Historical introduction

Christine A. Lee

University of London, London, UK
Oxford Haemophilia and Thrombosis Centre, Headington, Oxford, UK

*It was the best of times, it was the worst of times, it was
the age of wisdom, it was the age of foolishness, it was
the epoch of belief, it was the epoch of incredulity, it was
the season of Light, it was the season of Darkness, it
was the spring of hope, it was the winter of despair, we
had everything before us, we had nothing before us, we
were all going direct to Heaven, we were all going direct
the other way.*

Charles Dickens, A Tale of Two Cities *(1859)*

Early history

Hemophilia is probably one of the best examples in medicine
where basic scientific discovery has been rapidly translated
into clinical practice. Many patients with hemophilia have
been enthusiastic to participate in trials of new treatments,
and although such treatments have prolonged life, they have
also been associated with devastating side-effects.

Many people have been aware of this rare sex-linked disorder
because Queen Victoria, who reigned from 1837 to 1901,
was a carrier [1] (Figure 1). She had two carrier daughters,
Alice and Beatrice, and a son with hemophilia, Leopold [2].
Her daughter Alice was the grandmother of Alexis, the
Tsarevich, whose repeated hemophilic bleedings resulted in his
mother, Alexandra, coming under the influence of Rasputin.
It has been suggested that hemophilia may have had a profound
effect on Russian history [3]. Beatrice, born in 1856,
was the last child of Victoria and Albert. Her daughter Ena
became Queen of Spain and had two hemophilic sons,
Alphonso and Gonzalo. Beatrice had three sons, two of whom,
Leopold and Maurice, were affected with hemophilia. All
three sons served during World War I, when Maurice was
killed, but Leopold died in his late 20s following surgery [4].

Dr John Otto, a physician in the New York Hospital from
1796 to 1817, published the first medical description of hemophilia—this
was a case of a woman carrier, and the sex-linked
inheritance was noted as well as the occurrence of premature
death [5].

Bulloch and Fildes' *Treasury of Human Inheritance* has
been described "for students of haemophilia ... at once [their]

Shakespeare for its drama and human warmth and their bible
for its towering authority" with 1000 references and case
reports and 200 pedigrees of hemophilic families [1,6]. It
includes a description of seven generations of the Appleton-Swain
family, originating from a small town near Boston,
USA, from the early part of the eighteenth century to the later
years of the nineteenth. This family was first described by
Hay who noted, "None but males are bleeders ... whose
daughters only have sons thus disposed." The kindred was
re-investigated by William Osler in 1885. Many of the hemophilic
males died an early death from bleeding [6].

The natural history of hemophilia without treatment was
vividly reported in a monograph published by Carroll Birch
in 1937 from the USA. This was summarized by Biggs [7].
The cause of death in 113 patients was recorded—many died
from very trivial injury; 82 died before 15 years of age and
only eight survived beyond 40 years (Table 1).

Treatment

The first treatment for hemophilia was reported in 1840 in
The Lancet [8]. George Firmin, an 11-year-old boy, bled after
surgery for squint. Using the recently developed syringe by Dr.
Blundell (Figure 2), blood from "a stout woman" was directly
transfused and the child survived. The paper describes the
inheritance of hemophilia in the family.

Fractionation of human plasma was developed in response
to the challenges of World War II. The major components of
plasma were separated by the control of their solubility in a
multivariable system. The five variables were salt, protein,
alcohol, pH, and temperature [9]. Cohn's fraction 1 was rich
in factor VIII and fibrinogen.

McMillan pioneered the use of human factor VIII in the
USA and in 1961 he published his experience [10]. Replacement
therapy with Cohn's fraction 1 was used in 15 hemophilic
patients presenting with a variety of hemorrhagic and surgical
conditions. There was effective hemostasis in all patients.
However, mild and transient hepatitis developed in one patient
35 days after infusion (this was possibly hepatitis C).

