

Chapter 2

Blood Coagulation

Abstract This chapter is devoted to the description and modeling of the process of blood coagulation which is crucial for life and is the result of an intricate sequence of chemical reactions, involving an active role of platelets and of a surprisingly large number of blood born massive molecules performing a sequence of operations aimed at the formation of the clot and at its subsequent dissolution. Due to such a complexity there is an enormous variety of conditions leading to insufficient or excessive coagulation. We will discuss also the biological and mathematical aspects regarding these pathologies.

2.1 Introduction

Healing of a wound is a long process whose stages are more or less known to everybody. The first step, which has to be fast, is to stop bleeding (*hemostasis*). This is the purpose of blood coagulation, which creates a jelly material (*clot* or *thrombus*) sealing the lesion. On an external wound the clot surface dries up forming a solid crust, which protects the lesion site. Here the much longer process of tissue repair is initiated, which involves cells proliferation and (depending on the lesion size) revascularization. The soft clot core gradually dissolves (also at a slow pace) through a process called *fibrinolysis*, since it requires the progressive destruction of the clot skeleton, made of a polymer called *fibrin*.

What is less known is that lesions of small size may occur spontaneously in blood vessels. Such events, quite common at the scale of small vessels, trigger coagulation. A typical example is the rupture of a plaque formed beneath the *tunica intima* (internal epithelial layer) of an artery, but there are relatively frequent cases, not as much traumatic, in which e.g. the endothelial lining of a capillary breaks producing a submillimetric lesion. The consequences of internal bleeding can be very serious even if the leak is minimal (if it happens for instance in the brain). If we think that our capillary network has an estimated total length of 10^5 km with a surface of the order of 10^3 m² (these are not misprints!), we realize that the occurrence of small injuries is not a rare event and that such an impressive system needs a continuous surveillance and an efficient tool for repairing damages, so to prevent internal bleeding. Indeed, our body is equipped with a fantastic machinery which readily intervenes when epithelial tissue happens to be accidentally exposed

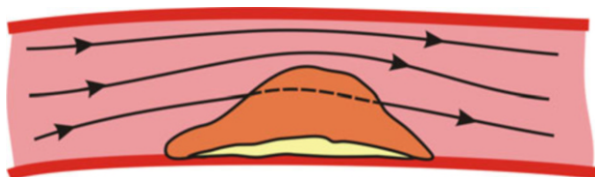


Fig. 2.1 A sketch of the two stages of hemostasis

to circulating blood and efficiently halts bleeding. Its complexity is as astonishing as fascinating.

In this chapter we will first illustrate the basic biological mechanisms of blood coagulation and how they have been discovered. Next we will describe some of the mathematical models that have been developed to predict clotting evolution and also the effects of therapies.

It is useful to anticipate that the formation of a clot over a lesion is a two step process: *primary* and *secondary* hemostasis. A very schematic sketch is shown in Fig. 2.1: the effect of primary hemostasis is the small platelets rich plug grown directly over the injury; the plug is then covered by the main body of the clot, produced by secondary hemostasis. The two stages involve very different physiological processes, as we shall see.

Another important feature emphasized by the figure is the strong interference between the clot formation and the blood flow. This is actually a crucial point in mathematical modeling, owing to the strong coupling of the two phenomena. Indeed, the clot, in which basically no flow takes place, modifies the geometry of the vessel lumen significantly altering the blood flow, which in turn has a great influence on the coagulation process. As a matter of fact, blood advection provides the needed cellular and chemical elements to the clotting site and at the same time the flow has a “washing” effect, taking them away downstream. Therefore, when we will enter the fantastic complexity of the biology and chemistry of blood coagulation, we must keep in mind its intrinsic link with fluid mechanics.

The same figure points out another question of dramatic importance: the clot has to stop growing before it creates a significant lumen reduction (*stenosis*) or even complete obstruction of the vessel (*embolism*). Thus the tremendously complex machinery of clots formation must also possess a built in self-regulation mechanism preventing excessive growth as well as the propagation of the clot along the vessel.

The main characters in primary hemostasis are *platelets*, the tiny and protean blood cells which constantly inspect blood vessels wall. When they detect the presence of a lesion they rapidly change their shape emitting pseudopods with which they bind to the injured epithelium and among themselves, readily forming a tight plug (the so called *white clot*) and even secreting growth factor to facilitate the subsequent repair of injured tissue. Secondary hemostasis is a much more complex process involving an impressive *cascade* of chemical reactions whose end product is the fibrin network entrapping blood. Unraveling that intricate chemical path took the effort of generations of hematologists. It had to wait not only for the

birth of modern medicine, but also for the development of biochemistry, taking a decisive acceleration only around the half of the twentieth century. The task was so difficult that what is believed today to be the correct biological model of secondary hemostasis has eventually been formulated only about 10 years ago.

