THE FRITSMA FACTOR
Your Interactive Hemostasis Resource

Hemophilia Therapy

From Rasputin to Recombinants

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Hemophilia
Rasputin to Recombinants

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www.PrecisionBiologic.com

- **Hemophilia case study**
- **Hemophilia in history**
- **Alexis and Rasputin**
- **Advances 1920–70**
- **AIDs and recombinants**
- **Future therapy**

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40 YO Af-Am Hemophilic

- Bleeding into ankle
  - Anatomic soft-tissue and joint bleeds
  - Tried RICE, DDAVP inhaler
- Ran out of factor VIII (FVIII) concentrate
  - Provided free by Medicare through hemophilia center
  - National Hemophilia Foundation: www.hemophilia.org
- Clinical path resident on-call; night tech
  - Determine patient residual factor VIII activity
  - Order antihemophilic factor (AHF) VIII concentrate
    - Dispensed by transfusion service
  - Compute and prepare dosage
  - Reconstitute with sterile water, administer as IV push
  - Subsequently determine therapeutic factor VIII activity

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Hemophilia A Symptoms
Spontaneous anatomic (soft-tissue) bleeds

- Bleeding at umbilical stump, circumcision
- Delayed bleeding following injury
  - Joints, muscles, body cavities, GI, soft tissue, tongue, kidney, testicles, CNS
- Often spontaneous bleeds, especially joints
- Inflammation, hematomas, hemarthroses

Mild	Moderate	Severe
15%	15%	70%
6-30% VIII	1-5% VIII	<1% VIII
Bleed after major trauma	Bleed after minor trauma	Spontaneous bleeding

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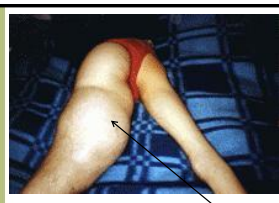

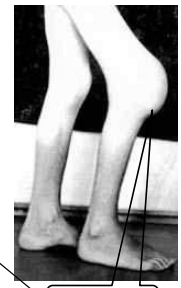

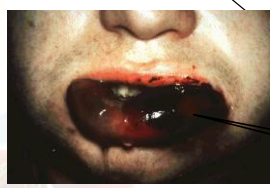

Hemarthroses

Airway Obstruction

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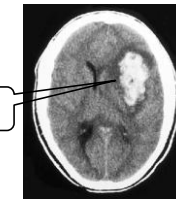
Other Complications

- Lifestyle
- Economic
- Vocational
- Neurologic
- Psychological
- Lack of insurance
- Narcotics addictions



Cerebral hemorrhage

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Calculating AHF Dosage

- One unit of factor = amount of activity in 1 mL normal plasma, same as 100%
- Determine plasma volume based on weight
  - Blood volume (BV, mL) = weight (kg) x 70 mL/kg
    - Use 60 mL/kg for obese, BMI 25–30
    - Use 50 mL/kg for morbidly obese, BMI > 30
  - Plasma volume (PV, mL) = BV x (100%–HCT%)
- Determine units of AHF required
  - Units of AHF required = (desired AHF in units/mL - initial units/mL) x PV (mL)

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40 YO Af-Am Hemophilic

- 80 kg, HCT 40%, 0 factor level
- Determine plasma volume
  - Blood volume (mL) = 80 kg x 70 mL/kg = 5600 mL
  - Plasma volume (mL) = blood volume (5600 mL) × (100%−40%) = 60% × 5600 = 3360 mL
- Determine units of factor required:
  - Wish to reach 80% factor level (0.8 U/mL), therefore…
  - Units of factor required = (0.8 U/ mL−0) x 3360 mL = 2688 (2700) U
  - Typical FVIII concentrate vial provides ~1000 U, use 3
- Avoid overdose: thrombotic and wasteful

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If Factor Assay not Available

- When factor assay not available and timing is critical, assume 0 activity or…
- Approximate factor VIII from PTT

Factor VIII	PTT
40%	35 s
30%	50 s
20%	65 s
10%	90 s
1 %	120 s
Example only, do not use	