In 1954, in the UK, Macfarlane speculated that:
*... maintenance therapy would be impracticable if only
human AHG (FVIII) were available, since it would need a
special panel of about 500,000 donors to treat the 500
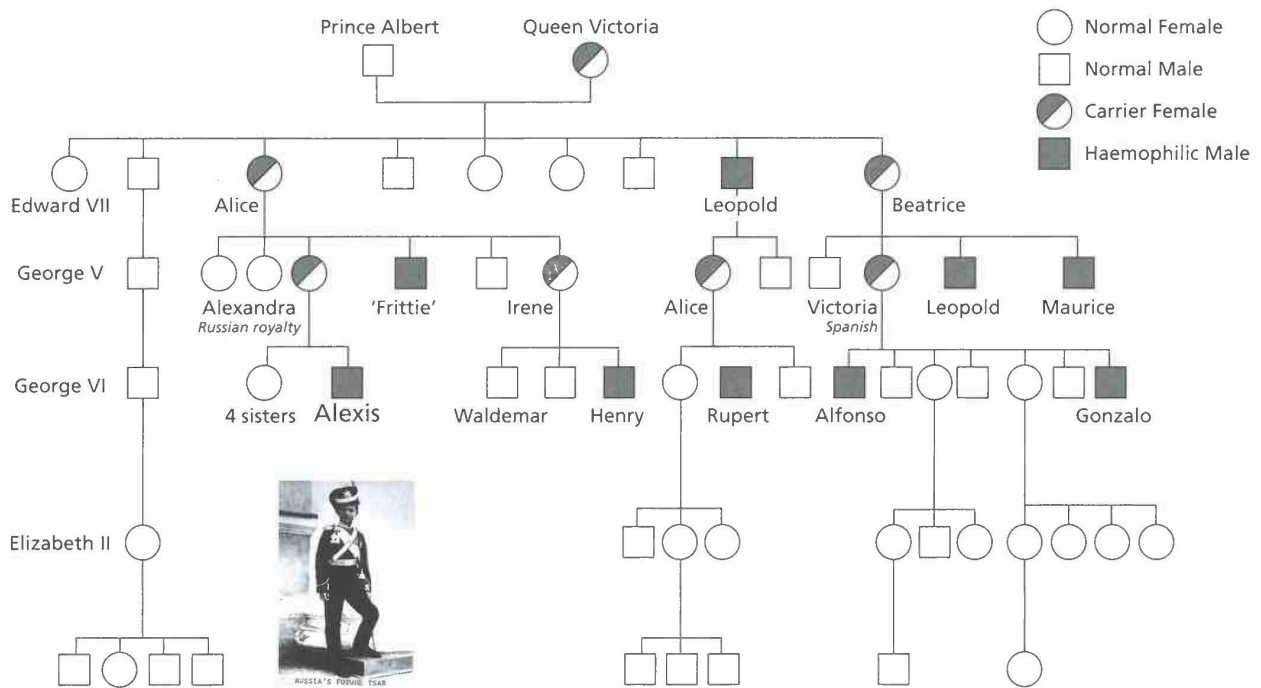hemophiliacs estimated to exist in the country (the UK) ...*

Figure 1 The family tree of Queen Victoria. Reproduced from [6] with permission.

Table 1 Cause of death for 113 cases of hemophilia by Carroll Birch.

Cause of death	No. of cases	Notes	
Operations	25	Circumcision	15
		Tooth extraction	6
		Vaccination	1
		Lanced hematomata	2
		Tonsillectomy	1
Trivial injuries	23	Cut lip, bitten tongue, injuries to forehead, finger, scalp, etc.	
Epistaxis	6	Four with serious injuries	
Hematuria	4		
Throat bleeding	3		
Cutting first tooth	1		
Fracture of leg	1		
Internal bleeding	21		
Central nervous bleeding	7		
Lung hemorrhage	5		
Intestinal bleeding	3		
Gastric bleeding	3		
Miscellaneous	4		
Birth trauma and umbilical bleeding	7		

Reproduced from [7] with permission.

Bovine blood has 16 times the anti haemophilic activity (FVIII) of human blood and enough would be available for the continuous treatment of the whole haemophilic population of this country [11].

