History of Drugs for Thrombotic Disease
Discovery, Development, and Directions for the Future

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The history of the antithrombotic agents—aspirin, heparin, warfarin, and the thrombolytics—is a rich and lively odyssey of serendipity, perseverance, vision, and conflict involving a number of striking personalities. The history of aspirin spans ages and continents from Hippocrates’ analogies for women in labor to the rediscovery of the white willow bark by English country scholar Reverend Edward Stone. Bayer chemist Felix Hoffmann reinvented aspirin for his ailing father; suburban physician L.L. Craven pioneered the prophylactic antithrombotic uses of aspirin; and Sir John Vane elucidated aspirin’s mechanism of action as the inhibition of prostaglandin synthesis. Heparin was discovered by McLean, working as a medical student in 1915 in search of a pure procoagulant in dog liver. His original impure material differed somewhat from today’s heparin, but purified heparin was rapidly accepted for a myriad of clinical uses; to this day, diverse new properties of this complex glycosaminoglycan continue to be elucidated. The oral anticoagulants emerged from veterinary research in the 1920s on a hemorrhagic disorder afflicting cattle that consumed spoiled sweet clover hay. Several chance encounters led Karl Link and his University of Wisconsin team to the identification of dicumarol as the offending agent in 1939 and its widespread therapeutic use by Wright and others in the 1940s. Link later developed warfarin as a rodenticide, but its use in humans soon followed in the 1950s. Vitamin K was discovered in the 1930s; its involvement in the mechanism of the anticoagulant agents was not delineated until the 1970s. The intrinsic ability of clotted blood to liquify and the fibrinolytic properties of normal urine were noted in the 1800s. Tillett and Sherry’s group stumbled on the fibrinolytic properties of streptokinase in the 1930s and pioneered the therapeutic use of streptokinase in the 1940s and of urokinase in the 1960s. Several teams found tissue-type plasminogen activator in various body sites beginning in the 1940s, leading to its cloning and widespread use in the 1980s; anisolated plasminogen–streptokinase activator complex is an example of rational drug design. The discoverers of these diverse agents have not only provided physicians with a potent armamentarium of antithrombotic drugs but also helped elucidate much basic science and vividly demonstrated the merits of perseverance, independent thought, and adherence to the scientific method. (Circulation. 1994;89:432-449.)

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The history of the discovery and development of agents for the treatment of thrombotic disorders (antiplatelet agents, anticoagulants, and thrombolytics) is rich in historical lessons; replete with fascinating, charismatic, and often eccentric personalities; and above all is a long odyssey of stunning medical advances. The stories begin with diverse settings, problems, and personalities, but all culminate in seminal advances in the understanding of vascular biology and hemostasis and in stunning therapeutic innovations.

Most of the sagas involve serendipitous findings, encounters, or twists of fate, which so often surround scientific advances. Many of the discoveries were made by individuals with little direct experience in the new fields where their research had led them. Rather than abandoning their work when faced with frustrating and inexplicable findings, they pursued interesting leads that often led them in a direction opposite to their initial goal (eg, many workers who discovered potent inhibitors of thrombosis were initially searching for procoagulants). Often, parallel work was proceeding in laboratories on either side of the Atlantic; in some cases, lines of discovery merged or diverged; in others, they continued ignorant of each others’ existence. Discovery and development were not linear but proceeded in fits and starts; many important findings were ignored, only to be rediscovered or unknowingly replicated decades (or centuries) later. Importantly, all of the discoveries in antithrombotic agents led to important advances in our understanding not only of antithrombotic processes but also of procoagulant systems.

The cast of characters come from Europe, Canada, and the United States; from esteemed and venerable urban centers of academia such as the Karolinska Institute, Johns Hopkins, Cornell, the Mayo Clinic, and Bellevue Hospital, as well as from unexpected locales such as the agricultural and veterinary facilities of Alberta, North Dakota, and Wisconsin; from the Oxfordshire countryside and nearby marshlands; and even from a private medical practice in Glendale, Calif. The innovators came from diverse backgrounds as country gentleman scholars and pastors, medical students, chemists and pharmacologists, veterinary pathologists and agriculturalists, or clinicians in medicine and surgery; working in academia, industry, or privately, they studied compounds ranging from simple organic acids and polycyclic compounds (Fig 1) to large sulfated carbohydrate chains to large glycoproteins. Their compounds originally came from plant, animal, human urine, cell culture, or bacterial broth sources; the current versions are prepared by simple chemical synthesis (eg, aspirin, warfarin), purification from animal tissues (eg, heparin and related compounds), bacterial cultures (eg, streptokinase [SK]...
and its variant anisoylated plasminogen–streptokinase activator complex [anistreplase]), human cell cultures (eg, urokinase [UK]), or by recombinant DNA technology (eg, tissue-type plasminogen activator [TPA]). Finally, the stories highlight the basic virtues of the scientist: clear rational thought, perseverance, hard work, and an open mind to new and unexpected findings and ideas. The end result of these adventures has been a diverse and steady stream of agents of protein importance to the public health and at least two Nobel Prizes in Medicine.

This review will be limited to the most important agents in current use; miscellaneous agents such as ticlopidine, dipyridamole, dextran (purified from bacterial cultures of Leuconostoc mesenteroides), and ancord and reptilase (natural snake vipers venoms) are not covered further given their sparse use and relatively brief histories.

Aspirin: Marsh Plants, Arthritic Fathers, and Corporate Intrigue at Bayer

The saga of one of the most commonly used drugs worldwide (and certainly the most commonly used antithrombotic agent) is the oldest in the history of these drugs. Aspirin is also the only antithrombotic drug discovered and developed completely outside the United States, although US clinical research in the past few decades has been instrumental in delineating the myriad clinical roles for this true and original “wonder drug.” The annual US production of aspirin in 1985 was estimated to be 28.2 million lb; in the United States alone, 20 to 30 billion pills are purchased annually. It is estimated that Americans use 100 tablets of aspirin per citizen per year; about 15% of the population takes aspirin at least once a week.

Unlike the anticoagulant and thrombolytic drugs, which are used only for thrombotic disorders, aspirin (ie, acetylsalicylic acid [ASA]) is true to its title of wonder drug, still being a gold standard for antipyretic, analgesic, antiplatelet, and anti-inflammatory agents. Hippocrates extolled the analgesic properties of salicylates, and subsequent ancient physicians reported their potent antipyretic and anti-inflammatory actions; recognition of their antiplatelet activity would not come until the 1940s.

The first recorded descriptions of therapeutic benefits of extracts of willow bark and other plant sources of salicylates were made by Hippocrates, the father of modern medicine, 2400 years ago. He recommended chewing willow leaves for analgesia in childbirth and the juice of the poplar tree for eye diseases. Subsequent reports on the analgesic effects of willow leaves include those by Dioscorides, a Greek surgeon in the Roman military service, in 75 AD (he also found it useful in treating gout); Pliny the Elder, a first-century AD Roman encyclopedia writer (who also detailed analgesic and other uses of poplar bark, leaves, and gum); and Galen, a Greek-educated Roman physician, in his second-century AD encyclopedia. Galen also first described the antipyretic and anti-inflammatory properties of willow leaves. Throughout the Middle Ages, medical references and writings detailing folk remedies described the medicinal value of willow leaves, roots, and bark; the wintergreen plant; the poplar tree; and other sources of salicylates.

Early use of salicylate-containing plants was not confined to Europe; in the New World, Native Americans used willow tree bark for antipyretic purposes. Early Pennsylvania Germans, possibly on the advice of Native Americans, used extracts containing salicin (a natural salicylate compound) made from a plant called Shepherd’s purse to treat fevers caused by dysentery. In 1798, Longmore reported that tea containing wintergreen was frequently used by Canadians in Quebec for antipyresis.