- Collect baseline plasma
  - Assay or freeze and confirm with assay next day shift
- Maintain patient database

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Plasma-derived FVIII Concentrates

- Risk of HBV, HCV, HIV transmission
  - Human and animal serum
  - Hemofil-M®, Monarc-M®, Monoclate-P®
  - Purification: immunoaffinity column, solvent-detergent, Pasteurization, viral filtration, combinations
  - $0.35–0.60/U
- Seroconversion per CDC surveillance: 0
  - Predicted risk, 1:60,000

MONARC-M
[Antihemophilic Factor (Human)]
Method M, Monoclonal Purified

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Recombinant FVIII Concentrates

- Serum in culture medium
  - Helixate®, Kogenate®, Recombinate®
  - $0.68–1.05/U: *some select for only previously untreated patients (PUPs)*
  - No HBV, HCV, HIV seroconversions
- No protein in culture or prep
  - Calculated viral risk=0, actual=0: Advate®
- B-domain-deleted FVIII concentrate
  - Human albumin: ReFacto®,
  - No protein: Xyntha®
  - Can't assay using clot-based factor VIII assay, use chromogenic

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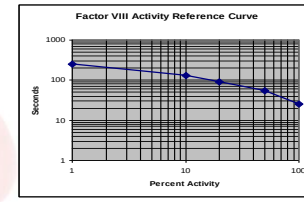
Factor VIII Assay

- Dilute plasma 1:10, add factor VIII-depleted reagent plasma 1:1
- Add PTT reagent, incubate 3 minutes
- Add $CaCl_2$, record interval to clot formation
- Compare result in seconds to dilution curve



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Factor VIII Assay Dilutions Parallelism Indicates No Inhibitor

Plasma Dilution	Seconds	Raw Factor VIII Activity	Computed Factor VIII Activity (× dilution)
1:10 "undiluted"	90 s	20%	20%
1:20	104 s	10%	20% (parallel)*
1:40	107 s	5%	20% (parallel)
1:80	110 s	2.5%	20% (parallel)

* <10% difference from undiluted indicates parallelism, no inhibitor

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40 YO Af-Am FVIII Concentrate Therapy

- Peak: 15 m after administration: 0.3 U/mL
  - Should have been 0.8, what happened?
  - Suspect anti-factor VIII inhibitor
  - If peak reaches expected value, go on to next administration
- Nadir: 12 h after administration
  - Reflects half-life, should reach 50% of desired activity
  - Administer new AHF, use half the dosage second time

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40 YO Af-Am, FVIII Inhibitor

- Inhibitor: alloantibody to FVIII concentrate
  - 30% incidence
  - Dose and severity response: mostly severe hemophilia
- Factor VIII assay
  - If non-parallel, reflex to Bethesda titer
- The "poor man's" Bethesda titer substitute
  - Dilute plasma 1:20 in normal plasma
  - If prolonged, presume >5 BU, treat as high titer
    - Corticosteroids and FEIBA® or NovoSeven® (VIIa)
  - If not prolonged, presume <5 BU, treat as low titer
    - Factor VIII concentrate
  - Confirm with full Bethesda titer
    - Larry D. Brace, PhD, Edward Hospital, Naperville, Il

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FVIII Assay Dilutions non-Parallelism Indicates Inhibitor

Plasma Dilution	Seconds	Raw Factor VIII Activity	Computed Factor VIII Activity (× dilution)*
1:10 (undiluted)	80 s	10%	10%
1:20	93 s	8%	16%
1:40	107 s	5%	20%
1:80	108 s	4%	32%

* > 10% difference from undiluted = non-parallel, implies inhibitor

Kasper CK. Laboratory diagnosis of factor VIII inhibitors. In Kessler C, Garvey MB, Green D, Kasper C, Lusher J. Acquired Hemophilia 2nd Edition. Excerpta Medica 1995

