THE FRITSMA FACTOR
Your Interactive Hemostasis Resource

Managing Hemophilia Therapy
Rasputin to Recombinants

George A. Fritsma, MS MLS
The Fritsma Factor; Your Interactive Hemostasis Resource
www.fritsmafactor.com

Hemophilia case study
Hemophilia in history
Alexis and Rasputin
AIDs and recombinants
Extended life recombinants
Gene transfer

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40 YO Af-Am Hemophilic

- Bleeding into ankle: midnight
  - Anatomic soft-tissue and joint bleeds
  - Tried RICE, DDAVP (Stimate®) 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, FVIII) 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	Mod	Severe
6–30% VIII	1–5% VIII	<1% VIII
Bleed after major trauma	Bleed after minor trauma	Spontaneous bleeds
15%	15%	70%

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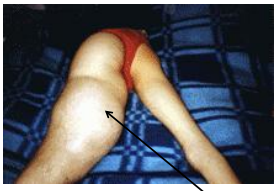

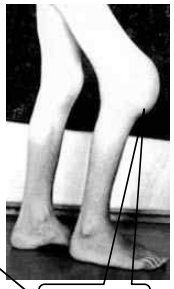

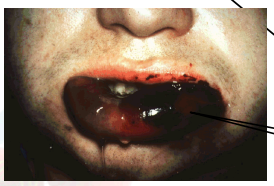

Hemarthroses

Airway Obstruction

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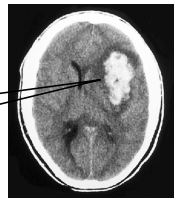
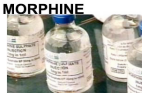
Other Complications

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



Intracerebral hemorrhage

MORPHINE



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Calculating FVIII Concentrate Dosage

- One unit of FVIII = amount of activity in 1 mL normal plasma, = 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 FVIII concentrate required

Units of FVIII required =
(desired FVIII in units/mL – initial units/mL) x PV (mL)

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40 YO Af-Am Hemophilic

- 80 kg, HCT 40%, factor level <1%
- 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 FVIII required:
  - Wish to reach 80% factor level (0.8 units/mL), therefore…
  - Units of FVIII required = (0.8 units/ mL−0) x 3360 mL = 2688 (2700) units
  - Typical FVIII concentrate vial provides ~1000 units, use 3
- Avoid overdose: thrombotic and wasteful

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If Factor Assay not Available

- When factor assay is not available and timing is critical, assume 0 activity or…
- Estimate 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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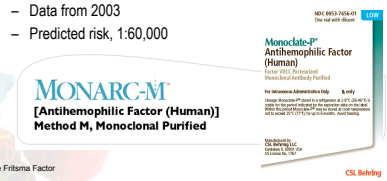
Plasma-derived FVIII Concentrates

- Theoretical risk of HBV, HCV, HIV transmission
  - Hemofil-M®, Monarc-M®(Baxter), Monoclate-P®
  - Human and animal plasma matrix
  - Purification: immunoaffinity column, solvent-detergent, Pasteurization, viral filtration, combinations
  - $0.35–$0.60/AHF unit
- Seroconversions per CDC surveillance: 0
  - Data from 2003
  - Predicted risk, 1:60,000



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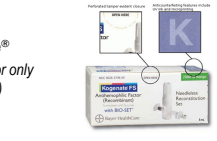
Recombinant FVIII Concentrates

- Serum in culture medium
  - Helixate®, Kogenate®, Recombinate®
  - $0.68–1.05/AHF unit: *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
  - Extended half-life: Eloctate
  - Can't assay using clot-based FVIII assay, use chromogenic FVIII



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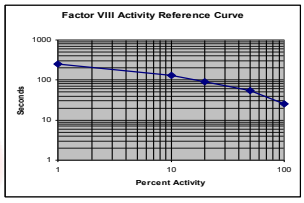
Factor VIII Assay

- 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 calibrator curve



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Factor VIII Assay Plasma 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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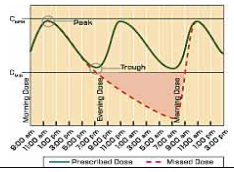
40 YO Af-Am FVIII Concentrate Therapy