Thus, bovine antihemophilic globulin (AHG, FVIII) was produced in Oxford, UK, and used to cover tooth extractions. The treatment was effective and the rise in FVIII was measured by the newly developed thromboplastin generation test [12]. However, the material showed some antigenic properties—an early recognition of inhibitor or antibody development. This led the Oxford group to develop an alternative animal source of FVIII—porcine FVIII [13].

The scientist Edith Bidwell led much of the early fractionation work at Oxford, and in 1961 the first patient to be treated with human FIX concentrate was reported [14]. A 4-year-old boy, with severe hemophilia B, had developed a large hematoma following a difficult venipuncture. The resulting hemorrhage had become infected resulting in osteomyelitis of the radius. A through-the-elbow amputation was performed in June 1960 under cover of FIX concentrate. The patient, aged 39 in 1995, qualified as an architectural technician, drove, and played golf [15].

The life of people with hemophilia was revolutionized by the development of cryoprecipitate. Judith Pool, in the USA, had discovered that if plasma was cooled to a very low temperature in the test tube a "cryoprecipitate" developed, which contained fibrinogen and FVIII. A method for making cryoprecipitate in a closed-bag system from a single blood dona-

Figure 2 Blundell's syringe for the direct transfusion of blood. Reproduced from [8] with permission.
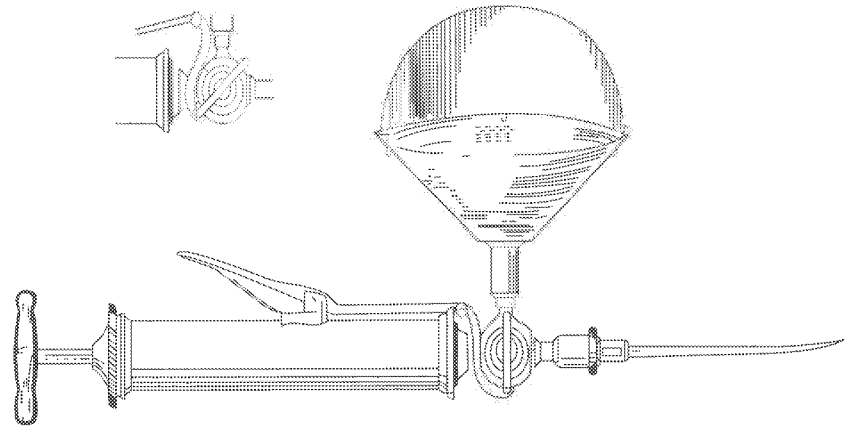
tion was described [16]. This meant that people with hemophilia could learn to treat themselves at home for the first time. Such treatment is still used in the developing world.

During the 1970s human freeze-dried (lyophilized) FVIII and FIX became available and patients were able to treat themselves more conveniently at home. The blood donors were British for the manufacture of NHS concentrates. Commercial products were manufactured from mostly American donor plasma. The donor pool size could be between 10 000 and 20 000 donations and the cryoprecipitate was produced from large-pool fresh-frozen plasma. The FVIII was extracted using ethanol and salt—Cohn's fractionation—and the final product was freeze dried or lyophilized. It was reconstituted by adding water and (self)-administered intravenously. Such products were not heated until 1985.

The availability of these products resulted in a dramatic increase in treatment. The lives of patients with hemophilia were improved because they could self-treat at home as soon as spontaneous bleeds occurred. However, they resulted in epidemics of human immunodeficiency virus (HIV) and hepatitis C virus (HCV).

Human immunodeficiency virus

The epidemic of HIV occurred during the years 1978–85 and was largely caused by USA-derived commercial concentrate. The first patient to seroconvert in the UK was treated in 1979 for abdominal bleeding and he developed non-A non-B hepatitis (HCV) followed by HIV [17]. When an HIV test became available in 1985 it was possible to retrospectively test stored samples from patients with hemophilia to establish the dates of seroconversion. In this way, a cohort of 111 patients with HIV with known dates of seroconversion was identified (Figure 3) [18]. The median age was 22 years (range 2–77) and the median date of infection was January 1983 (range December 1979 to July 1985). All these patients were coinfected with HCV either at or before the time of HIV infection.