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FVIII Inhibitor Therapy

- Plasma-derived factor IX complex
  - Activated prothrombin complex concentrate (PCC)
  - Prepared by $BaSO_4$ extraction
  - Available since 1980
  - FEIBA®, Autoplex ®
- FEIBA dosage
  - 50 Units/kg/12 h standard
  - 70 Units/kg/8 h in hemorrhage
  - Limit 200 U/kg/24 h to avoid DIC risk: fatal
  - Cannot monitor: only general evaluation with PTT

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Recombinant FVIIa Concentrate

- Dose: 90 μg/kg
  - Repeat every 3–6 h
  - 6 h FVII half-life
- $0.83/μg
  - For our 80-kg patient, one dose = ~$6000
- Cannot monitor
  - General evaluation using PTT
  - No risk of DIC

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Hemophilia

- Anatomic bleeding caused by congenital single-factor deficiencies
- 85% factor VIII deficiency (hemophilia A)
  - 1 in 10,000 male births
- 14% factor IX deficiency
  - Hemophilia B or Christmas disease
  - 1 in 30,000 male births
- 1% XI (autosomal, Rosenthal syndrome)
- Rare autosomal recessive single factor deficiencies
  - Prothrombin, V, VII, X, XIII

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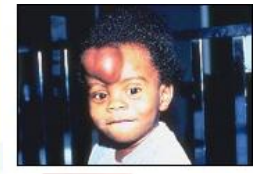
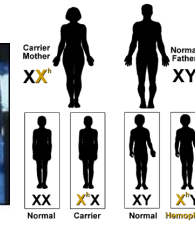
Hemophilia A Inheritance

- Sex-linked recessive, 1/10,000–20,000
- 25–30% spontaneous mutations





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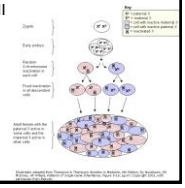
Hemophilia A Genetics

- 186 kb gene on X chromosome
  - Deletions, stop codons; missense and nonsense point mutations
  - 25−30% spontaneous mutation rate
  - Predominantly quantitative deficiency
  - Male hemizygotes are affected
- Hemophilia A in females is rare
  - Acquired anti-coagulation factor VIII
  - Autosomal hemophilia or VWD type N (Normandy)
  - Random "excess Lyonization"
  - Hemophilic father, carrier mother



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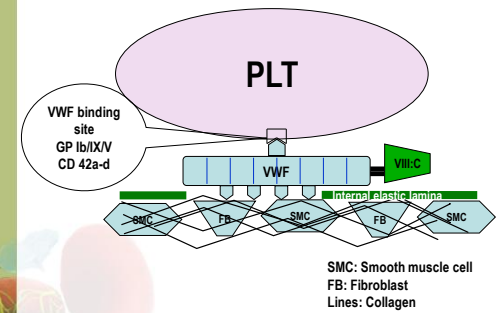
VIII/VWF-Platelets Interaction



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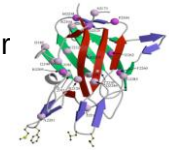
Factor VIII is a Glycoprotein Cofactor



- 285,000-D heterodimer
  - Translated from the X chromosome
- Cleaved by thrombin, leaving a Ca^{++}-dependent portion that detaches from VWF and binds factor IXa and phospholipid
- Stabilizes IXa in the "tenase" reaction
- Deficiency slows thrombin production
- In vitro, deteriorates 5%/h at 18–24°C

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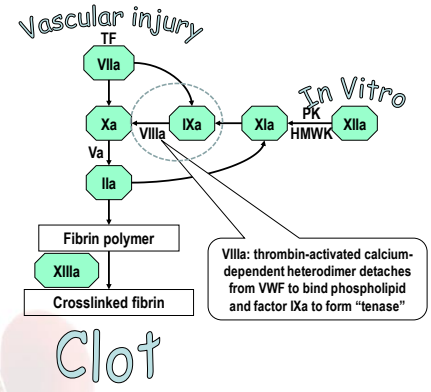

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1st Documented Bleeder's Disease

2nd century: Talmudic ruling of *Rabbi Judah the Patriarch* exempts a woman's 3rd son from circumcision if two elder brothers had died of bleeding after circumcision

2nd century: *Rabbi Simon ben Gamaliel* forbade a boy to be circumcised after sons of his mother's three elder sisters had died after circumcision

11th century: Arabic surgeon *Albucasis* describes village males who bled to death from "trivial" wounds

Ingram GIC. The history of haemophilia. J Clin Pathol 1976; 29: 469-79.