- Peak: 15 m after administration: 0.3 units/mL
  - Should have been 0.8 units/mL, what happened?
  - Suspect anti-factor VIII inhibitor
  - If peak reaches expected value, go to next administration
- Nadir (trough): 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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FVIII Assay Plasma 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 & rising, 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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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, perform PTT
  - If prolonged, presume >5 BU, treat as high-titer inhibitor
    - Rx with corticosteroids and FEIBA® or NovoSeven® (VIIa)
  - If normal, presume <5 BU, treat as low-titer inhibitor
    - Rx with factor VIII concentrate
  - Confirm with full Bethesda titer
    - Larry D. Brace, PhD, Edward Hospital, Naperville, Il

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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 units/kg/24 h to avoid DIC risk: fatal
  - Cannot monitor: only general coag evaluation with PTT
  - $0.78/unit, about $4300/dose for the 80 kg patient

Ludlam DA, Morrison AE, Kessler C. Treatment of acquired hemophilia. Semin Hematol 1994;31 (Suppl 4) 16–19

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Recombinant FVIIa Concentrate

- Dose: 90 μg/kg for inhibitor
  - Repeat every 3–6 h
  - 6 h FVII half-life
- $0.83/μg
  - For our 80-kg patient, one dose = $6000
- Cannot monitor
  - General coag 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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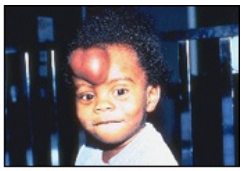
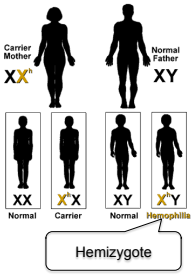
Hemophilia A Inheritance

- Sex-linked recessive, 1/10,000 males
- Carrier mother, hemizygous son





Hemizygote

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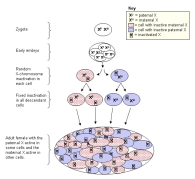
Hemophilia A Genetics

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



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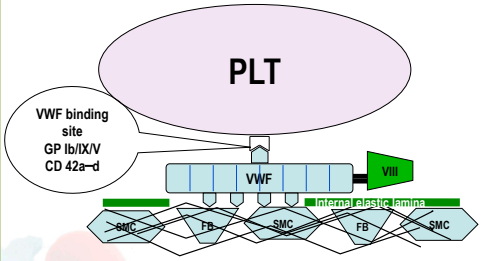
FVIII Requires VWF Carrier



SMC: Smooth muscle cell
FB: Fibroblast
Lines: Collagen

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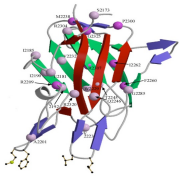
Factor VIII is a Glycoprotein Cofactor



- 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%/hour at 18–24°C

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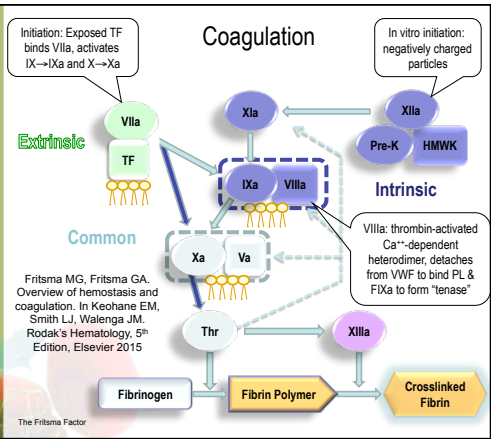
Coagulation



Fritsma MG, Fritsma GA. Overview of hemostasis and coagulation. In Keohane EM, Smith LJ, Walenga JM. Rodak's Hematology, 5th Edition, Elsevier 2015

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1st Documented Bleeder's Disease

2nd century: Talmudic ruling of *Rabbi Judah the Patriarch* exempts a 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: 465-79.

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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.

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1800–28: Documented Names

- Haemorrhoea
- Bleeding disease
- 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.

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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

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Alexandrina Victoria; May 24, 1819–Jan 22 1901, was *Queen of the United Kingdom of Great Britain and Ireland* from her coronation at the age of 18, June 20, 1837 until her death, altogether 63 years and 7 months. The Victorian era was a time of United Kingdom industrial, political, imperial, and military progress.