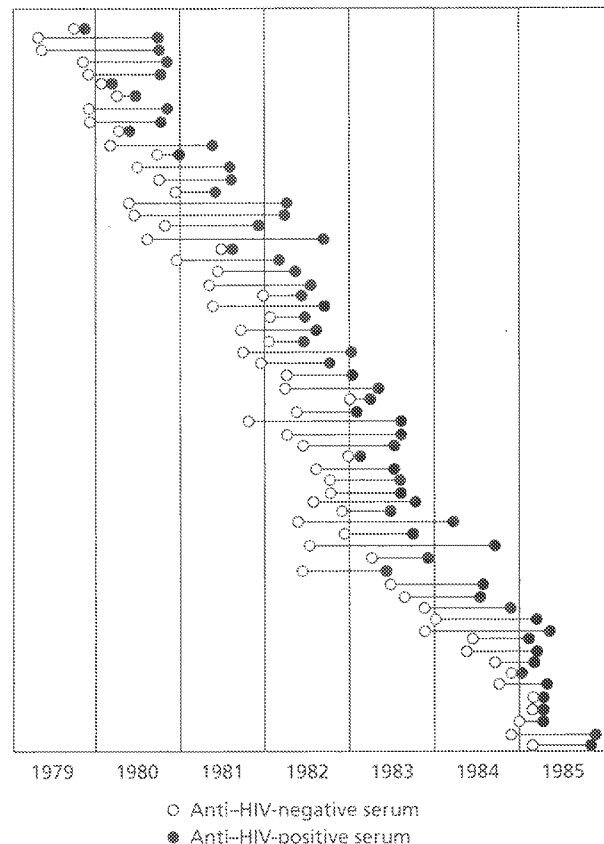○ Anti–HIV-negative serum
● Anti–HIV-positive serum

Figure 3 Patients with hemophilia and estimated dates of seroconversion. Reproduced from [18] with permission.

This cohort was closely monitored clinically, and serial CD4 counts were assessed regularly from December 1982. It was established that there was a linear decline of CD4 count from the normal of 800/μL and on average acquired immunodeficiency syndrome (AIDS) developed when the CD4 count
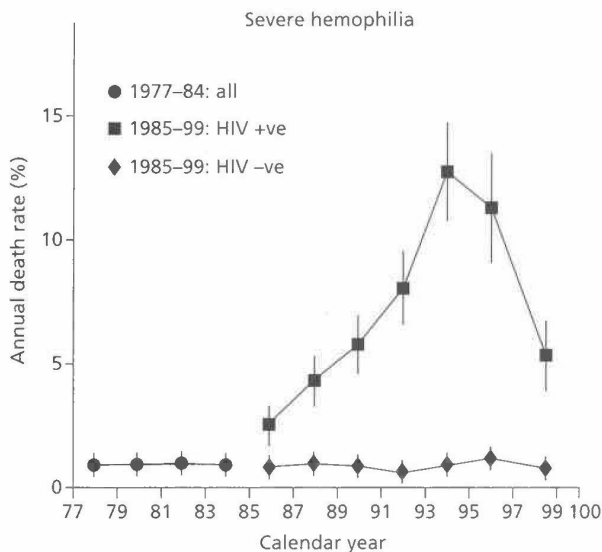
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Figure 4 Impact of HIV on mortality rates in the UK hemophilia population. Reproduced from [25] with permission.

reached 50 [19]. It was thus possible to model for each patient in the cohort the time from seroconversion when he would develop AIDS [20]. Predictions from this study in 1994 suggested that one-fourth of people infected with HIV would remain free of AIDS (without treatment) for 20 years or more. At that time this aroused much publicity because previously it had been thought that HIV inevitably resulted in rapid death.

The epidemic of HIV in hemophilic patients in the USA showed an increase in deaths per million from 0.50 in the 1970s to 60 by 1990 [21]. In the UK, 1246 of 7250 patients with hemophilia were infected with HIV. Observations on this well-characterized cohort resulted in a series of publications charting the course of the epidemic [22–25]. Highly active antiretroviral therapy became available in the early 1990s and as a result the deaths from HIV were reduced. This was shown very clearly in the patients with hemophilia in the UK (Figure 4) [25].