1791–1803: British & American Families

- 1000–1800: Several references to "bleeders"
- 1791 (Britain), Zoll: 6 brothers bled to death after minor injuries
  - Half-siblings by a different mother were unaffected
- 1803 (Philadelphia), Otto: "A hemorrhagic disposition existing in certain families"
  - Recorded males in his own family with symptoms and recognized transmission through asymptomatic women
  - Traced pedigree to a woman named Smith in Plymouth, 1720–30

Otto quoted in Bulloch W, Fildes P. Treasury of human inheritance, parts V & VI, section XIVa, Haemophilia, 1911.

1800–28: Documented Names

- Bleeding disease
- Haemorrhoea
- Idiosyncrasia haemorrhagica
- Hereditary haemorrhagic diathesis
- 1828: first use of "haemophilia" (blood-loving) appears in the title of a treatise by Hopff at University of Zurich

Hopff F. Cited by United States Surgeon General's catalogue, 1st series: Hemophilia, 1828.

Bulloch and Fildes

Bulloch W, Fildes P. *Treasury of human inheritance, parts V and VI, section XIVa, haemophilia*.

Published as Eugenics Laboratory memoirs XII, Francis Galton Laboratory for National Eugenics, University of London; 1911, Dulau and Co, 37 Soho Square, London.

- 1000 references and case reports
- 200 pedigrees
- Identified haemophilia as sex-linked, but carrier status not understood
- Meticulously traces the current spread of the mutation throughout Queen Victoria's family

Alexandrina Victoria; May 24, 1819–Jan 22 1901, was *Queen of the United Kingdom of Great Britain and Ireland* from June 20, 1837 until her death, altogether 63 years and 7 months. The Victorian era was a time of UK industrial, political, imperial, and military progress.

Queen Victoria

- Presumed spermatogenesis mutation in father; Edward, Duke of Kent, who was in his 50s when Victoria was conceived
- Victoria's seventh child, Leopold, was hemophilic
  - Stigmatized as a "weak" invalid by his mother
  - Married at 29
  - Died of cerebral hemorrhage following a fall at 31
- Two daughters, Alice (2nd) and Beatrice (8th) turned out to be carriers

Massie RK. Nicholas and Alexandra. (1968). Gollancz, London.

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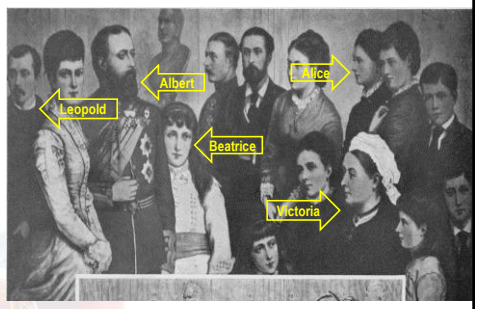
Queen Victoria and Family



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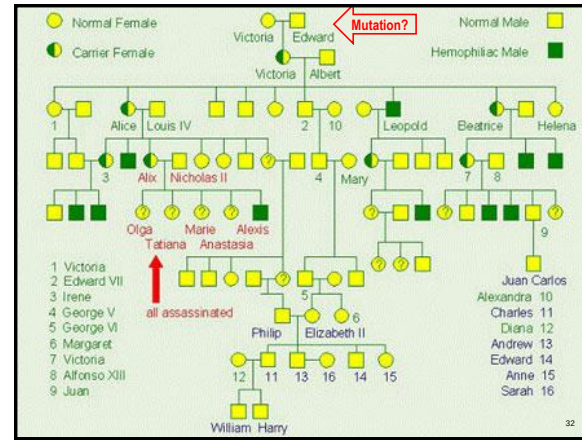