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Queen Victoria: Hemophilia Carrier

- 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 of Victoria, Alice (2nd) and Beatrice (8th) turned out to be carriers

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

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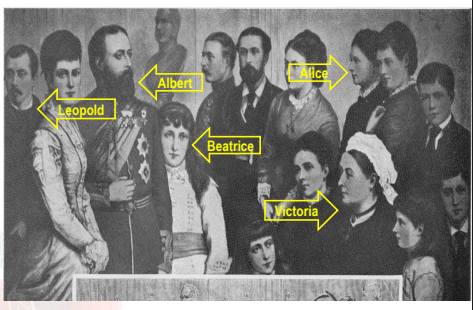
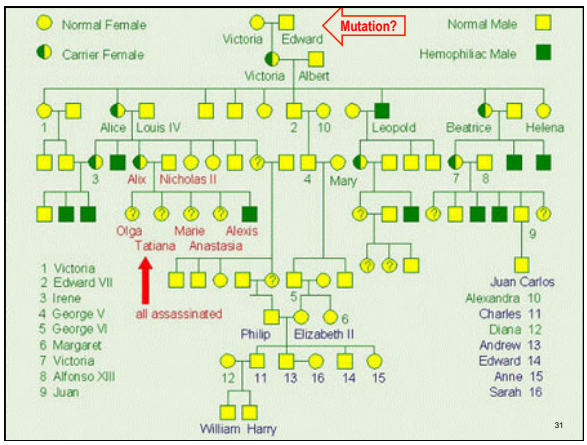
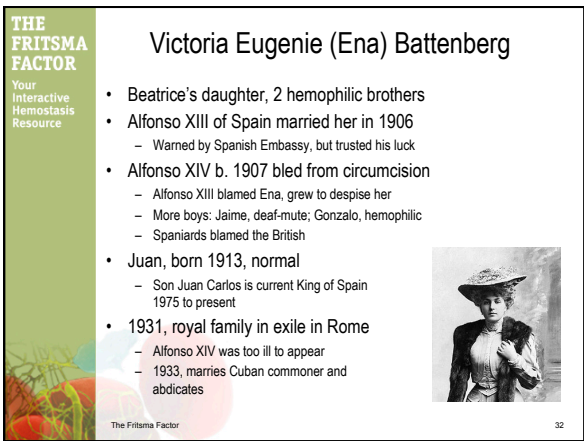
Queen Victoria and Family




