protease</td>
</tr>
<tr>
<td>------</td>
<td>High-molecular-weight kininogen (Fitzgerald factor)</td>
<td>cofactor</td>
</tr>
</tbody>
</table>

**Factor VI** was at one time used to designate activated Factor V.
...... but nobody really knew how all those factors interacted to turn liquid plasma into a solid fibrin clot

That’s why the roman numerals aren’t in order in the coagulation cascade - thus making it is hard for us to remember
In the 1960’s the coagulation factors were organized into a “cascade” or “waterfall” model. This evolved into the current cascade model ...

Eventually the many coagulation factors were organized into a cascade model...
Fig. 1. Tentative mechanisms for the initiation of blood clotting in mammalian plasma in the intrinsic system. Abbreviations: F., factor; Act., activated; P.T.A., plasma thromboplastin antecedent. The term “Act. Proaccelerin” is probably a misnomer but was used in this figure instead of accelerin or prothrombin converting principle. Accelerin refers to a thrombin-modified form of proaccelerin; prothrombin converting principle, a term we have used elsewhere, does not identify the precursor of this enzyme. Hageman factor, Christmas factor, and Stuart factor are clotting factors named after the patients who were among the first observed in which the clotting deficiency was seen. This scheme does not represent all views held on the mechanism of blood coagulation (32).
The “cascade” model evolved into what my generation of medical students was taught ….
Intrinsic Pathway

aPTT

Factor XII/HMK/PK

Factor XI → Factor Xla

Factor IX

Extrinsic Pathway

PT

Factor IXa

Factor VIIIa

Factor XIa

Factor Xa

Factor Va

Prothrombin → Thrombin

Fibrinogen → Fibrin

aPTT = long abbreviation for test = long pathway

deficiency in one of the factors will give you a longer time in the aPTTT or the PT, depending on the factor

T = short abbreviation for test short pathway

factor III = tissue juice

these reactions almost always happen on a phospholipid surface - this is important bc we don't want this rxn to spread throughout the body - we want them to be localized to a surface

surface for this rxn is a cell that has tissue factor on it outside the vessel

most of these factors also require Ca - this is imp't bc if we take out calcium, we can decoagulate blood

on this slide ->
top= protease, bottom= cofactor

Prothrombin

aPTT = activated partial thromboplastin time

PT = prothrombin time

hemophiliacs have a normal PT - they have factor VIII or IX deficiencies
Homologous Coagulation Factors

• Vitamin K-Dependent Serine Proteases:
  - Factors II, VII, IX & X
  - Structurally similar
  - Circulate as inactive zymogens
  - Activated by proteolysis
  - Work best in complex with a protein cofactor on lipid surface containing phosphatidyl serine
  - Activity is calcium dependent

these guys are all very closely related- probably arose by gene duplication
prothrombin
you do not need to be able to recognize structures

Prothrombin

Factor VII

Factor IX

Factor X

Protein C

some are activated by one cleavage, others are activated by two cleavages - these cleavages allow them to rearrange into an active conformation

Why should I care about the biochemistry of coag factors?
Why should I care about the biochemistry of coag factors?

• It helps explain some things that are very useful
  – How does Coumadin (Warfarin) work?
  – How do calcium chelators act as anticoagulants?
Things that are necessary for coagulation proteases to work

- Post-translational modification to produce gamma-carboxy glutamic acid (Gla) residues, which is vitamin K – dependent
- Calcium to bind to Gla’s and hold the protein in the active conformation
- Phospholipid surface for the proteases to bind to along with their cofactors
Vitamin K-dependent factors contain Gla-residues

1. Glutamates in the Gla domain get carboxylated by carboxylase
2. Vitamin K is oxidized in this reaction
3. Warfarin inhibits this reductase, keeping the vitamin K from being reduced back to the usable state.
4. Pts given warfarin take a while to become "anticoagulated"
Warfarin: Commonly Used Oral Anti-Coagulant

- Warfarin alters synthesis of vitamin K-dependent factors by preventing vitamin K-dependent carboxylation of
  - Factors II, VII, IX, X
  - Protein C & Protein S
- **Result:** no longer bind calcium
- New proteins must be synthesized to overcome the warfarin effect
Coag proteins work as protease/cofactor complexes
Vitamin K-dependent proteases (FII, VII, IX and X)

The Coagulation Cascade

- Helps us interpret clinical laboratory tests
  - Prothrombin time (PT)
  - Activated Partial Thromboplastin Time (aPTT)
The Coagulation “Cascade” Doesn’t Explain How Blood Clots in vivo

- Patients lacking FXII, HMK, or PK have a long aPTT but no bleeding.
- Patients lacking FXI have a long aPTT and may or may not have bleeding.
- Patients lacking FVIII or FIX have an equally long aPTT and serious bleeding.
Intrinsic Pathway

aPTT

Factor XII/HMK/PK

Factor XI

Factor XIa

Factor IX

Factor IXa

Factor VIIIa

Factor X

Factor Xa

Factor Va

Prothrombin

Thrombin

Fibrinogen

Fibrin

moral of the story:
aPTT and PT tests tell you if your pt is
deficient in one of these factors (but not
which one), but does not predict the bleeding
tendency of the patient - it happens
differently on cells in the body than it does in
a test tube.