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Victoria Eugenie (Ena) Battenberg

- Beatrice's daughter, 2 hemophilic brothers
- Alfonso XIII of Spain married her in 1906
  - Warned by Spanish Embassy, but trusted his luck
- Alfonso XIV b. 1907 bled from circumcision
  - Alfonso XIII blamed Ena, grew to despise her
  - Jaime, deaf-mute; Gonzalo, hemophilic
  - Spaniards blamed the British
- Juan B. 1913, normal
  - Son Juan Carlos is current King of Spain 1975 to present
- 1931, royal family in exile in Rome
  - Alfonso was too ill to appear
  - 1933, marries Cuban commoner and abdicates

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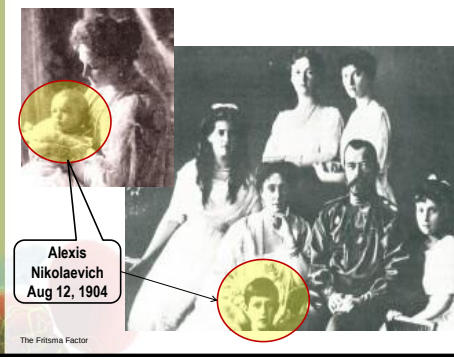
The Romanovs: Tsar Nicholas II, Alexandra (Alix, granddaughter of Victoria) and family



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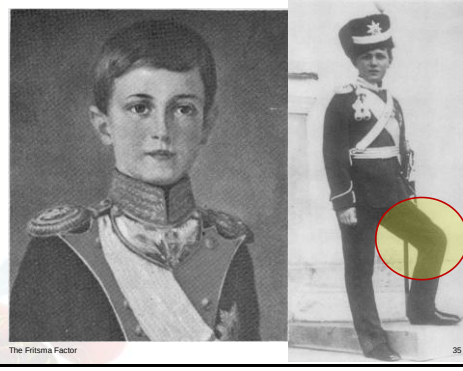
Prince Alexis, 1912



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Grigori Yefimovich Rasputin 1869–1916

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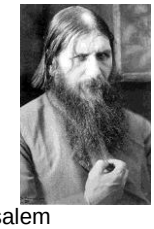
Rasputin



- 1869, Pokrovskoye, Siberia
- Two sibs drowned
- 1887: three months in Verkhoturye Monastery
- 1901: *strannik* (pilgrim) wandered through Greece, Jerusalem
- 1903: Saint Petersburg, *starets* (holy man) with healing & prophetic powers
- 1905: Alexandra introduced by Anna Vrubova to get help for 1 YO Alexis

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Rasputin's Power Rises
1912 Belovezhski Forest Holiday

- Faith healer, hypnotism?
- Leeches?
- Calming influence, distraction?
  - 'God has seen your tears and heard your prayers. Don't be sad, the little boy is not going to die. Don't let the doctors frighten him.'

Rasputin became the czar's primary adviser and gatekeeper, used his power for financial gain and debauchery, and was increasingly hated by the Russian nobles, though loved as a mythical figure by some of the peasants.

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The Romanovs in 1912

Pierre Gilliard, Alexis' tutor, wrote: "*The illness of the Tsarevich cast its shadow over the whole of the concluding period of Tsar Nicholas II's reign. Without appearing to be, it was one of the main causes of his fall, for it made possible the phenomenon of Rasputin and resulted in the fatal isolation of the sovereigns who lived in a world apart, wholly absorbed in a tragic anxiety which had to be concealed from all eyes*."

Historians have since disputed the contribution of Alexis' hemophilia to Russian politics, but the strain on the royal household is clear enough.