Normal Female
Carrier Female
Victoria
Edward
Mutation?
Normal Male
Hemophiliac Male
Victoria
Albert
1
Alice
Louis IV
2
10
Leopold
Beatrice
Helena
3
Alix
Nicholas II
4
Mary
7
8
Olga
Marie
Alexis
Tatiana
Anastasia
9
1 Victoria
2 Edward VII
3 Irene
4 George V
5 George VI
6 Margaret
7 Victoria
8 Alfonso XIII
9 Juan
all assassinated
5
Philip
6
Elizabeth II
12
11
13
16
14
15
William
Harry
Juan Carlos
Alexandra 10
Charles 11
Diana 12
Andrew 13
Edward 14
Anne 15
Sarah 16
31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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
More boys: Jaime, deaf-mute; Gonzalo, hemophilic
Spaniards blamed the British
Juan, born 1913, normal
Son Juan Carlos is current King of Spain 1975 to present
1931, royal family in exile in Rome
Alfonso XIV was too ill to appear
1933, marries Cuban commoner and abdicates
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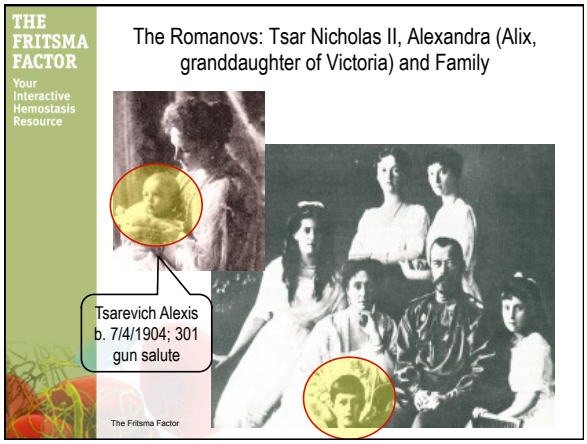
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The Romanovs: Tsar Nicholas II, Alexandra (Alix, granddaughter of Victoria) and Family
Tsarevich Alexis b. 7/4/1904; 301 gun salute
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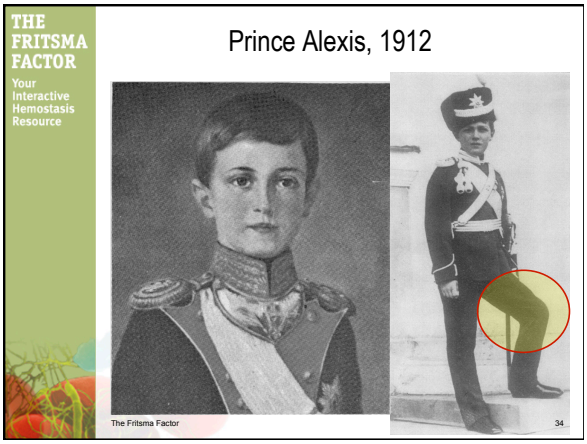
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Prince Alexis, 1912
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Grigori Yefimovich Rasputin
1869–1916
1869, Born Pokrovskoye, Siberia
Two sibs drowned
1887: 3 months in Verkhoturye monastery
1901: strannik (pilgrim), wandered Greece, Jerusalem
1903: Saint Petersburg, starets (holy man) with healing & prophetic powers
1905: Introduced to Alexandra by Anna Vyrubova to help with Alexis' hemophilia
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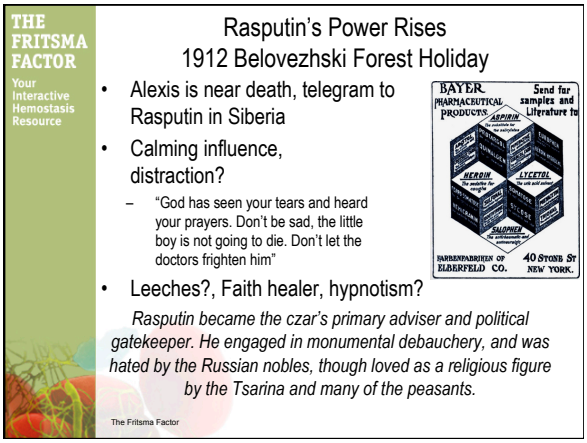
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Rasputin's Power Rises
1912 Belovezhski Forest Holiday
Alexis is near death, telegram to Rasputin in Siberia
Calming influence, distraction?
"God has seen your tears and heard your prayers. Don't be sad, the little boy is not going to die. Don't let the doctors frighten him"
Leeches?, Faith healer, hypnotism?
Rasputin became the czar's primary adviser and political gatekeeper. He engaged in monumental debauchery, and was hated by the Russian nobles, though loved as a religious figure by the Tsarina and many of the peasants.
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40 STONE ST NEW YORK.
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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 that 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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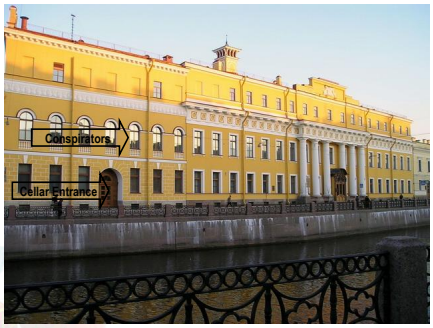
Yusupov Palace, St. Petersburg



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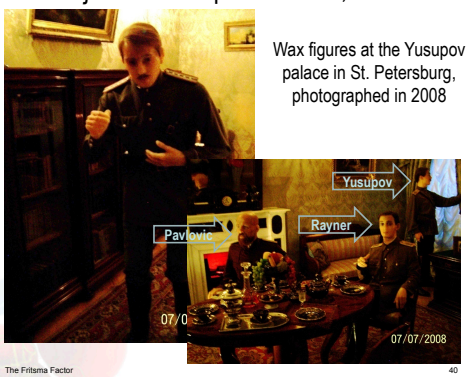
Maj. Felix Yusupov: Dec 16, 1916



Wax figures at the Yusupov palace in St. Petersburg, photographed in 2008

The Fritsma Factor 40

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Poisoning Rasputin

The Fritsma Factor 41

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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 withdrawn and injected therapeutically
  - "Auto-hemotherapy"
- Female hormone therapy
  - in the belief that femininity prevents expression of the hemophilic gene

The Fritsma Factor 42

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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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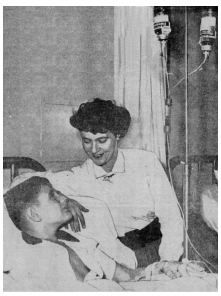