The modern history of salicylates begins with Reverend Edward Stone of Chipping-Norton in Oxfordshire, England, around 1757. At the time, the preeminent antipyretic drug was quinine, a derivative of the bark of the cinchona tree (“Jesuit’s bark” or “Peruvian bark”). The cinchona tree was indigenous to South America and a coveted and expensive import to the Old World; all attempts at cultivation in the Old World failed. Cinchona bark was found to be successful in treating malaria in 1630 and was then used successfully in other febrile disorders. A cheaper, locally available alternative agent was greatly needed in Europe, especially during disruptions in supply that occurred during the wars of the period.
Reverend Stone set out in search of a cinchona substitute, guided by some simple principles. The first was the prevailing view at the time that many cures for illnesses were to be found right where the illness itself most commonly occurred. Aware of the old wives' tale about the white willow tree bark's use, Stone tasted it in 1757 and was impressed by its severe bitterness, reminiscent of Jesuit's bark. He wrote, "As this tree delights in a moist or wet soil where agues [fevers] chiefly abound, the general maxim that many natural maladies carry their cures along with them, or that their remedies lie not far from their causes, was so very apposite to this particular case that I could not help applying it. That this might be the intention of Providence had some little weight with me." In line with the then-popular "Doctrine of Signatures," fevers were thought to be more common in marshy areas where the white willow tree, *Salix alba vulgaris* (Fig 2), abounds, providing an appealing, vaguely theological rationale for its use.

Stone spent the next 6 years performing detailed, carefully planned clinical studies that would impress modern clinical trialists on approximately 50 patients suffering from inflammatory disorders and agues (fevers), with uniformly encouraging results. He reported his findings in a letter (Fig 3) to the Earl of Macclesfield, president of the Royal Society, dated April 25, 1763, entitled "An account of the success of the bark of the willow in the cure of agues." While Stone's account first called the medical community's attention to the willow, it is clear that the lay public was already quite aware. Buchner reported in 1838 that in Germany, the use of willow bark as an antipyretic was long known, and in 1858, Cazin reported similar familiarity with willow bark's antipyretic effects among French peasants.

Additional medical reports on the willow's antipyretic and analgesic effects emerged in 1772 by Gunzius; in 1778 by van Geuns; in 1792 by Samuel James, a surgeon from Hertfordshire; in 1798 by William White, an apothecary from Bath who made partial inroads into isolating the active principle; and in 1803 by G. Wilkinson, from Sunderland. James and White reported similar effects of a willow related to Stone's white willow, *Salix latifolia*. Stone's impressive accounts provided scientific credence to the already prevalent use of willow bark for fevers and inflammatory illnesses and provided the impetus for the next step, that of isolating and purifying the active principle for further study and production for clinical use. This next phase in aspirin's development had to wait a half-century until chemistry and pharmacology could catch up to the clinical and observational acumen of Stone and others.

Due to the superiority of continental scientists in chemistry and pharmacology, the setting for most of the next phase in the story of aspirin shifts back and forth...
between the competing French and Germans. The earliest attempts at isolation of an active principle from a natural source of salicylates were made by Fontana and Brugnatelli while working with willow bark in Italy between 1826 and 1829; they isolated small amounts of the glycoside compound salicin. In Munich in 1828, Buchner also isolated salicin. Leroux achieved the same feat in Paris in 1829 and demonstrated its antipyretic activity. Merek also isolated salicin from willow bark in Darmstadt in 1833. On hydrolysis, salicin yields glucose and salicylic alcohol; the latter can be converted into salicylic acid either in vivo or in the chemistry laboratory. Pagene-stecher, a Swiss pharmacist, distilled salicylaldehyde from the meadowsweet or queen of the meadow plant (Spiraea ulmaria, now Filipendula ulmaria) in 1831. In 1835, the Swiss chemist Lowig was the first to prepare salicylic acid (which he named "Spisaure"; Spir is for the genus Spiraea, and saure is German for acid) from salicylaldehyde. Shrubbs of the Spiraea genus (Fig 4), related to the rose, were found to contain salicylates in greater quantities than the willow. Salicin occurs in the willow and poplar trees and in the black haw plant. Methyl salicylate occurs in beech and birch trees, coffee, licorice, olive, wintergreen, and various shrubs and grasses. Small amounts of salicylates are found in orange, apple, strawberry, cherry, plum, raspberry, grape, and numerous plants and shrubs. Even one animal product, beaver castor (a glandular secretion), contains salicylates.

Piria, working in Paris, isolated salicylic acid from the willow bark glycoside salicin in 1838. In 1839, Dumas showed that Lowig's and Piria's compounds were identical; they both prepared salicylic acid, one working with salicin from willow bark, and the other working with salicylaldehyde from meadowsweet. Another plant source would yield salicin; in 1843, the American William Procter and the Frenchman Auguste Cahours isolated methyl salicylate from oil of wintergreen (Gaultheria procumbens and G. hispidula), another old folk remedy for "agueish disorders." The following year Cahours would hydrolyze methyl salicylate to salicylic acid, thus devising a third route to this compound. Advances were now steady in the isolation and purification of salicylates from natural sources, but widespread use still awaited the advent of synthetic salicylates, which would allow for mass production.

The next phase, that of synthetic production, occurred mainly in Germany due to German preeminence in synthesis at the time. Gerland was the first to synthesize salicylic acid in 1852. Although a French chemist, Charles Gerhardt, was the first to synthesize aspirin in a crude form in 1853, the compound was ignored, and Gerhardt has always remained obscure in the aspirin story despite being the first of several "inventors" of aspirin. Aspirin briefly resurfaced in 1859 when it was prepared by von Gilm and again in 1869 when the German Karl Kraut found an improved synthetic method of making ASA. Again, it was ignored, as was Kraut. In 1860, Kolbe and Lautemann at Marburg University developed a practical method of preparing salicylic acid from phenol in large quantities; Kolbe would later join the Bayer Company.
one of his students, von Heyden, set up the first large factory dedicated to synthetic salicylate production in Dresden. This allowed for bulk production, resulting in widespread commercial use and the rekindling of clinical research to follow-up on Stone’s classic observations. Some of the first commercial uses of salicylic acid were as a surgical antiseptic and for preservation of food, milk, and beer. Fig 5 summarizes the complex chemistry and history of the various salicylate derivatives and their plant precursors.

In 1875, Buss reported the antipyretic effects of synthetic salicylic acid. Separate clinical reports in 1876 by Stricker and Riess in Berlin and by Maclagan in Dundee described successful treatment of acute rheumatic fever, typhoid fever, and miscellaneous inflammatory conditions with salicylic acid and salicin, respectively. In 1877, the Frenchman See reported similar success in treating gout and chronic arthritis with sodium salicylate. Part of aspirin’s efficacy in gout was explained by Campbell’s 1879 report on its uricosuric properties. As with its antipyretic action, the use of salicylates in inflammatory disorders was new to physicians but not to the layman. Soon after the publication of his report, Maclagan received a letter from a Dr Ensor at Cape of Good Hope, in which he noted that the Hottentots of Africa had long used willow bark in treating rheumatic diseases. As early as 1865, a lay book on home remedies noted that David Mowry of Ohio recommended an elixir containing yellow poplar bark for treating rheumatism. Use of salicylate, sodium salicylate, phenyl salicylate, and methyl salicylate grew but was marked by often severe gastric toxicity. Methyl salicylate was also used as a flavoring for soft drinks and candies and as a topical analgesic; salicylate is still used as a topical keratolytic for warts and corns. The stage was set for a dramatic turning point involving serendipity, rediscovery, intrigue, obstinence, and, finally, unimagined success.

The salicylate trail led to a small dye and pharmaceutical firm, the Bayer Company. Founded by the chemist Friedrich Bayer in 1863 in Barmen, Germany, the company moved to Elberfeld, Germany, after Bayer’s death and developed aniline-based dyes, derived from coal tar products. Bayer’s drug division was headed by Carl Duisberg and divided into a pharmaceutical branch headed by Arthur Eichengrun and a pharmacologic branch headed by Heinrich Dreser from the University of Bonn. Eichengrun chose to develop a much needed version of salicylic acid with a better toxicity profile. He happened to assign the job to 29-year-old chemist Felix Hoffmann (Fig 6) in 1893, who had previously been assigned the task of finding new yellow aniline dyes. It is now legend that Hoffmann’s father suffered from crippling chronic arthritis and was intolerant of salicylic acid; he begged his son to find him a remedy. Hoffmann searched the literature on salicylate derivatives, found the works of Gerhardt and Kraut, and developed an improved synthetic pathway to ASA between 1893 and 1897. On October 10, 1897, Hoffmann described the new synthetic process in his laboratory notebook. Hoffmann reportedly tested the rediscovered agent on himself and then on his father. His father improved markedly, and a legend was born. Eichengrun enthusiastically passed ASA on to Dreser, who rejected it due to an erroneous belief that it was cardiotoxic. The drug was again shelved; Dreser was preoccupied with his recent successful cough suppressant for Bayer, heroin (incidentally, it too was a rediscovered drug).