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Rasputin and Admirers, 1914

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Yusupov Moika Palace, St. Petersburg

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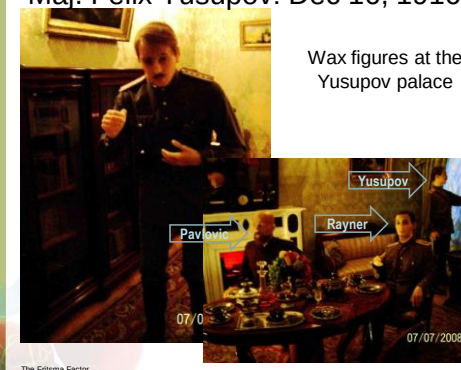
Maj. Felix Yusupov: Dec 16, 1916



Wax figures at the Yusupov palace

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Poisoning Rasputin

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Treatment Attempts 1901−1942

- Lime
- Gelatin
- Oxygen
- Splenectomy
- Bone marrow
- Sodium citrate
- Calcium lactate
- Witte's peptone
- Hydrogen peroxide
- Induced anaphylaxis
- Antidiphtheric serum
- The 'galvanic needle'
- Animal and human sera
- Adrenaline
- Bird's muscle
- IV oxalic acid
- Vitamin therapy
- X-ray irradiation
- Serum from the mother
- Tissue fibrinogen by mouth
- Bromide extract of egg white; sedative
- Blood—both injected and withdrawn therapeutically, auto-hemotherapy
- Female hormone therapy
  - in the belief that femininity prevents the expression of the hemophilic gene

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Effective Treatments

- 1926, Surgeon General: 12 referenced attempts at whole blood transfusion
- 1934, McFarlane: topical application of Russell viper venom
- 1937, Patek and Taylor first characterization of anti-hemophilic globulin
- 1938, McFarlane: fresh whole plasma
- 1950s: EJ Cohn fractionation of whole human and animal plasma
  - Animal: Biggs and Macfarlane, 1954; Bidwell, 1955
  - Kekwick and Wolf, 1957; Soulier, Gobbi, Larrieu, 1957; Blomback, Blomback, Nilsson, 1958

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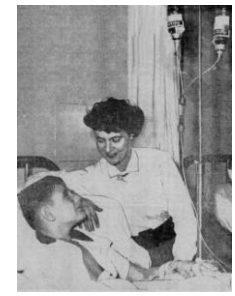

1952: Stephen Christmas
Canadian Hemophilia Society:
Delineation of factor IX deficiency (Christmas disease) from factor VIII deficiency

Nine-year-old Donald Burns smiles on father's knee. His is believed to be first successful appendectomy operation to a haemophiliac in North America.

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Breakthroughs

- 1964, Judith G. Pool (1919−75, U of Chi)
  - Developed cryoprecipitate
  - First opportunity for hemophilic home care
- 1968, Kenneth M. Brinkhous
  - (1908−2000, UNC Chapel Hill)
  - First to chemically characterize factor VIII in 1938
  - Developed AHF with hemophilic dog experiments
  - AHF released through Hyland in 1968

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Advances in the 1960–70s

- Dental extractions and minor procedures using Kohn fractions
- Orthopedic correction of hemarthroses using AHF
- Prothrombin complex concentrate, II, VII, IX, X for inhibitor
- Home therapy
- Hemophilia treatment centers 1973
- By 1980, life expectancy was 60
- But high hepatitis rate, 20,000 donors/pool
  - No viral inactivation

Rev. Robert Massie, Jr

Susan Massie

Robert K. Massie

Massie R, Massie K. *Journey*. Knopf, USA 1973

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AIDS

- Fall 1980: *Pneumocystis carinii* pneumonia and Kaposi sarcoma in homosexual males
  - Searched for non-infectious causes such as amyl nitrite "poppers," anti-sperm antibodies or anal intercourse
- Spring 1982: CDC recorded three cases of PCP in hemophilics receiving AHF, all died
  - Reports of similar symptoms in Haitian hemophilics and drug abusers
  - No homosexual behavior or illegal drug use
  - Led to concept of blood-borne viral infection

Evatt BL. The tragic history of AIDS in the hemophilia population, 1982–1984. J Thrombos Haemost 2006; 4: 2295–301.