1952: Stephen Christmas, Canadian Hemophilia Society: *Delineation of factor IX deficiency (Christmas disease) from factor VIII deficiency (Alexis was factor IX deficient)*

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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

- 1960: dental extractions and minor procedures using Kohn fraction
- 1960: orthopedic correction of hemarthroses using AHF
- 1970: home therapy
- 1973: hemophilia treatment centers
- 1980: prothrombin complex concentrate
  - Proplex®, II, VII, IX, X for IX deficiency
- 1980: activated PCC, FEIBA® for inhibitors
- 1980: life expectancy was 60
  - But high hepatitis rate, 20,000 donors/pool
  - No viral inactivation

Rev. Robert K.Massie III

Susan Massie

Robert K. Massie

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

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AIDS

- Fall 1980: *Pneumocystis carinii* pneumonia (PCP) and Kaposi sarcoma (KS) in homosexual males
  - Searched for non-infectious immunodeficiency 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
  - Paralleled reports of similar symptoms in Haitian hemophilics and in US 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 CDC 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 Dr. 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.*"
- The National Hemophilia Foundation, however, alarmed, had contacted plasma manufacturers in December, 1982

American Red Cross

National Hemophilia Foundation
for all bleeding and clotting disorders

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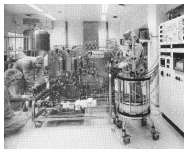
NHF Initiative: 1983

- 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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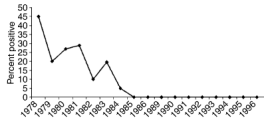
Virus Isolation: 1983

- July, 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

genesis genetics institute

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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Market Efforts and Inhibitor Formation

- B-domain deleted: ReFacto®
- No human or animal albumin in final formulation: Advate®
- Stabilizing matrix of sucrose, glycine, histidine: Kogenate FS®
- Hemostatic efficacy: 90% cessation of bleeding
- Risk of inhibitor formation in PUPs is approximately double the risk from plasma-derived FVIII
- Risk of inhibitor formation in response to rFVIII in previously treated persons (PTPs) is <1%
- 2013: Biogen Idec long half-life FVIII, Eloctate, FIX, Alprolix

Pipe SW. The promise and challenges of bioengineered recombinant clotting factors. J Thromb Haemost 2005; 3: 1692–1701.
Barnes C, Lillicrap D, Pazmino-Canizares J, et al: Pharmacokinetics of recombinant factor VIII (Kogenate-FS®) in children and causes of inter-patient pharmacokinetic variability. Haemophilia *2006;2* (Suppl 4): 40–9.

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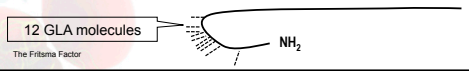
rFIX

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



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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 vs OD: 0.52 vs 1.08 total bleeds and 0.2 vs 0.52 joint bleeds/patients/month
- 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 clotting; cost, compliance, no extended duration data
- Inhibitor formation in severe hemophilics
  - Meta-analysis of 20 trials: 1248 patients, 798 severe, 632 treated exclusively pdFVIII and 616 with rFVIII, median age 12
  - Combined data: 14% inhibitors in pdFVIII, 25% in rFVIII
  - High titer inhibitors: 8.8% in pdFVIII, 12.3% in rFVIII

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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On-demand Versus Primary Prophylaxis

- 19 hemophilics 30−45 YO, all <1 unit/dL
  - Mean 2 bleeds/month, no additional bleeding disorder
  - ≥3 bleeds in elbow, ankle or knee, prior 6 months
  - Bayer Kogenate FS: sucrose-stabilized rFVIII
  - Prospective, open-label
- Six months on-demand Rx per bleed
  - Rx 20−100 units/kg every 12−24 h to cessation
- Seven months' prophylaxis
  - Month 7 (1st month of prophylaxis) was run-in
  - Home: 20−40 units/kg 2 mL/min infusion 3x/week
- Outcomes
  - Primary: patient self-assessed joint bleeds
  - Secondary: all bleeds, joint function, quality of life, health economics and safety

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Results

Median of 19 subjects	6 mo on-demand	6 mo prophylaxis
Infusions	29	88
Total consumption, units	70,421	211,933
Joint bleeds	15	0
All bleeds	20.5	0
Spontaneous bleeds	13.5	0
Trauma bleeds	2.5	0

- Baseline: median joint bleeds: 14.0; 24% mild, 63% moderate, 13% severe
- Fourteen used rFVIII, two used plasma-derived FVIII, three used both
- Prophylaxis median trough: 48 h: 6 units, 72 h: 4 units
- Mean total Gilbert joint function score (pain, swelling, atrophy, deformity, range of motion, instability): on-demand, 25.3%, prophylaxis 19.8%
- Safety: no Rx-related adverse events

Collins P, Faradji A, Morfini M, Enriquezs MM, Schwartz L. J Thromb Haemost 2010;8:83–9.