As it often happens, a great help in understanding a process comes from the observation of the ways it can fail. In the case of blood clotting there are two categories of failures: excessive coagulation and defective coagulation. The former case produces the unwanted formation of clots (*thrombosis*) with the risk of embolism. Thromboembolic diseases can affect various blood vessels, damaging different organs, generally with serious consequences. Well known infamous examples of organs which may host thromboembolic events are: limbs (Deep Venous Thrombosis or DVT), lungs (pulmonary embolism), brain (stroke) and heart (myocardial infarction). Defective coagulation gives rise to severe bleeding after injuries or even to spontaneous hemorrhages, of which the hemorrhagic brain stroke is the most dangerous. Therefore it is not surprising that the studies on blood coagulation had always gone in parallel with the investigation of bleeding disorders and the way of curing them. For this reason a specific section on this important subject (also from the point of view of mathematical modeling) will be included in the present chapter. Not surprisingly, the complexity of this topic reflects the one of the derailed physiological process.

There are many books on hemostasis. We quote [125, 153, 166, 239, 288]. The review papers [35, 71] have been used as a basis for the present chapter, which however covers a much larger spectrum. The literature both on the medical and on the mathematical side is immense and no review of reasonable size can really be comprehensive.

We want to deal first with the historical aspects, in order to realize how difficult it was to acquire the tools to emerge from the maze and eventually see the light.

2.2 Historical Remarks

In this section we will shortly review the history of observations and studies on blood coagulation preceding the twentieth century.

Thrombosis was one of the first reported clotting disorders, though just through its symptoms, namely limb swelling, most frequently in pregnant women.¹ For a

¹A widespread belief, still alive in the nineteenth century, was that limb swelling accompanying pregnancy was due to the accumulation of excess milk which found its way to reach the legs. The name “phlegmatia lactea” was coined for this condition by the French physician and botanist **François Boissier de Sauvages de Lacroix** (1706–1767) (known as Sauvage) in his *Nosologia Methodica* (1768). Pregnancy may increase the risk of thrombosis in various ways. The swollen uterus can compress pelvic vessels, reducing blood circulation in the legs. Hormonal changes can also produce hypercoagulability by increasing the concentration in blood of pro-coagulant factors and reducing the concentration of anticoagulant factors. A more serious condition sometimes related to pregnancy is the Antiphospholipid Antibody (or Hughes, or sticky blood) Syndrome,

review on pregnancy and post-partum related thrombosis see [245]. Gender specific studies on thrombosis are presented in [56, 273].

A very well written historical review on thrombosis in general is [13], which contains many important references. Symptoms attributable to arterial thrombosis have been described in the old traditional Chinese medicine. We recall from Chap. 1 that in Chinese medicine blood motion was provided and controlled by Qi. An excessive action of Qi would lead to an acceleration of blood, which could result in bleeding, while a weak action generates stasis and the insurgence of the above mentioned symptoms. A long list of herbal medicaments was available to cure both kinds of disorders.

As to the Egyptians, the topic of blood coagulation is not mentioned in the known medical papyri. Coming to western civilization, again we have to deal with the central figure of **Hippocrates** (ca. 460 BC–370 BC), whom we have mentioned in Chap. 1. He observed limb swelling (particularly during pregnancy) and coined for it the term *leucophlegmatia*, though the same term was later used to indicate symptoms of various origins. **Galen of Pergamon** (131–201) (see the same chapter) was the first to use the term *thrombosis* in connection with limb swelling (from Greek *thrombos*, meaning clot).

Particularly interesting was the point of view of **Aristotle** (384–322 BC) about blood clotting, which he claimed to be caused by *heat loss*. This looks of course a very naïve claim,² but he also said that a *fibrous material* was necessary for clotting.³

Throughout middle ages and even for most of the eighteenth century, still under Galen's influence, derailed “humors” were considered responsible for limb swelling.

Coming closer to our times, while a lot of humors trash was still invading the medical literature, fundamental discoveries were made in the seventeenth century, laying the foundation for a scientific approach to biology. These were mainly related to the development of microscopy, particularly in the Netherlands. We have already mentioned the contributions by **Anthony Leeuwenhoek** (1632–1723) and others that in the course of a couple of centuries lead to the knowledge of blood composition, which is clearly at the basis of any theory on coagulation. That was just a first step. The famous French surgeon **Jean-Louis Petit** (1674–1750) recognized that blood clotting was part of the process of hemostasis (Fig. 2.2).

A description of vein occlusion by blood clots was provided, Fig. 2.3 (1676) by the celebrated English surgeon **Richard Wiseman** (1601–1686).⁴ Much before the so-called Virchow triad became famous (see below) he recognized two different causes of thrombosis: stasis and hypercoagulability.

due to the autoimmune production of antibodies against a cell membrane substance called phospholipid, eventually producing clots.