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July 27, 1982

- CDC reported to blood and plasma industries, gay organizers, hemophilia organizations, FDA and NIH
- Consensus to not act
  - Evidence of three patients too weak to conclude hemophilics were a risk group
  - Concern it would stigmatize homosexual community
  - Lose homosexual donors to blood and plasma services
  - Millions of dollars to change manufacturing policies
- Fall of 1982: six more cases, including two non-hemophilic blood recipients
  - Donor services would not reveal donor identities to avoid embarrassing and subsequently losing donors
  - One definite identification

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January 4, 1983

- CDC reported the statistical prevalence of hepatitis B was identical in hemophilics and AIDS risk groups (surrogate association)
- CDC reported to the same groups including ARC, AABB, National Hemophilia Foundation, National Gay Task Force, Pharmaceutical Mfrs Association, Council of Community Blood Centers, State and Territorial Epidemiologists, and individuals.
- Again, consensus to not act, debate was irrational, acrimonious and public, harshly critical of Evatt and CDC

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January 13, 1983

- CCBC and AABB: "*transfusions are life-saving procedures; some adverse reactions are acceptable to save lives. The rare disorder affecting nine cases is not enough to force a policy change*."
- ARC head Dr Cumming wrote: "*It has long been noted that CDC increasingly needs a major epidemic to justify its existence… In short, we can not depend on the CDC to provide scientific, objective, unbiased leadership*."
- NHF, however, already alarmed, had contacted plasma manufacturers in December, 1982

American Red Cross

National Hemophilia Foundation
for all bleeding and clotting disorders

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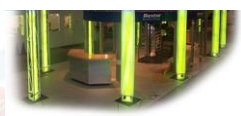
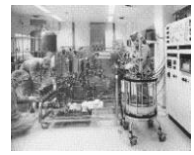
NHF Initiative: 1983

- Dec 1982: *Alpha Therapeutics* began to screen donors
- 20% of commercial plasma came from donor services who refused to screen donors for sexual orientation
- US Public Health Service guidelines, March 4, 1983
  - CDC bypassed FDA, sent guidelines direct to PHS
  - Donor screening and surrogate testing: hepatitis markers
- March, 1983, Baxter Hyland began heat treating plasma
- August, 1983, 26 confirmed cases of transfusion transmitted AIDS, including one F IX deficiency





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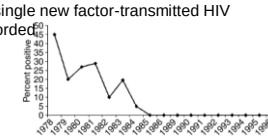
Virus Isolation: 1983

- Mid-1983: Pasteur Institute isolates virus from lymphadenopathy patients
- Feb, 1984: Pasteur Institute isolates virus from AIDS samples provided by CDC
- Sep, 1984: Alpha and Cutter demonstrate heat treatment is safe and does not increase immunogenicity
- Oct, 1984: CDC/PHS screening and heat treatment guidelines published and adopted
- By 1984, 63% of 15,500 US hemophilia patients had HIV
- Since 1/1/1985, not a single new factor-transmitted HIV infection has been recorded



Frequency of HIV infection in US hemophilia birth cohorts. (From medical records)

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Recombinant Clotting Factors

- 4/7/1976: *Genentech* incorporated
- 1981: *Genetics Institute* incorporated
- 1982: rFIX cloned by both (small molecule)
- Aug, 1984: Both cloned rFVIII gene and produced the protein
- 1985: rVWF coexpressed

Pipe SW. Recombinant clotting factors. Thromb Haemost 2008; 99: 840–50.

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rFVIII Barriers

- Extensive post-translational modification
  - Need for mammalian cloning system
  - Chinese hamster ovary cells
- rFVIII affinity for phospholipid
  - CHO membrane binds and degrades FVIII
  - CHO cells transfected with FVIII and VWF genes
  - Resolved by rVWF coexpression—higher affinity
- Need for serum-free cloning system
  - Eliminate animal viruses, ease purification
- High-volume fermentation process
  - Immunoaffinity, ion-exchange, pasteurization, solvent-detergent, nanofiltration reduces 7000 L to 1 L.