In 1898, Eichengrun reportedly tested ASA on himself, disproving the notion of cardiotoxicity, and surrep-
titiously gave it to Berlin physicians to test on their patients. Their reports were glowing, and one of the physicians, Felix Goldmann, gave Bayer management enthusiastic feedback. Dreser still scoffed at ASA, but Duisberg himself intervened and submitted the drug for independent testing. When the results were again glowing, Dreser wrote a paper promoting ASA in 1899 (and described his pharmacokinetic studies of the drug on himself) and ignored the contributions of Hoffmann and Eichengrun. Within months, the first reports from physicians regarding the therapeutic use of aspirin were published by Witthauer (who reported salutory effects on pain and rheumatic fever) and Wohlgemut (who also reported benefit in rheumatic fever). Also in 1899, F.C. Floeckinger of Texas published the first English-language account of ASA.

Dreser, faced with instant and widespread success, turned to the next problem. The cumbersome name “acetyl salicylsäure” was not only hard to remember, but as a generic name it could not be patented and thus had to be replaced by a new trade name. Hoffmann preferred “a-salicin” or “a-salicyclic” (abbreviating the acetyl substituent); Dreser again rejected Hoffmann and introduced the name “aspirin” (a for acetyl, spir for Spirsaure or the genus Spiraea, and -in as a popular suffix for drugs of the times) in January 1899. The drug was introduced commercially 5 months later, and the drug’s merits carried it to the top of sales charts worldwide, displacing older, more gastrotoxic salicylates such as salicylic acid, sodium salicylate, and phenyl salicylate as well as nonsalicylate analgesic/antipyretics such as phenacetin and antipyrine.

Bayer rose to the heights of the pharmaceutical world and was the sole distributor of aspirin in the United States through its subsidiary Bayer Company of New York until 1917. In that year, the US patent expired, and the Monsanto Company of St Louis began making aspirin. During World War I, the New York branch of the Bayer Company (and their state-of-the-art factory in Rensselaer, NY) fell out of German control when the US government seized the firm as being held by enemy aliens and put it up for auction in 1918. Sterling Products (now Sterling Drug Inc, a division of Sterling-Winthrop), then a small patent medicine firm based in West Virginia, purchased the rights to the US Bayer. However, in 1921, they lost control of the name “aspirin” and the compound itself in court; both permanently entered the public domain in the United States. Today, five other US firms make aspirin in addition to Sterling. The parent Bayer Company became a subsidiary of the
German dye and pharmaceutical giant I.G. Farben in 1925 and is known today as Farbenfabriken Bayer GmbH or Bayer AG, of Leverkusen, Germany.2 The United States, United Kingdom, and France are the only nations in the world where aspirin has become a generic drug name, made by several different manufacturers under the name “aspirin.” Elsewhere, Bayer AG retains trademark rights to the name of Aspirin and is the sole manufacturer of “Aspirin” in 70 countries, whereas Sterling-Winthrop, Inc, owns similar trademark rights in Canada; other manufacturers may market the compound only as ASA or a brand name other than aspirin.3

Aspirin’s use soared due to its antipyretic, analgesic, and anti-inflammatory properties, not due to its antithrombotic effects. As early as 1891, Binz in Germany noted that salicylic acid enhanced mucosal bleeding in patients,2 but it would be a half-century before ASA’s antithrombotic properties were first exploited. Interest in its antithrombotic effects did not resurface until 1940, when Karl Link, the discoverer of the oral anticoagulants dicumarol and warfarin, discovered a remarkable coincidence that intertwined the histories of ASA and the oral anticoagulants: dicumarol spontaneously breaks down into salicylic acid. Experimenting on himself, he showed that ASA can reduce prothrombin activity and was thus a weak anticoagulant; he published several reports in 1943 on ASA’s anticoagulant effect, but he considered this a dangerous side effect rather than a therapeutic effect and returned to dicumarol research in the mid-1940s.13 Goven14 also described a hypoprothrombinemic effect of ASA in 1946.

Gibson15 in 1948 was the first to propose that ASA be tried as a treatment for vascular diseases; he treated five patients with thrombophlebitis, temporal arteritis, or angina with success, reported this small series in 1949,16 and called for extensive trials of ASA for thrombotic disease. The next step was taken by L.L. Craven, an otorhinolaryngologist in private practice in Glendale, Calif, in the late 1940s. He noted that when he gave his tonsillectomy patients Aspergum (containing ASA) for analgesia, only those who used the gum excessively bled excessively. In 1948, he began treating all his older male patients with ASA to prevent myocardial infarction (MI). In 1950, he reported to the Annals of Western Medicine and Surgery17 in a brief letter to the editor that none of 400 patients taking ASA developed MIs. In two subsequent articles in the obscure Mississippi Valley Medical Journal,18,19 Craven extended the claim of no MIs to more than 8000 patients in this uncontrolled yet astonishing and prescient series. Interestingly, Craven also described a subset of patients treated with ASA as secondary prophylaxis in addition to the primary prevention cohort; they, too, were protected without exception. Finally, Craven reported complete protection from major strokes in his series.19 At the time, there was great debate about the role of dicumarol for MI; due to the crudeness of Craven’s data as well as the obscurity of his journals, his work was largely ignored. Ironically, Craven died of an MI the following year, despite adhering to his own advice regarding ASA.2

Even these early proponents of the use of ASA as an antithrombotic had no idea of the mechanism of any of its actions. Quick20 had noted as early as 1944 that aspirin prolongs the bleeding time, but Link’s work on its weak hypoprothrombinemic effects was given wider notice. In 1967, Weiss et al21 found that ASA’s bleeding diathesis was due to inhibition of platelet activation by collagen; this was confirmed by O’Brien.22 However, the mechanism of the platelet dysfunction still remained unexplained until John Vane’s historic report in 1971.23 Vane and coworkers found that aspirin inhibited prostaglandin synthetase, explaining most of its antipyretic, anti-inflammatory, and antiplatelet effects. Vane (now Sir John Vane) received the Nobel Prize in Medicine for this work in 1982.


Likewise, evidence began to mount regarding ASA’s efficacy in secondary prophylaxis of MI with a series of studies in the 1970s and 1980s, beginning with the 1974 report of Elwood et al26 and that of the Coronary Drug Project aspirin study in 1976.27 Evidence favoring ASA’s benefit in primary prophylaxis of MI (although still considered controversial by some) began with an obscure geriatric study with negative results by Heikinheimo and Jarvinen28 reported in 1971 and continued with the positive results of the Boston Collaborative Drug Surveillance Group report in 1974.29 In 1988, the ISIS-2
study proved ASA's role in treating MI in the acute phase. Similarly, the 1983 report by a Veterans Administration Cooperative Study\textsuperscript{31} on ASA in unstable angina extended the proven benefits of the drug.

ASA was granted "pre-1938" exemption status to the US Food and Drug Administration (FDA) approval process when the agency was established; therefore, it was never subjected to FDA scrutiny for its use as an antipyretic, analgesic, and anti-inflammatory agent. However, its use as an antithrombotic did have to undergo FDA approval for labelling for new indications such as TIA prevention (1980) and secondary prophylaxis for MI and for unstable angina (1985). Oddly, despite much supporting evidence and obvious public health implications, FDA approval is lacking for use in acute MI, primary prophylaxis of MI, and use after balloon angioplasty or coronary bypass surgery. Finally, several studies are currently examining the role of ASA in preventing thromboembolism associated with atrial fibrillation. Other current research is attempting to define the optimal or lowest effective dose of ASA for its various antithrombotic indications and the possible effects of ASA with or without oral anticoagulants in preventing progression of atherosclerotic disease.