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Extended Half-life Factors VIII and IX

- Recombinant B-domain deleted Fc fusion factor VIII
  - Biogen Idec Eloctate, FDA-approved 2014
  - 96 adult hemophilic males with > 12 annual bleeds, 3–4 doses/week
  - Extended by Fc receptor and IgG recycling pathway
  - rFVIIIFc half-life 19 h versus rFVIII 12; 1.6–3.6 annual bleeds
  - Prophylactic Rx frequency up to 5 days versus 3–4 doses/week
  - Monitor using clot-based FVIII assay with kaolin-based PTT
- Recombinant Fc fusion factor IX
  - Biogen Idec Alprolix, FDA-approved 2014
  - Monitor using FIX assay with kaolin-based PTT or chromogenic assay
  - rFIXFc half-life 70–80 h versus 24 h, 7–10 day intervals

biogen idec

Shapiro AD[1], Ragni MV, Kulkarni R, et al. Recombinant factor VIII Fc fusion protein: extended-interval dosing maintains low bleeding rates and correlates with von Willebrand factor levels. J Thromb Haemost. 2014;12:1788–800.
Mancuso ME, Mannucci PM. Fc-fusion technology and recombinant FVIII and FIX in the management of the hemophilias. Drug Des Devel Ther. 2014 28;365–71.

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Cell-based Hemophilia Therapy

- Genetic delivery targets: liver, skeletal muscle, hematopoietic tissue, endothelial cells
- Vectors: adeno-associated virus (AAV) and liver sinusoidal endothelial cells (LSEC)
- Barriers: T-cell immune response to vector, FVIII molecule too large for vector, inefficient transduction

- Fomin ME, Togarriti P, Muench MO. Progress and challenges in the development of a cell-based therapy for hemophilia A. J Thromb Haemost 2014; 12: 1954–64.
- Chuah MK, Nair N, Vandendriessche T. Recent progress in gene therapy for hemophilia. Hum Gene Ther 2012; 23:557–65.
- Lozier J. Gene therapy of the hemophilias. Semin Hematol 2004; 41:287-96.

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FIX Gene Transfer: Landmark Study

- Adenovirus-associated virus vector (AAV)
  - Codon-optimized human factor IX transgene
  - scAAV2/8-LP1-hFIXco
- Six hemophilia B (<1% FIX) patients
  - 29–64 YO, 2 with FIX null
  - Four with missense mutations (type II deficiency)
  - All receiving prophylactic FIX 3X/week
  - No inhibitors, no antibodies to AAV
- Vector administration dosages
  - $2x10^{11}$ vector genomes/kg body weight (vg/kg)
  - $6x10^{11}$ vg/kg
  - $2x10^{12}$ vg/kg

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FIX Transfer Study Outcomes

- 4 of 6 required no FIX Rx
- 2 required on-demand FIX Rx
  - One ran the half-marathon
- High-dose subjects had rise in ALT
  - Immune response to hepatocytes?
  - Controlled with prednisolone
- No anti-FIX antibodies
- Did not detect cellular immunity to vector

Nathwani, AC, Tuddenham GD, Rangarajan S, et al, Adenovirus-associated virus vector–mediated gene yransfer in hemophilia B. NEJM 2011;365: 2357–65.

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Current Factor VIII Cellular Transfer Trial

- FVIII gene is 7 kb, AAV capacity 4.6 KB
- B-domain-reduced FVIII with minimal glycosylation
- Codon optimization: AAV8 variant has capacity to serve as a vector
- Successful in mice and macaques
- Human trials in progress

McIntosh J, Lenting PJ, Rosales C, et al. Therapeutic levels of FVIII following a single peripheral vein administration of rAAV vector encoding a novel human factor VIII variant. Blood. 2013 25;121:3335-44
Ward NJ, Buckley SM, Waddington SN, et al. Codon optimization of human factor VIII cDNAs leads to high-level expression. Blood 2011;117:798–807.