

**THE FRITSMa FACTOR**  
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## Hemophilia Therapy

From Rasputin to Recombinants



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## Hemophilia Rasputin to Recombinants

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

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### 1791–1803: British & American Families

- 100 AD–1800: Written 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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### 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 spread of the mutation throughout Queen Victoria’s family

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### Queen Victoria: 1837–1901

- 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 an invalid by his mother
  - Married at 29
  - Died of cerebral hemorrhage following a fall at 31
- Two daughters, Alice and Beatrice were carriers. Alice was second, Beatrice ninth

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

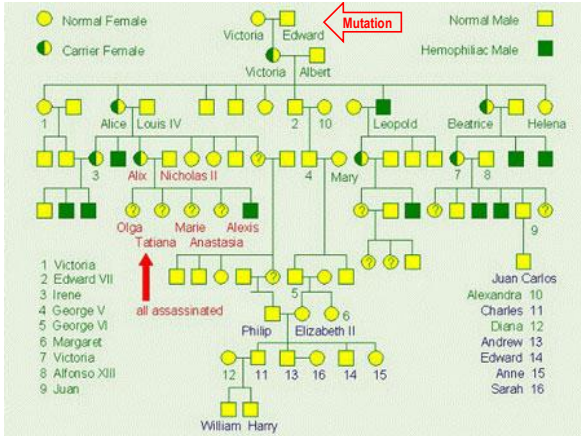
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### Queen Victoria and Family

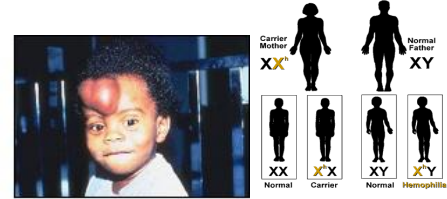


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### Hemophilia A Inheritance

- Sex-linked recessive, 1/10–20,000
- 25–30% spontaneous mutations
- Multiple mutations
  - Some qualitative, most quantitative



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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, 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

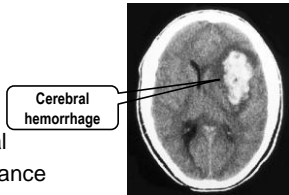


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### Other Complications

- Lifestyle
- Economic
- Vocational
- Neurologic
- Psychological
- Lack of insurance
- Narcotics addictions
- Social: Russian revolution, for example

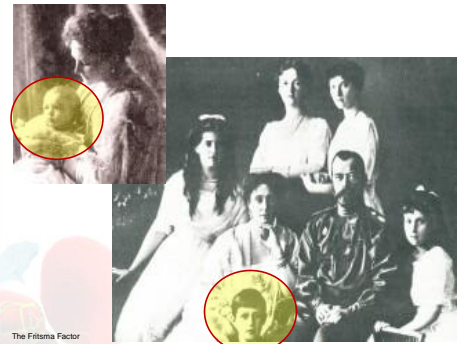


Cerebral hemorrhage

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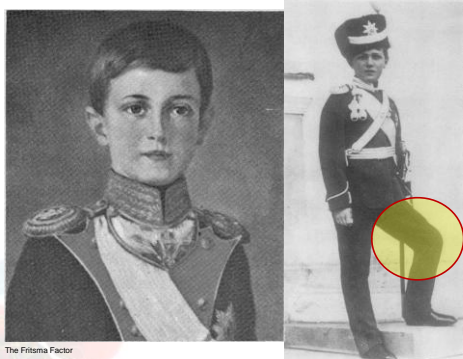
### The Romanovs: Tsar Nicholas II, Alexandra and Family



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Prince Alexis



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Grigori Yefimovich Rasputin  
1869–1916



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Rasputin's Power: 1905–16

- Calming influence?
  - "Don't let the doctors bother him too much; let him rest"
  - Distraction, causing Alexis to relax?
- Aspirin?
- Leeches?
- Faith healer, hypnotism?



*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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Rasputin and Admirers, 1914



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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' haemophilia to Russian politics, but the strain on the royal household is clear enough.

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Yusupov Moika Palace, St. Petersburg





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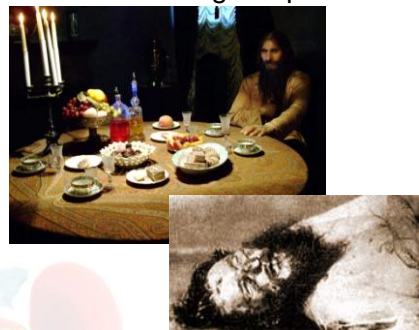
Maj. Felix Yusupov: Dec 29, 1916



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Poisoning Rasputin



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Effective Treatments Before 1960

- 1926, Surgeon General: 12 referenced attempts at whole blood transfusion
- 1934, McFarlane: topical application of Russell viper venom
- 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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Breakthroughs

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



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Further Advances in the 1960s

- Delineation of Christmas disease, factor IX deficiency, from factor VIII deficiency
- Dental extractions and minor procedures using Kohn fractions
- Orthopedic correction of hemarthroses
- Hemophilia treatment centers 1973
- Prothrombin complex concentrate, II, VII, IX, X for hemophilia with inhibitors



Rev. Robert Massie, Jr



Robert K. Massie



Susan Massie

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

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AIDS

- 1980: *Pneumocystis carinii* pneumonia (PCP) and Kaposi sarcoma in homosexual males
  - Searched for non-infectious causes such as amyl nitrite "poppers" or anal intercourse
- Spring 1982: CDC recorded three cases of PCP in hemophiliacs receiving AHF, all died
  - No homosexual behavior or illegal drug usage
  - 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, hemophilia associations, gay organizers, FDA and NIH
- Consensus to not act
  - Evidence of three patients too weak to conclude hemophiles were a risk group
  - 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
  - One definite identification

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January 4, 1983

- CDC reported the statistical prevalence of hepatitis B was identical in hemophiles and AIDS risk groups
- 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 CDC

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January 4, 1983

- Donor services: *“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, contacted plasma manufacturers



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NHF Initiative: 1983

- Dec 1982: Alpha Therapeutics began to screen donors
  - Clotting factor concentrates made from 20,000 donors, certain to have the virus
- 20% of commercial plasma came from donor services who refused to screen donors for sexual orientation
- US Public Health Service guidelines, March 4, 1983
  - 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

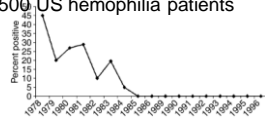


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Virus Isolation: 1983

- 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 alter product
- Oct, 1984: CDC/PHS screening and heat treatment guidelines published and adopted
- By 1984, 63% of 15,500 US hemophilia patients had HIV



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 had 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 generated 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® approved 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:  $\gamma$ -carboxylation of 12 glutamic acids at amino terminus
  - Vitamin K
  - Coexpressed  $\gamma$ -glutamyl carboxylase
  - Coexpression of furin
- 90% bleeding cessation using 1-2 infusions
- PUPs—3% high titer inhibitors



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### 1999: rFVIIa

- Same  $\gamma$ -carboxylation issue
- For inhibitors: 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

- Abundant safe rFVIII
- Randomized control trials
  - 2005: Joint outcome study: 25 IU/kg every other day generates 6X decrease in joint deterioration by MRI compared to on-demand Rx up to 6 YO
  - Italian evaluation study on prophylaxis: 10 Y f/u on 25 IU/kg 3X a week showed fewer bleeding episodes
- Barriers
  - Venous access, cost, compliance, duration
  - Inhibitor formation in severe hemophilics
    - Molecular prediction
    - Evidence that formation is lower in prophylaxis than on-demand Rx

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.

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## Future

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

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

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