Compared with Stone's willow bark powder and other plant sources of salicylates, current commercial ASA is prepared by chemical synthesis. Beginning with coal tar or light petroleum fractions, salicylic acid is synthesized and then acetylated.\textsuperscript{3}

Aspirin is, of course, not the only antiplatelet agent; several novel and potent antiplatelet agents that capitalize on diverse mechanisms recently have been developed. They include thromboxane synthesis inhibitors and/or receptor antagonists (sulotroban, dazoxiben, ridogrel, and others), antibodies to glycoprotein (Gp) IIb/IIIa, synthetic or natural snake venom-derived "RGD" peptide fragments (or "disintegrins") that block fibrinogen-Gp IIb/IIIa interactions, serotonin antagonists, and prostacyclin analogues. However, none of the scientifically elegant, newer drugs have in any way displaced aspirin.

The history of aspirin spans more than two centuries, two continents, and several blind alleys as well as fortuitous breakthroughs, culminating in ubiquitous use for myriad indications, including a broad range of thrombotic disorders. Aspirin continues to be a billion-dollar industry, and its diverse actions continue to fascinate scientists and the lay public alike. The wide public interest is exemplified by the success of the 1991 book detailing much of the aspirin story, The Aspirin Wars.\textsuperscript{2}

**Heparin: Dog Livers, Procoagulants That Turned Anticoagulant, and Mr Schulte**

The story of the discovery and development of heparin and related glycosaminoglycan anticoagulants is not as old as that of ASA, but it is no less fascinating, instructive, or important. Like the ASA story, the heparin saga involves many brilliant scientists on two continents, several chance findings, and many obstacles to success. Unlike aspirin, heparin involves a natural animal source rather than a plant source, and early workers were studying hemostatic effects rather than other properties, such as antipyresis or analgesia. However, in some ways, the heparin story is even stranger than that of aspirin since its discoverer was not looking for a natural anticoagulant substance; in fact, he set out to characterize natural procoagulants. Although many would have been discouraged by findings opposite to their goal, Jay McLean had the insight and perseverance to pursue strange leads. What he discovered as a medical student working in William Howell's laboratory at Johns Hopkins in 1915-1916 was a powerful, natural anticoagulant that is still used universally for a variety of venous and arterial thrombotic disorders and is today under great scrutiny for its promise as a cellular growth factor modulator in a variety of vascular cell types.

The heparin story begins decades before McLean's historic medical student laboratory project with early studies on the physiology of hemostasis in the late 1800s. The basic details of hemostasis were just beginning to emerge, and most attention was first placed on procoagulant systems; endogenous anticoagulant systems were to be discovered and exploited later. In 1880, Schmidt-Mulheim\textsuperscript{32} reported on "peptone shock," an early experimental model for the study of hemostasis. An endogenous anticoagulant originating in the liver was released after experimental injection of peptone in the dog; later workers would identify the water-soluble substance as heparin.\textsuperscript{33} Pavlov\textsuperscript{34} also described a water-soluble tissue extract as an anticoagulant in 1887 but was unable to characterize it further. Morawitz\textsuperscript{35} noted in 1905 that extractions of tissues by organic solvents yielded procoagulants, whereas aqueous extracts of the residue after extraction with organic solvents showed anticoagulant activity. Biedl and Kraus\textsuperscript{36} reported in 1909 that similar to peptone shock, the blood became incoagulable in anaphylactic shock due to release of an endogenous anticoagulant. In 1911, Doyon et al\textsuperscript{37} also isolated a water-soluble dog liver anticoagulant after peptone shock.

In 1912, William Howell of Johns Hopkins was the world's most prominent expert in coagulation, and he was studying the procoagulant effects of a dog brain thromboplastin extract he called cephalin. The stage was set for a fortuitous, historic chain of events.\textsuperscript{38} Perhaps the most important step was the first; medical student Jay McLean, a San Francisco native, decided to leave the University of California and transfer to Johns Hopkins to prepare for a planned career as an academic surgeon. Despite being denied admission to Johns Hopkins, McLean left California, worked at several menial jobs to earn money, made the long journey to Baltimore, and presented himself to a stunned admissions office at Johns Hopkins requesting admission to his chosen school. He offered to work in a research laboratory for a year awaiting admission to the medical school; this offer was accepted the next day. McLean immediately called on Howell and announced his desire for an academic career in surgery and for a year (1915-1916) of physiological research in Howell's laboratory. He was given the job of isolating the true active thromboplastin principle of the crude brain mixture cephalin.

Cephalin was a mixture of phospholipids; McLean found that when it aged, it lost its procoagulant activity. He prepared phosphatides from other tissues, including Erlandsen's courin (from dog heart) and Baskoff's hep-araphosphatide (from dog liver) in an effort to find a purer procoagulant from which to start his isolations.
Instead, in January or February 1916, he found that aged cephalin, curcin, and especially heparphosphatide not only lost their thromboplastic action but, with time, became actively anticoagulant. McLean had discovered heparin, although as will be discussed, this substance may be different from what we today call heparin.

Stunned by the discovery, he told his mentor, “Dr Howell, I have discovered antithrombin.” Howell was skeptical; McLean placed a beaker of blood on Howell’s desk and added “heparphosphatide” (the name “heparin” had not yet been invented); the blood never clotted. Howell remained unconvinced, thinking that any antithrombin present must be a protein, not a lipid. Nonetheless, Howell dropped all his other work to take up research on the natural anticoagulant.

McLean announced his discovery in an oral presentation on February 19, 1916, before the Society of the Normal and Pathological Physiology at the University of Pennsylvania and reported his discovery in print in 1916 in the only paper he would write on what was later to be called heparin, innocently entitled “The Thromboplastic Action of Cephalin.”/!

Amazingly, Howell was against reporting anything about the discovery of the anticoagulant either at the meeting or in the paper. McLean, seemingly devoid of his fervor for Johns Hopkins, accepted a research fellowship at the University of Pennsylvania the following year and dropped the work. He later tried to return to work in the field but was too busy with his clinical surgical practice (inexplicably, he never went into the academic career he sought in 1915)./!

Looking back, the world benefitted immeasurably from McLean’s brief dabbling with research. McLean died suddenly in 1957 at the age of 66 while he was in New York at the invitation of Irving Wright (a pioneer in the clinical use of both heparin and warfarin) for the purpose of participating in a historical symposium on the discovery of anticoagulants. The partially completed memoirs of his work at Johns Hopkins were included in the symposium’s proceedings, which were published in Circulation in 1959.

Meanwhile, Howell seized on the substance he initially shunned (much like Dreser, the Bayer “propo-
nent” of aspirin). In 1918, Howell and Holt/ renamed heparphosphatide “heparin” and confirmed its solubility in organic solvents. They also discovered that heparin could be found in numerous other organs. However, in 1922, Howell/ reported under the same name “heparin” an anticoagulant from dog liver that was water soluble; this was in retrospect a new and very different substance than McLean’s phospholipid (soluble in organic solvents) or Howell’s 1918 heparin, as first pointed out by Reinert and Winterstein in 1939. Silver et al/ demonstrated in 1959 that McLean and Howell’s original phosphatide “heparin” was likely a mixture of phospholipids, inositol phosphates, sphingomyelin, and phosphatidylserine; all turn out to have anticoagulant activity. Howell’s “new” heparin was strangely similar to Doyon’s 1911 water-soluble anticoagulant from peptone shock. Further purification by Howell and Holt between 1922 and 1928/ revealed that the new heparin was not a lipid of any kind but in fact a sulfated carbohydrate (consistent with its aqueous solubility) that was present in peptone shock blood. Neither McLean nor Howell bothered to obtain a patent for heparin; Howell turned over his techniques without charge to the local Baltimore pharmaceutical firm Hyn-
son, Westcott, and Dunning. The firm was unable to produce dog liver heparin with better than 1% to 2% purity; the material was too toxic and of too low activity for even animal use. Baltimore suddenly became a dead end for heparin.

Others tried to administer crude heparin to animals and humans; all encountered limiting toxicity. Mason reported in 1924 that heparin used to anticoagulate transfused blood produced severe toxic effects in humans/; in animals, they were able to demonstrate the prevention by heparin of experimental intravascular thrombosis induced by injection of thromboplastin. Reed/ reported in 1925 that heparin was an effective anticoagulant in the dog. In 1927, Shionoya/ found that heparin prevented in vitro blood clotting. Godilowski/ also treated humans with heparin in 1933 but found his preparation to be of low potency.