Hepatitis C virus

The epidemic of HCV was a much longer one, from 1961 to 1985. The first patients became infected from the first large-pool plasma-derived FIX concentrates, used in 1961, and the epidemic ended with the dry heating of concentrates in 1985. Thus, all patients with HIV were coinfected with HCV either at the time of HIV infection or before. The natural history of HCV in a population of 310 patients whose date of infection was known showed that 25 years after infection with HCV 19% had progressed to death from liver disease and that HIV was a cofactor for progression [26].

However, the first recognition that hepatitis was a hazard of blood transfusion was a publication in 1943 [27], reporting seven cases of jaundice occurring 1–4 months after transfusion of whole blood or plasma, and a publication in 1946 [28], showing the increased risk of pooled plasma. Thus, it was not surprising that large-pool clotting factor concentrates should cause hepatitis; however, this was difficult to characterize in the absence of a test for HCV until 1991. There was also enthusiasm for the new concentrates among both patients and their treaters. In a historical interview Dr. Rosemary Biggs explained, "The next thing that started to crop up was that patients started to get jaundice, and we felt at that time that they were better alive and having jaundice than dead with haemophilia" [29]. In an anonymous leader it was recognized that "In some cases early death from liver disease may be the price paid by haemophiliacs for the improved quality of life afforded by the easy availability of clotting factor concentrates" [30].

The high risk, approaching 100%, of non-A non-B hepatitis following a first exposure to plasma-derived clotting factor concentrate (irrespective of whether the donors were of NHS or USA commercial origin) was demonstrated, although the hepatitis from commercial product was more severe, with a shorter incubation period [31]. Once testing had become available it was possible to characterize the HCV epidemic in hemophilic patients more clearly [26]. Approximately one-third of those infected with HCV were also infected with HIV. It was found that the relative hazard of death for those coinfected with HIV and HCV was 19.47 compared with those infected with HCV alone [26].

Many patients with hemophilia have been "cured" or "cleared" of HCV with interferon-based therapies, most recently with pegylated interferon and ribavirin. In an international multicenter cohort study, 147 patients maintained a sustained viral response up to 15 years after treatment whereas in 148 unsuccessfully treated patients the cumulative incidence of end-stage liver failure was 13% after 15 years [32].

The ultimate cure for end-stage liver failure is liver transplantation, and a small number of transplants have been performed in hemophilic patients. A report in 2002 described 11 hemophilic patients who were monoinfected with HCV and who had been successfully transplanted. Since the liver is the site of synthesis of clotting factors, on average, 36 hours post transplant the patients no longer needed treatment with clotting factor concentrate—liver transplantation is essentially "gene therapy" for hemophilia [33].

New products

The epidemics of HIV and HCV were the stimuli to achieve safe plasma-derived products using viral inactivation processes. These were effectively introduced in 1985 and no HIV or HCV transmissions following exposure to clotting factor concentrates have occurred since that time. The first-generation products were conventionally fractionated and heated in

lyophilized state ("dry heated"). These have now been withdrawn. Second-generation products involve dry superheating at 80°C for 72 h; solvent detergent; pasteurization; and heating in hot vapor. Third-generation products are prepared by monoclonal immunoadsorption directed to either FVIII or von Willebrand factor, the carrier protein for FVIII [34].

In 1984, a series of landmark papers were published in *Nature* describing the structure of FVIII and the cloning of the gene [35]. This enabled the manufacture of recombinant FVIII and the investigation of such products in worldwide trials. The results of a study in 107 patients, including pharmacokinetics, treatment for home therapy, surgery, and in previously untreated patients (PUPs), who were mostly children, demonstrated that it had biologic activity similar to plasma FVIII and was safe and efficacious in the treatment of hemophilia [36]. This meticulous study showed, for the first time, the natural history of the treatment in PUPs and the development of inhibitors (antibodies to FVIII)—6 of 21 children developed inhibitors. It soon emerged that the three recombinant products, two full-length FVIII, and one B-domain deleted, had similar inhibitor incidences of 25% [37,38]. Inhibitors have now emerged as the biggest challenge in the treatment of hemophilia.