Prolonged aPTT only

really long

Prolonged aPTT only

Variable bleeding

Prolonged aPTT

Severe bleeding

not as long as if you are XII deficient

no bleeding
tendency - so prob
no role in
hemostasis, more
likely to play a role
in inflammation,
probably in lysi ng
clots, and maybe in
thrombosis

only severe
bleeding in some
sort of trauma or
surgery
How does it really work in the body?
Cells are important in the body, but aren’t included in the coagulation cascade or the clinical lab tests.
Hemostasis Occurs on Two Surfaces: TF-bearing Cells and Platelets

1. Initiation
   - tissue factor bearing cell initiates the coagulation process by making a little bit of thrombin, which then activates platelets stuck down at the site of injury

2. Amplification

3. Propagation
   - platelets generate lots of thrombin - the bulk of thrombin responsible for forming the fibrin clot
TF forms a “hemostatic envelope” around the vessel
however, when factors are there, the thrombin can help activate the platelets. This priming amount of thrombin helps set the stage for the platelets to help take over the coagulation process.
Activated Platelet
Fibrin Clot Formation

Fibrinogen → IIa → fibrin

then that thrombin converts fibrinogen to fibrin
fibrin forms polymers that are cross linked by factor XIII
Factor XIII

- Activated by thrombin during coagulation
- Has transglutaminase activity
- Covalently crosslinks fibrin strands to stabilize the clot
TF-Bearing Cell

A Cell-Based Model of Hemostasis

Problem area in hemophilia. You get initiation, but the platelet thrombin generation "fizzles".

PT: measures extrinsic/initiation pathway

EXTRINSIC PATHWAY

VIIa
TF
X
Xa
Va
lipid
II
fibrinogen
fibrin

Tissue Factor Bearing Cell

TF

VIIa
Xa
Va
Xa
II
IIa

IX
IXa

*short name (PT) = short pathway

aPTT: measures intrinsic/platelet pathway*

*long name (aPTT) = long pathway

The intrinsic and extrinsic pathways are not redundant, but have distinct roles in hemostasis *in vivo*.
Fibrinolysis

TISSUE PLASMINOGEN ACTIVATOR (tPA)
activates plasminogen to plasmin and is released by endothelial cells. certain vascular beds produce a lot of tPA if they tend to get a lot of clots - ex: lungs

STREPTOKINASE
UROKINASE
FACTOR XIIa
KALLIKREIN

PLASMINOGEN
contact factors that don't play act in hemostasis but play a role in converting plasminogen to plasmin

FIBRIN CLOT

PLASMIN

FIBRIN CLUT

FIBRINOLYSIS
when the wound heals and we have to dissolve the fibrin clot = fibrinolysis

Amicar (EACA)
used to dissolve clots in people, comes from bacteria
found in urine, released by WBCs

Aprotinin
used to block binding of tPA to plasminogen and plasminogen to fibrin in pts who are bleeding.
no longer on the market, but inhibited plasmin
FIBRINOGEN

+ 2 FRAGMENTS A,B,C

PLASMIN

FRAGMENT X

PLASMIN

FRAGMENT Y

PLASMIN

FRAGMENT D

FRAGMENT E

plasmin cleaves the rod of fibrinogen and degrades the fibrin clot into smaller pieces.
Control of Clot Formation

Separation of Initiation & Propagation
- Extrinsic pathway and platelet are separated by the vessel wall - only get large scale thrombin generation if these interact

Presence of plasma coagulation inhibitors
- Antithrombin (AT or ATIII)
  - Activity increased by heparin & LMWH
- Tissue Factor Pathway Inhibitor (TFPI)

Anti-thrombotic mechanisms on healthy vascular endothelial cells
- Thrombomodulin (TM)/Protein C/S system
- Heparan sulfates that bind AT
## Hemostasis Sets the Stage for

<table>
<thead>
<tr>
<th>Inflammatory Cell Influx &amp;</th>
<th>Effective Wound Healing</th>
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<tbody>
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<td>Thrombin has many biological activities</td>
<td>Fibrin is the matrix for healing</td>
</tr>
<tr>
<td>Platelets release cytokines &amp; growth factors</td>
<td>Hemostatic defects can lead to defective wound healing</td>
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- Thrombin:
  - has many biological activities
  - set the stage for healing

- Fibrin:
  - is the matrix for healing
  - make scaffold for healing to occur on

- Platelets:
  - release cytokines & growth factors

- Hemostatic defects can lead to defective wound healing
Bleeding Disorders

- We are only going to talk about inherited (not acquired) disorders
  - Platelet problems
  - Coagulation factor problems
Clinical Bleeding