The next critical step obviously was that of purifying heparin adequately; otherwise, it seemed to be doomed as a potential therapeutic agent. The void was filled by three teams of scientists working independently in Toronto, Stockholm, and Copenhagen. C.H. Best (of “Banting and Best” fame, the team that isolated insulin) led the Toronto team, beginning their studies on heparin in 1929; Charles and Scott/ from their team produced pure crystalline heparin in 1933 and further perfected their techniques from 1933 through 1936. Interestingly, Jaques/ claims that once again, the heparin of Charles and Scott was a new heparin, different certainly than McLean’s phosphatide but also different than Howell’s 1922 carbohydrate heparin. The source was different (beef lung), most likely explaining the difference in properties from Howell’s dog liver heparin. Schmitz and Fischer of Copenhagen also purified heparin, also in 1933. In Stockholm in 1935, Jorpes/ also purified heparin and solved its chemical structure, confirming it to be a highly sulfated glycosaminoglycan.

Hedenius and Wilander/ injected Jorpes’ purified heparin into human volunteers successfully with no ill effects in 1935, marking the dawn of the clinical era of heparin. The critical technical advances in purification pioneered in Toronto and Copenhagen were essential for clinical investigations, which followed rapidly.

A young Stockholm surgeon from Sabbatsberg Hospital, Clarence Crafoord, had a reputation for his skill in performing pulmonary embolectomies in severe cases of pulmonary embolism. His experience with this disease spurred him to approach Jorpes in 1929, when Crafoord proposed clinical trials of the new anticoagulant in preventing venous thromboembolism in surgical patients. Jorpes sadly turned him away after explaining that the crude extracts of the time were too toxic. But in 1935, Jorpes remembered Crafoord’s interest, and this time he approached the surgeon with purified heparin. In August 1935, Crafoord began to treat general surgery patients postoperatively with intravenous heparin to prevent the disease he was noted for treating surgically.

In May 1935, similar studies of heparin for prophylaxis of venous thromboembolism had begun at the University of Toronto under the surgeon Gordon Murray; each found almost complete prevention of thrombosis in separate reports published in 1937. Similar glowing reports of prophylaxis of venous thrombosis in Swedish gynecologic and obstetric series were reported.
by Wetterdal and Leissner in 1938 and 1939. Finally, heparin had been shown in humans to be well tolerated, effective hematologically in anticoagulating the blood, and effective in preventing thrombosis.

All that remained to secure its role as a miracle drug for myriad thrombotic conditions was to demonstrate therapeutic efficacy in patients with established thromboses. Anecdotal but impressive clinical reports began to emerge very soon after the stunning (although uncontrolled) prophylaxis data. Holmin and Ploman, each reported single cases of successful treatment of central retinal vein thrombosis with heparin in 1938. Also in 1938, Magnusson used heparin successfully in a case of posterior inferior cerebellar artery thrombosis. Soon, larger but still uncontrolled series of patients with venous thromboembolism and impressive responses to heparin were reported by Murray and Best in 1938, by Crafoord in 1939, by Magnusson in 1940, and by Bauer in 1941.

All of the above patients had been treated in Canada and Sweden. The first patient in the United States to receive purified heparin was Arthur Schulte, a young man with “malignant recurrent thrombophlebitis,” in 1939. His physician was Irving Wright, a pivotal clinician in the stories of both heparin and dicumarol. Dr. Wright was among the first clinicians to use these anticoagulants, and he treated Mr. Schulte first with heparin, later with dicumarol, and then later still with warfarin. In fact, both Dr. Wright and his patient are still alive and well; Mr. Schulte is still on warfarin (he has been anticoagulated longer than anyone in the world), and whenever he is briefly off warfarin (due to bleeding), his thrombophlebitis recurs. Dr. Wright was among the first to report on clinical success with dicumarol in 1941, was the first clinician to treat MI patients with dicumarol in the mid-1940s, and ran the American Heart Association’s trial of dicumarol for MI in the late 1940s (later becoming its president).

Like Crafoord before him, personal experience with venous thromboembolism prompted Wright’s initial involvement with heparin. Wright developed appendicitis in 1938 and then postoperative thrombophlebitis of the legs; he was very ill for months due to the lack of any effective treatment in the United States at the time. When Schulte consulted Wright in late 1938, Wright contacted Best in Toronto, who graciously came to New York in 1939 with a precious supply of purified heparin. It was given by continuous intravenous infusion to Schulte for 16 days until the supply ran out; Schulte recovered fully.

The late 1930s saw further basic science advances regarding heparin. The experimental circle from the 1880s was finally closed when Wilander demonstrated that the anticoagulant of peptone shock was indeed heparin. Waters et al. showed in 1938 that the anticoagulant of both peptone and anaphylactic shock was completely inhibited by protamine; Chargaff and Olson had shown that heparin was dramatically neutralized by protamine in 1937. In 1936, Holmgren demonstrated that the cellular source of heparin was the ubiquitous mast cell; this finding explained the presence of heparin in a wide variety of organs. Brinkhous et al. reported in 1939 that heparin did not prevent clotting of isolated thrombin and fibrinogen; there was a serum cofactor whose activation was necessary for heparin’s action.

The use of heparin for a variety of venous or arterial thrombotic disorders became standard in the 1940s; large randomized and controlled clinical trials followed later and established the role of heparin in thromboembolic disease. Similar to McLean’s original dog liver heparin phosphate, current commercial heparins are still prepared from natural sources, beef lung or pork gut. At present, 10 pharmaceutical firms produce calcium or sodium heparin in the United States. In 1968, Abildgaard identified antithrombin III (ATIII) as the heparin cofactor protein; in the 1970s, other groups described the exact molecular mechanisms of heparin’s activation of ATIII and its resultant inhibition of thrombin and other coagulation proteins. Recombinant ATIII recently became available in the United States to treat thromboses in patients with ATIII deficiency.

Currently, low molecular weight fractions of heparin are undergoing rapid clinical development as growing information supports their superiority to standard heparins with respect to bleeding and antithrombotic efficacy. The existence of naturally occurring low molecular weight fraction has been known for decades; however, synthetic preparations of these fractions from standard fractions began only in the mid-1970s, when it was discovered that the low molecular weight fractions had a much higher ratio of anti-factor Xa activity to antithrombin activity than does standard heparin. Clinical trials in preventing and treating venous thromboembolism followed in the 1980s and continue today; these agents are widely available for clinical use abroad, and FDA approval was granted in early 1993 for Rhone Poulenc Rorer’s enoxaparin fragment.

Heparins are also being studied for their ability to enhance endothelial function, promote angiogenesis, suppress inflammatory responses, modulate proteases, inhibit complement proteins and the activation of mast cells, and suppress vascular smooth muscle activity and proliferation. These effects may greatly enhance heparin’s usefulness in a diverse array of vascular and nonvascular conditions. Finally, heparinoids and related nonheparin glycosaminoglycans are being developed as antithrombotic and growth factor-modulating agents.

**Oral Anticoagulants: Spoiled Sweet Clover, Hemorrhagic Chicken Feed, and Farmer Carlson’s Cows**

The story of the oral anticoagulants is also epic and, like heparin, again centers mainly in Canada and the United States. The source of the agent is again a plant, but it was the animals consuming it who alerted scientists’ attention, beginning with rural Northern Plains veterinarians. Like ASA, the natural plant compound has been superseded by a superior congener prepared by chemical synthesis.

Around the turn of the century, resourceful farmers in northern prairie states began planting melilot or sweet clover plants (*Melilotus alba, M. officinalis*; Fig 7) imported from Europe; the overfarmed soil and harsh climate would not support standard animal feed crops any longer. Sweet clover provided abundant silage for cattle but within two decades brought a new disease that decimated cattle herds and horrified farmers: sweet
clover disease, in which affected cattle developed relentless and fatal spontaneous bleeding. It was first thought to represent "hemorrhagic septicemia." Schofield, a veterinary pathologist in Alberta, first observed the mysterious condition in 1921 and reported it in 1922 and 1924.\(^7\)\(^5\)\(^7\)\(^4\) He found that the disease was due to neither pathogens nor nutritional deficiency but instead traced it to the consumption of spoiled (not fresh) sweet clover hay and noted a prolonged clotting time. He also showed that the disease could be cured by immediate withdrawal of the spoiled clover feed and with transfusions from healthy cattle.