Variant Creutzfeldt–Jakob disease

Even though plasma-derived concentrates are very safe with respect to HIV and hepatitis transmission, and also recombinant products are used predominantly in the developed world, there remains the possibility of variant Creutzfeldt–Jakob disease (vCJD), particularly in the UK.

The peak exposure of the UK population to vCJD through the food chain was in 1998 when nearly 400 000 cattle were infected with bovine spongiform encephalopathy (BSE). There has been almost no BSE since 2000 and the small epidemic of vCJD in humans has peaked with a total 166 cases (www.cjd.ed.ac.uk).

However, there have now been four cases of transmission by blood from donors incubating vCJD [39]. Thus, surveillance of the UK hemophilia population is ongoing because many were treated with plasma-derived concentrates manufactured from UK-derived plasma between 1980 (when the epidemic of BSE began) and 2001 (when concentrates derived from non-UK plasma were used exclusively) [40]. At the time of writing, abnormal prion protein has been demonstrated at post mortem in the splenic tissue of a patient with hemophilia who died from other causes [41].

The future

The outlook for people with hemophilia is now very good. In a study of 6018 people with hemophilia in the UK between 1977 and 1998, who were not infected with HIV, the median
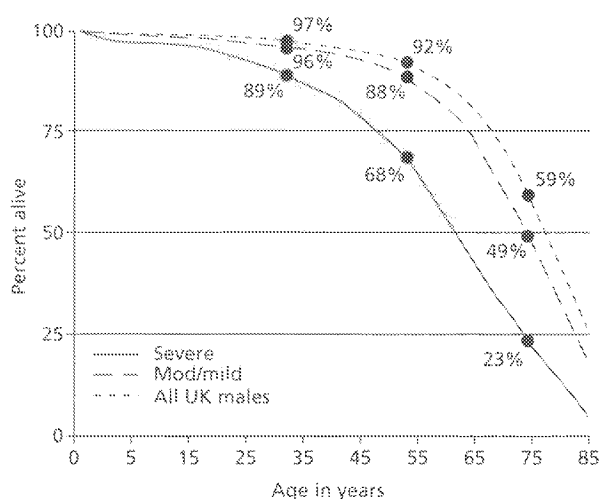

Figure 5 Survival in 6018 men with hemophilia not infected with HIV between 1977 and 1998 and in the general male UK population. Mod, moderate. Reproduced from [42] with permission.

life expectancy was 63 years for those with severe hemophilia and 75 years for those with nonsevere hemophilia. This approaches that for the normal male population (Figure 5) [42].

This new edition of *The Textbook of Hemophilia* gives a perspective on the "state of the art" in 2010. There are still many challenges, but as the history of hemophilia shows, there is no doubt that future advances in basic scientific discovery will be rapidly translated into clinical practice.

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The Tsarevitch Alexei Romanoff was a descendant of Queen Victoria who had severe haemophilia with bleeding from an early age. By the time of this photograph he already had a flexion deformity of his left hip and knee due to bleeding. As all descendants of Queen Victoria affected by her mutation had died before factor VIII and factor IX were differentiated, the type of haemophilia in the royal families of Europe was unknown until in 2009. Forensic DNA analysis of bones of the murdered Russian Royal family, recovered from graves near Yekaterinburg, prove that the royal disease was Haemophilia B, due to single base substitution creating a novel out of frame splice acceptor site at the 5′ end of intron 3. The molecule in the background is factor IX which Alexei lacked, causing the tragic bleeding that changed the course of history.

Reference: Genotype Analysis Identifies the Cause of the "Royal Disease" Evgeny I. Rogaev, Anastasia P. Grigorenko, Gulnaz Faskhutdinova, Ellen L. W. Kittler, Yuri K. Moliaka. *Science*: 326; 817 (2009).