• Platelet Problem
  – Petechiae & purpura
  – Mucocutaneous bleeding

• Coagulation Factor Problem
  – Bruises (ecchymoses)
  – Soft tissue hemorrhage
Platelet storage organelles. Predominant are the α-granules of which there are upwards of 50 per platelet. A large number of proteins are stored and released from these organelles; in the figure proteins are grouped by category for convenience, and this is not meant to signify a physiological storage organization. There is, however, some evidence that subpopulations of α-granules may contain discrete populations of proteins (14).
Descriptors of Bleeding

- Petichiae are < 3 mm,
- Purpura are 0.3-1 cm,
- Ecchymoses are > 1 cm

Clusters of palpable, pruritic petechiae on the thigh of a patient with Henoch-Schönlein purpura. These lesions could be mistaken for thrombocytopenic petechiae.
Thrombocytopenia leads to endothelial changes

- The top shows an EM of a capillary from a thyroid perfused with PRP for 5h.
- The bottom shows a capillary from a thyroid perfused with PPP for 5h. Note the disruption in the endothelium.

Thrombocytopenia leads to endothelial changes

This picture shows a RBC extravasating from a capillary of a thrombocytopenic mouse (arrow).

RBC appear to traverse small channels in the endothelial cells.

Factor Deficiencies: General Considerations

Deficiencies of each of the following exist:

- Factor VIII
  - hemophilia A
- Factor XI
- Prothrombin
- Factor V
- Fibrinogen
- Factor IX
  - hemophilia b
- Factor VII
- Factor X
- Factor XII
- Factor XIII

deficiency of tissue factor does not exist - you can't survive without tissue factor. vascular system will not develop normally in the absence of TF or its inhibitor
Factor Deficiencies: General Considerations

• Inheritance: Most are inherited as autosomal recessive disorders

• Factors VIII and IX are encoded on the X chromosome and their deficiencies are sex-linked recessive

• While bleeding is the hallmark of these disorders, its severity and pattern vary depending on the involved factor
Congenital Bleeding Disorders

• vonWillebrand Disease
• Hemophilia A & B
• FXI deficiency
von Willebrand Disease
von Willebrand Disease

- Autosomal
- Most common inherited bleeding disorder
- vWF mediates platelet adhesion under high shear - bleeding is typical of platelet defects
- vWF is the carrier for FVIII - FVIII level may be reduced and aPTT may be prolonged
- Subdivided into several types based on multimer pattern and antigen level

We will see this. It has a wide range of clinical manifestations. May not show up until surgery, trauma, or if the pt begins taking an anticoagulant. Can also be very severe bleeding.
Hemophilia A & B

Deficiency of FVIII or FIX
Hemophilia A and B

- X-linked
- Up to 30% from de novo mutation i.e. no family history
- Mild, moderate and severe forms
- Dysfunctional molecules – Cross-reacting material positive (CRM+)
- Reduced level of a normal molecule - Cross-reacting material negative (CRM-)
Hemophilia Is a Failure of **Platelet Surface Thrombin Generation**

Much early coagulation research was driven by the presence of hemophilia in the royal families of Europe.
she probably suffered a de novo mutation
Inheritance of Hemophilia

- Hemophilia A (FVIII deficiency) 1 in 10,000 live male births
- Hemophilia B (FIX deficiency) 1 in 30,000 live male births
- Inherited as sex linked recessive traits

An affected male will produce only normal males and carrier females (with a normal female)

A carrier female will produce offspring of which half the females are carriers and half the males are affected (with a normal male)
# Inheritance of Hemophilias

<table>
<thead>
<tr>
<th></th>
<th>Normal male (XY)</th>
<th>Hemophilic male (X^hY)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal female (XX)</td>
<td>(Normal female) (XX)</td>
<td>(Carrier female) (XX^h)</td>
</tr>
<tr>
<td>Carrier female (X^hX)</td>
<td>(Carrier female) (X^hX)</td>
<td>(Normal male) (XY)</td>
</tr>
</tbody>
</table>

- All daughters will carry the condition.
- Half of daughters will carry.
- 50% of sons affected.
Hemophilia A and B

- Clinical picture is identical in A & B
- Prolonged aPTT in both, need factor assays to distinguish
- Severely affected have spontaneous soft tissue and joint hemorrhage
- Severely deficient may develop antibody inhibitors
FXI deficiency

long aPTT but bleeding isn't as bad as hemophilia
FXI Deficiency

• Autosomal
• Common in certain populations - Ashkenazi Jews, some Arab populations
• Bleeding with trauma or surgery, especially if on aspirin
• Bleeding risk not predictable from aPTT or FXI level

populations where you have consanguinity bc that has a tendency to concentrate recessive genes

a specific factor X deficiency has been traced to consanguinity in the mountains of North Carolina.

otherwise its usually mild