Roderick, a veterinary pathologist in North Dakota, first observed the disorder almost simultaneously with Schofield in 1921 and subsequently reported in 1929 and 1931 that affected cattle were profoundly deficient in prothrombin.\(^7\)\(^5\)\(^7\)\(^6\) Also in 1929, Dam\(^7\)\(^7\) reported another strange disease: dietary hemorrhagic chick disease, associated with chicken feed prepared by a process that extracts all sterols. In 1935, he also found prothrombin deficiency in the chickens and postulated the existence of a sterol "vitamin K" that prevented bleeding.\(^7\)\(^8\) Dam\(^7\)\(^8\) as well as Almquist and Stohstad\(^7\)\(^9\) isolated vitamin K in 1935; Doisy (Thayer et al\(^7\)\(^9\)) then solved its structure, and Doisy and Dam shared the Nobel Prize in Medicine in 1943.

Meanwhile, spoiled sweet clover disease raged on. Once again, a turning point emerged: Karl Paul Link, PhD (an agriculturalist) was offered a job in late 1932 at the University of Minnesota by Ross Gortner, who told the young Link about sweet clover and offered it as a project for study if he accepted the job.\(^8\)\(^1\) Gortner's laboratory workers had tried to extract the "hemorrhagic agent" but, like Schofield, Roderick, and others, had
failed. Link instead accepted a position at the Agricultural Experiment Station at the University of Wisconsin in Madison under Drs Brink and Smith; his task there also involved sweet clover but ignored the bleeding problem entirely. He was asked to develop a strain of sweet clover low in or free from coumarin, which is responsible for the melilots’ sweet smell when freshly cut, yet bitter taste that cattle disdained. Coumarin was even used commercially to scent inferior tobacco and as an ingredient in artificial vanilla and some perfumes.82 No one suspected that the sweet-smelling, bitter-tasting coumarin was related to spoiled sweet clover disease.

A serendipitous, legendary encounter ensued,81,82 launching Link on a personal crusade to address the real sweet clover problem—the deadly bleeding it caused when it spoiled. In February 1933 during a howling blizzard, a farmer named Ed Carlson from Deer Park, Wisc, appeared.83 His cattle were decimated by the disease; told to go to the Agricultural Experiment Station to get advice, he found it closed, and chance led him to Link’s Biochemistry Building, where Carlson produced a dead cow, a milk can containing blood that would not clot, and 100 lb of spoiled sweet clover. Link could only tell the desolate Carlson to avoid the hay and transfuse the ill cows; nothing else could be done. The crushed farmer, too poor for either treatment, drove home into the blizzard. Like Hoffmann’s encounter with his father and Wright’s battle with phlebitis, the direction of Link’s work was forever changed.

In 1935, Quick et al83 developed the prothrombin time (PT) test, which was critical to the work. In 1937,84 he showed the PT to be elevated in both the sweet clover and hemorrhagic chick diseases. At dawn on June 28, 1939, Link’s associate Harold Campbell saw a pure, crystalline hemorrhagic agent (H.A.) on a microscope slide (Fig 8); when Link came in that day, Campbell was asleep on the couch, and a lab technician was drunk, stating, “I’m celebrating, Doc. Campy has hit the jackpot.” Like McLean who repeated his work before announcing his discovery to Howell, Campbell waited 2 days before giving Link a vial and stating, “This is H.A.”82 Unlike the doubtful Dreser or Howell before him, Link was confident of his associate’s work, and the successes mounted. They reported the hemorrhagic agent to the world in 194085; colleague Huebner (Stahmann et al86) solved the hemorrhagic agent’s structure as 3,3'-methylenedioxy-4-hydroxycoumarin) and synthesized it in April 1940.87 The compound received the name “dicumarol,” which would serve in the United States as both generic and trade name.

The mystery of why only spoiled hay caused the disease was solved; Link had earlier found that none of the 60 or so natural coumarins (including coumarin itself) were pathogenic; however, in moldy hay,
coumarin is oxidized to 4-hydroxycoumarin and then coupled with formaldehyde and another coumarin moiety (catalyzed by Aspergillus hay mold) to form dicumarol. Link had already deduced that coumarin had to somehow be involved: Smith, Brink, and Roberts showed in 1934 through 1935 that spoiled Melilotus dentata, low in coumarin, did not cause hemorrhagic disease when fed to cattle. Likewise, when coumarin was added to alfalfa hay that was allowed to spoil, cattle succumbed.82 Link also found that dicumarol had been previously prepared by Anschütz in 1903 and then promptly ignored86; he also sensed a connection with vitamin K, showing that it completely reversed the actions of either spoiled clover or dicumarol.81 Link's laboratory personnel synthesized more than 100 3-substituted, 4-hydroxycoumarins between 1942 and 1944; meanwhile, Link gladly supplied dicumarol to clinicians who read his stunning reports in 1940. Link quickly assigned the patent rights to his research benefactors, the Wisconsin Alumni Research Foundation, who applied for a patent on dicumarol in 1941. The foundation then licensed three drug firms—Lilly, Squibb, and Abbott—to produce dicumarol. Currently, only Abbott still produces dicumarol in the United States.

Link first gave dicumarol to O. Meyer at Wisconsin General Hospital in late 1940; by January 1941, Meyer became the first to document its anticoagulant effects in human volunteers (Link was conducting the animal work). He was the first to report its clinical use at a meeting in February 194188; however, the first published report came in June 1941 from the Mayo Group headed by Allen.89 who first gave dicumarol to humans in May 1941. Meyer's report was published in October 1941.90 That month, a group from Cornell in New York City headed by none other than Irving Wright presented its report on human use91; their published article appeared in 1942.92 Dr Wright became the first to use dicumarol therapeutically, immediately treating his pioneering thrombosis patient Arthur Schulte and others, again with success. Reports from three other groups followed in 1942,93 and the drug became widely available in 1944.

Nichol, in 1942, was the first to propose treating patients with acute MI with dicumarol, a radical idea at the time given that the role of thrombosis in MI was controversial. He began treating MI patients in 1943 and reported successful results in 1944; he published his findings in 1946.94 Wright also began treating MI patients before Nichol, issuing a preliminary report of success in 1946.95 followed by the final results of a landmark randomized study sponsored by the American Heart Association in 1948.96 Oral anticoagulant treatment of MI became widespread in the 1950s and then fell out of favor in the 1960s and 1970s; currently, it is undergoing a resurgence as a result of recent favorable data.

Meanwhile, Link developed tuberculosis in 1945 and retreated to a sanitarium. Away from his laboratory, he occupied his boring days by reading about, of all things, the history of rodent control. Again, a chance encounter led Link to greater things. Returning to the laboratory in 1946, he was determined to develop the ideal rat poison. Having found dicumarol weak and unreliable when he tried it as a rodenticide in 1942,81 he searched through the compounds synthesized by Ikawa from 1942 through 194497; his colleague Scheel found that compounds 42 and 63 were more potent and reliable.81 Scheel favored compound 63, but Link settled on compound 42 (3-phenylacetyl ethyl, 4-hydroxycoumarin) in 1948. Again, patent rights were given to the Wisconsin Alumni Research Foundation, and compound 42 was dubbed “warfarin” (from the first letters of the foundation, with the suffix -arin) by Link.81

Ironically, in 1948, warfarin was launched as the ideal rat poison, not as a human therapeutic agent. At the same time, several European coumarin congener started to compete with the problematic dicumarol as a human medication. Faced with this challenge, in 1950, the resourceful Link asked Shapiro in New York and Meyer at the University of Wisconsin to try warfarin in humans; Link was amused that after the clinicians snatched his “cow poison” from him in 1941, he was now handing over rat poison to them.81 In 1951, a navy recruit unsuccessfully attempted suicide with 567 mg warfarin; his surprising full recovery induced Shapiro and Meyer to follow Link’s earlier advice.86 They tested warfarin in human volunteers in 1953 and reported it to be far superior to dicumarol in 1953 through 1954.98,99

Clinicians quickly discarded dicumarol in favor of the rat poison warfarin; it was introduced commercially in 1954, and Pollock reported the first clinical series using warfarin in 1955.100 Notably, when President Eisenhower suffered an MI that year, he was treated with warfarin. Warfarin is now the standard treatment for all venous or arterial thrombotic conditions requiring long-term treatment. Compared with Link and colleagues’ dicumarol, which was isolated from spoiled sweet clover hay, current commercial warfarin and other oral anticoagulants are prepared by chemical synthesis. In the United States in 1993, dicumarol is still produced by Abbott; phenprocoumon, acenocoumarol, and other coumarin derivatives still in widespread use in Europe are no longer available in the United States. Currently, DuPont, Abbott, and Lemmon produce warfarin sodium in the United States; Purdue Frederick’s warfarin potassium and DuPont’s injectable warfarin sodium are no longer marketed.