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rFVIII to Market

- Regulatory uncertainty
  - Sterility, consistency questions generate 600 tests/lot
- Baxter Hyland and Genetics Institute produced Recombinate®
- Bayer Miles Lab/Cutter Biological and Genentech produced Kogenate®
- 3/27/1987: UNC Chapel Hill, first phase II infusion into a 39 year-old hemophilic
- Recombinate® cleared 1992, Kogenate® 1993

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Post-market Advances

- B-domain deleted: ReFacto®
- Removal of human and animal protein additives (albumin): Advate®
- Hemostatic efficacy: 90% cessation of bleeding
- Risk of inhibitor formation in previously untreated persons (PUPs) is approximately double plasma-derived FVIII (pdFVIII)
- Risk of inhibitor formation in PTPs < 1%

Pipe SW. The promise and challenges of bioengineered recombinant clotting factors. J Thromb Haemost 2005; 3: 1692–1701.

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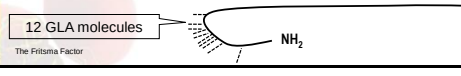
rFIX

- 1998: Genetics Institute BeneFix®
- Barrier: γ-carboxylation of 12 glutamic acids at amino terminus
  - Vitamin K
  - Coexpressed γ-glutamyl carboxylase
  - Coexpression of furin
- 90% bleeding cessation using 1-2 infusions
- PUPs—3% high titer inhibitors



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1999: rFVIIa

- Same γ-carboxylation issue
- For inhibitors: generates no DIC compared to activated prothrombin complex concentrates
- Activates through tissue factor and platelet surface binding
- Activates thrombin activatable fibrinolysis inhibitor (TAFI) to control fibrinolysis
- Novel variants in animal models

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Primary Prophylaxis in Children

- 2005: Joint damage outcome study
  - 25 IU/kg every other day generates 6X decrease in joint deterioration by MRI vs on-demand (OD) Rx up to 6 YO
- 2009 Italian study on prophylaxis
  - 10 Y f/u on 25 IU/kg 3X a week showed 0.52 vs 1.08 total bleeds and 0.2 vs 0.52 joint bleeds/patients/month in OD
- 2009 Danish/Russian study
  - Orthopedic issues 15.6 in OD vs 2.2 in prophylaxis

Franchini M, Coppola A, Molinari AC, et al. Forum on the role of recombinant factor VIII in children with severe haemophilia A. Haemophilia 2009; 1–9.
Gringeri A, Lundin V, von Mackensen S, et al. Primary and secondary prophylaxis in children with haemophilia A reduces bleeding frequency and arthropathy development compared to on demand treatment; a 10-year, randomized clinical trial. J Thromb Haemost 2009;7
Ingerslev J, Lethagen S, Poulsen L, et al. A case-controlled Danish-Russian comparative study of clinical outcomes in younger severe haemophilia patients treated with prophylaxis compared to those managed with on-demand treatment. J Thromb Haemost 2009;7

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Primary Prophylaxis in Children

- Encouragement
  - Abundant safe rFVIII
- Barriers
  - Venous access with clotting, cost, compliance, duration
- Inhibitor formation in severe hemophilics
  - Meta-analysis of 20 trials
  - 1248 patients, 798 severe, 632 treated exclusively with plasma-derived and 616 with recombinant FVIII, median age 12
  - Combined trials data: 14% inhibitors in PD FVIII, 25% in rFVIII
  - High titer: 8.8% PD, 12.3% recombinant

Iorio A, Marcucci M, Marchesini E, Mannucci P. Plasma derived and recombinant factor VIII concentrates include a different rate of inhibitor development in hemophilia A patients. A systematic review of the literature. J Thromb Haemost 2009;7

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Future

- rVWF
- rXIII
- Gene transfer
  - One human trial was negative
  - Animal trials in progress
- Need for new bioassays
  - Chromogenic FVIII
  - Activated FVII
- Need for humane public policies

Lozier J. Gene therapy of the hemophilias. Semin Hematol 2004; 41:287–96.