Even as clinical success was achieved, the precise mechanism of warfarin was not elucidated until 1974, when Stenflo et al101 described the post-translational carboxylation of vitamin K—dependent clotting factors. In 1978, Whitton et al102 and Bell103 independently found that warfarin acts by inhibiting the enzyme vitamin K epoxide reductase, thereby closing the historical loop begun by observations on cattle and chickens killed by lethal feed that completely blocked the action of vitamin K.

Finally, intense current interest in anticoagulants centers around the rapid development of novel small peptide and nonpeptide inhibitors of thrombin and other key enzymes in the cascade (factor Xa, tissue factor, and so on) that, unlike heparin, can access clot-bound thrombin or inhibit factor Xa bound to platelets. These novel agents include the hirudin family, argatroban, argipidine, PPACK, DuP 714, tick anticoagulant peptide, antistasin peptides, activated protein C, tissue factor pathway inhibitor, factor X fragments, and others.

Hirudin itself has a long, fascinating history.104 Medicinal leeches (Hirudo medicinalis) have been used throughout history; their saliva contains hirudin, a
direct and potent selective thrombin inhibitor that allows the leech to feed on flowing blood. It was discovered by Haycraft in 1884\textsuperscript{104} (decades before the discovery of the standard antithrombin agent heparin). Jacoby coined the name hirudin in 1904\textsuperscript{104}; its action as an antithrombin and its peptide nature were described in 1957 by Markwardt.\textsuperscript{104} The amino acid sequence was reported by Petersen in 1976,\textsuperscript{104} and it was subsequently prepared by recombinant DNA techniques. Hirugen is a C-terminal fragment of hirudin that binds the fibrinogen-binding site of thrombin, and hirulogs are synthetic bifunctional fragments with the C-terminal fragment of hirudin and its N-terminal fragment that binds to and blocks thrombin’s catalytic site. Hirudin and its derivatives are being shown to be effective anticoagulants (usually safer and more effective than heparin) in a variety of venous and arterial thrombotic disorders and will likely be approved for use within several years. They probably represent the class of agents that will finally end the long reign of heparin as the fast-acting anticoagulant of choice.

**Thrombolytic Agents: ‘Unstable’ Clots, ‘Plasma Lysing Factor,’ and Clot-Busting Streptococci, Urine, and Melanoma Cells**

Although the thrombolytic agents (SK, UK, TPA, and their derivatives) are new clinical tools compared with the previous drugs, their history reaches as far back as the 1860s. However, basic knowledge of the natural fibrinolytic system lagged far behind that of the coagulation cascade, leading to a lag in the development of therapeutic agents that manipulate the system. In a sense, these drugs have no direct effect on fibrinolysis but are cofactors or secondary enzymes that activate endogenous fibrinolysis. As with the history of the previous compounds, the development of thrombolytics was likewise marked by fortuitous findings and charismatic personalities. And as with the previous agents, fundamental advances in the understanding of basic science and mechanisms of thrombotic diseases catalyzed the race to develop these agents and fostered the widespread use they enjoy today. Both European and US workers contributed significantly not only to the discovery of the agents but also to their development and to the generation of supporting clinical data. All of the thrombolytics are large proteins, yet their current sources are diverse: SK and its congeners anistreplase from bacterial cultures, UK from human kidney cell tissue cultures, and TPA from recombinant DNA (derived from human melanoma cell lines) expressed in Chinese hamster ovary cell cultures.

The history of thrombolytic agents begins in 1861 with the report by von Brucke\textsuperscript{105} on the proteolytic activity of human urine. In 1886, Sahli\textsuperscript{106} noted urinary proteolytic activity with some specificity for fibrin. Purification and isolation of the fibrinolytic enzyme in urine came much later, in 1947 by MacFarlane and Pilling\textsuperscript{107} and in 1951 by Williams.\textsuperscript{108} Sobel et al\textsuperscript{109} coined the name “urokinase” in 1952. He and others demonstrated that UK is not a direct fibrin-digesting enzyme but rather an activator of endogenous plasminogen, thereby generating plasmin, which then consumes fibrin, fibrinogen, and other coagulation proteins. This mechanism was found to be shared by all current thrombolytic agents.

The next agent to be discovered was SK. In the early 1930s, William Tillett, a bacteriologist and Chief of Medicine at the New York University School of Medicine and its division at Bellevue Hospital, was studying acute-phase reactants (he was not interested at the time in coagulation or fibrinolysis).\textsuperscript{110} Noting that plasma from patients with an acute febrile illness could agglutinate hemolytic streptococci but serum could not, Tillett suspected that fibrinogen was the mediator of agglutination. He reasoned that if plasma (containing fibrinogen) was added to streptococci, the fibrinogen would be tied up in agglutinating the bacteria. If correct, the resultant plasma would be predicted to not be coagulable when removed. Once again, serendipity intervened, and a strong intellect would respond creatively to an unexpected situation. To Tillett’s initial disappointment, the treated plasma from the acute-phase patient clotted as well as the plasma from normal subjects. Just before discarding the test tubes, Tillett happened to look at them again. Strangely, the tube with the added culture had liquefied, although it had clotted initially. Changing gears to pursue the odd finding outside of his area of interest, a new experiment enabled Tillett and Garner\textsuperscript{111} to report in 1933 that the $\beta$-hemolytic, group C streptococci, produced a fibrinolytic agent. Tillett called it “fibrinolysin.”

Old blood clots lysing with time was not a new finding. Dastre\textsuperscript{112} reported in 1893 that clots would spontaneously liquify; he correctly attributed this to fibrinolysis, but the agent responsible was a mystery. Yudin\textsuperscript{113} exploited this phenomenon to prepare incoagulable blood for transfusion in 1936. Ultimately, the more advanced techniques and basic hemostasis knowledge of Tillett’s time enabled his colleague Milstone to show in 1941\textsuperscript{114} that the streptococcal fibrinolysin did not directly dissolve fibrin in vitro; rather it required a plasma protein cofactor he called “plasma lysing factor.” This factor later proved to be plasminogen, of course. In 1944, Christensen and MacLeod\textsuperscript{115} from New York University and Kaplan\textsuperscript{116} independently discovered this; Christensen coined the terms “plasminogen” and “plasm” and renamed fibrinolysin “streptokinase.” They also partially purified both plasminogen and SK. In 1946, Tillett recruited one of Bellevue’s residents, Sol Sherry, to join the team researching SK; he would become a pivotal figure in the development of not only SK but UK and even TPA in ensuing years.\textsuperscript{117}

Sherry got to work quickly; his team became the first to administer crude SK to humans in early 1947.\textsuperscript{117} They treated patients with chronic empyemas, hemithorax, and parapneumonic loculated effusions with intrapleural injections of SK with dramatic results (break-up of fibrin septations, allowing for easy aspiration of fluid); they also treated chronic abscess cavities with local injections of SK, reporting their results in 1949.\textsuperscript{118} As with early heparin preparations, the New York University SK was crude and too toxic for systemic use. Lederle Laboratories assisted by supplying them with improved preparations that were pure enough for intravenous use. By 1952, Johnson and Tillett\textsuperscript{119} reported that experimental rabbit ear vein thrombi were lysed by peripheral intravenous administration of the purer SK, thus pioneering the use of SK in the vascular tree.
The stage was set for intravenous use in patients; Sherry et al.\textsuperscript{120} (now in St Louis with a new team) reported in 1957 a regimen for sustained intravenous infusions of SK based on work with human volunteers. In 1958, Sherry and colleagues, including Fletcher and Alkjaersig,\textsuperscript{12,121} reported the first administration of SK (by intravenous infusion) to patients with acute MI, with anecdotal clinical success. They followed this report with several publications in 1959, all of uncontrolled studies, detailing their use of intravenous SK in treating MI.\textsuperscript{12,122,123} Back at New York University, Johnson and McCarty\textsuperscript{124} reported in 1959 that intravenous SK could lyse experimental human venous thrombi successfully. Lederle ran into problems with quality control of the SK preparations and pulled out of the field completely. Because of the temporary lack of a source of SK, Sherry's team turned to developing the recently described UK. They reported its successful use in maintaining a fibrinolytic state with intravenous infusion in human volunteers in 1965,\textsuperscript{125} paving the way for later use in acute MI by others. This advance in bringing UK to clinical use came with the help of the Abbott and Sterling-Winthrop (the same Sterling that purchased the US Bayer division in 1918) drug firms, which supplied purified UK. The first studies of intravenous UK in pulmonary embolism began in 1967, and large, randomized, controlled trials demonstrated the efficacy of thrombolitics in pulmonary embolism beginning with the 1970 report from the UPET study.\textsuperscript{126}

Meanwhile, SK was resuscitated in the 1960s by the drug firms Behringwerke AG and Kabi Pharmacia.\textsuperscript{117} As a result, between 1960 and 1979, 18 trials of intravenous SK in acute MI were published, and meta-analyses by Stampfer et al.\textsuperscript{127} and Yusuf et al.\textsuperscript{128} revealed that these early, small, primitive studies provided unequivocal evidence of SK's effect on reducing MI mortality. The famed GISSI and ISIS-2 studies of the 1980s provided the final convincing proof of the great usefulness of intravenous SK in acute MI.

Meanwhile, the usefulness of both intravenous SK and UK in venous thromboembolism was shown in several studies in the 1960s and 1970s, leading to FDA approval of both agents for intravenous use in 1977. SK was approved for deep venous thrombosis, pulmonary embolism, thrombosed dialysis fistulas, and arterial thrombosis; UK was approved only for pulmonary embolism and thrombosed intravenous catheters. Further FDA approvals for other indications came in 1982 for intracoronary use of both SK and UK in treatment of acute MI and in 1987 for intravenous use of SK in acute MI (UK remains unapproved for intravenous use in acute MI, due to a relative lack of studies). Current commercial SK is still prepared by purification from bacterial cultures. Two brands are produced for US use: one is manufactured by Behringwerke AG in Germany and distributed in the United States by the Swedish firm Astra AB (Hoechst-Roussel had distributed this brand until recently), and the other is made and distributed by the Swedish firm Kabi Pharmacia AB. UK, currently produced only by Abbott, is no longer prepared from human urine but instead by purification from human kidney cell cultures.

Variants of SK made brief appearances around 1960. Actase and Thrombolysin, produced by Ortho and Merck, respectively, were trade names for fibrinolysin, a crude mixture of human plasmin and SK. A series of reports in 1960 reported anecdotal success with intravenous fibrinolysin in treating experimental or human MI.\textsuperscript{129-131} Fibrinolysin preparations were abandoned in the 1960s as the European laboratories picked up production of pure SK.

Despite the success of early experience with intravenous SK for acute MI in the 1960s, clinicians used it rarely due to the paucity of large-scale trials of efficacy; concern over the significant bleeding incidence encountered with early, very long infusion protocols; confusion over widely differing dosing regimens; and prevailing skepticism about the role of thrombosis in acute MI. The thrombolytic era we now enjoy thus had to wait for final proof of the role of thrombosis and then the rediscovery of thrombolytic therapy, first with intracoronary use and finally coming full circle with intravenous use. This unfortunate delay of several decades in accepting the use of thrombolysis as the cornerstone of treatment of acute MI carried a heavy toll in lives. Despite the demonstration by Herrick as early as 1912 of the central role of thrombosis in acute MI, doubt lingered about the issue until the landmark angiographic demonstration of DeWood et al.\textsuperscript{132} in 1980 of acute thrombosis in most cases of acute MI.

With the medical community finally convinced of the importance of coronary thrombosis, enthusiasm greeted the wave of studies rediscovering thrombolysis. Chazov et al.\textsuperscript{133} in the USSR in 1976 were the first to report intracoronary use of SK in acute MI along with angiographic demonstration of thrombolysis; however, his report was in an obscure foreign-language journal and attracted interest only in retrospect. The much better known report of Rentrop et al.\textsuperscript{134} in 1979 of intracoronary SK led to a number of reports of uncontrolled small series of intracoronary SK, which would be followed by larger, randomized, controlled studies of intracoronary and then intravenous SK.

In 1979, Smith et al.\textsuperscript{135} reported the rational design of anistreplase, a combination of SK and its target, plasminogen. This agent, which spontaneously deacylates slowly after injection and thus can be administered as a single bolus, was first used in humans with acute MI by Been et al.\textsuperscript{136} and others in 1985, and it was approved for intravenous use in acute MI by the FDA in 1989. Anistreplase is manufactured by Beecham-Wulfling of Germany and distributed in the United States by Smith-kline Beecham; it is prepared by combining human lys-plasminogen (isolated from pooled plasma) with chemically acylated SK harvested from bacterial cultures.

Turning to TPA, tissue slices were shown by Astrup and Permin\textsuperscript{137} to lyse fibrin in 1947. Sherry's group briefly dabbled with TPA, reporting in 1964\textsuperscript{138} the partial purification of TPA from pig heart. Fully purified TPA was prepared by Rijken et al.\textsuperscript{139} first from uterine tissue in 1979 and then from human melanoma cell cultures in 1981.\textsuperscript{140} Binder et al.\textsuperscript{141} also reported the isolation of TPA from blood vessel perfusates in 1979. Pennica et al.\textsuperscript{142} reported the cloning of TPA DNA derived from human melanoma cells and its expression by \textit{Escherichia coli} in 1983. Clinical use evolved rapidly, seizing on the new preparations. Van de Werf et al.\textsuperscript{143} reported the first use of natural TPA (harvested from the Bowes human melanoma tissue culture) in an
uncontrolled series of acute MI patients in 1984, with clinical and angiographic success. The same group also became the first to use recombinant TPA (this time in dogs) with experimental MI, also in 1984.144

The same year, Colleen et al145 pioneered the use of recombinant TPA in patients with acute MI. Randomized, controlled trials followed, with striking success, leading to FDA approval of intravenous TPA for acute MI in 1987 and for pulmonary embolism in 1990. Unlike the early TPAs derived from a variety of human tissues or from melanoma cell culture, current commercial TPA is prepared from recombinant DNA derived from human melanoma cells and expressed in the Chinese hamster ovary tissue culture system. Currently, only Genentech produces TPA; Burroughs Wellcome has abandoned development of its TPA, which differs from the native molecule by a single amino acid and by its double-chained structure. Genentech’s product shares native TPA’s amino acid sequence, single-chained structure, and possibly its glycosylation.

Current research into new thrombolitics centers around modified or fibrin-antibody–bound TPAs with improved fibrin specificity, prourokinase, chimeric molecules combining TPA and prourokinase, and entirely novel and potent thrombolitics such as vampire bat TPA and staphylokinase.

In conclusion, the stories of the discovery and development of the major agents used for thrombotic diseases are fascinating, triumphant landmarks in the annals of medicine. The long odysseys share recurrent themes of good fortune; unexpected breakthroughs, often by the most unlikely individuals; international competition and cooperation; unheeded visionaries; and false leads and blind alleys. But above all, they are vivid examples of the merits of maintaining an open, skeptical, and active mind. The rewards to the innovators have at times been fame and accolades; the rewards to the world have been a small, diverse group of potent, invaluable antithrombotic agents.

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