²Nevertheless, we must consider that in this context “heat” was not meant just as a physical quantity, but as a vital entity carried by blood.

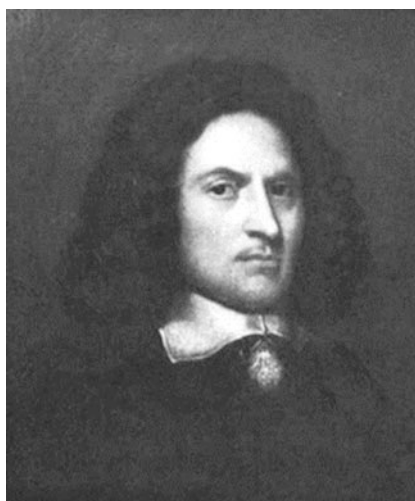
³Such a fibrous component was isolated much later by **Marcello Malpighi** (1628–1694).

⁴Despite his fame (testified by the great success of his book *Several Chirurgical Treatises*—London, Flesher 1676) as member of the Company of Barber-Surgeons his social rank was inferior to the one of physicians.

Fig. 2.2 Jean-Louis Petit
[Noé André Legrand, Les
collections artistiques de la
Faculté de Médecine de Paris,
Paris: Masson, 1911]



Fig. 2.3 Portrait of Richard
Wiseman [Royal College of
Surgeons of England
(Wellcome Library)]



Attention towards blood composition in the interpretation of the coagulation process was brought by **William Hewson** (1739–1774), who isolated a “coagulation lymph” responsible for the process (identifiable with what is known today as *fibrinogen*).

In the first part of the nineteenth century it became clear that limb swelling had to be attributed to veins obstruction, though the struggle against the humors theory (as we have seen) was still going on, surviving till the beginning of the next century! One of the most revered physician of the nineteenth century was the

Fig. 2.4 Rudolph Virchow

German pathologist⁵ **Rudolph Carl Virchow** (1821–1902), today still remembered in the field of blood coagulation for his *Virchow Triad*, emphasizing three elements contributing to thrombosis (Fig. 2.4).

In the modern language we can list them as hypercoagulability, alterations of hemodynamics (stasis), and endothelial injuries.⁶ He introduced the term *embolia*. Of course there were many other physicians who had similar views, but for lack of space we will not dwell on such details any further.

Instead, let us briefly mention some historical remark about bleeding disorders. It is natural that such phenomena have been important to people practicing circumcision.⁷ Babies whose blood could not coagulate properly could bleed to death. In the second century AD Rabbi **Judah haNasi** exempted from circumcision babies when two sons of the same mother had previously died after the operation (Babylonian Talmud, [213]). The Islamic physician **Albucasis (Abu al-Qasim**

⁵Though he was one of the founders of pathology, it is reductive to call him just a pathologist, since this great man was also a biologist, the editor of the *Archiv für pathologische Anatomie und Physiologie und für klinische Medizin* (known as the Virchow's journal), the founder of the *Gesellschaft für Anthropologie, Ethnologie und Urgeschichte* (very influential in German archaeology), and the leader of a democratic party. He wrote about many different subjects. He was such a strong opponent of Bismarck, that he was challenged by him to a duel in 1865. The legend says that, having to choose the weapons, he selected two pork sausages, one of them charged with trichinosis larvae, thus discouraging his adversary. Not everything he did was perfect: he was skeptic about Darwin's evolution theory.

⁶Virchow described the mechanism of thromboembolism [253], a phenomenon that was by no means clear at his time (inflammation was considered by many physicians the real cause of thrombosis: this was the subject of a famous dispute with the French pathologist Jean Cruveilhier). Curiously, he did not formulate the famous “triad”, which for some reason found a firm place in the literature much after his death (apparently not before 1950!). See the interesting review [22].

⁷Circumcision is a very old practice, already found in ancient Egypt and that was widely adopted also in the Islamic world. Its origin in Egypt was probably as an initiation practice to religious offices. The *Book of the Dead* describes self-circumcision by the sun-god Ra: *Blood fell from the phallus of Ra after he had finished cutting himself*.

Fig. 2.5 Queen Victoria
[photo: Alexander Bassano
(1882)]



Khalaf ibn al-Abbas Al-Zahrawi, 936–1013, dates are uncertain) who lived in Andalusia, reported cases of what today is known to be hereditary hemophilia. Accounts of bleeding disorders of hereditary type can be found in many later sources and with different names.

The term hemophilia was used for the first time by **Friedrich Hopff** in his 1828 treatise *Über die Haemophilie oder die erbliche Anlage zu todlichen Blutungen* (Zurich).

We refer to the historical review [116]. We know today that some classes of disorders known as hemophilia (namely hemophilia A and B) are due to a defective gene in the X-chromosome.⁸ That explains why it is extremely rare in women (having two X-chromosomes),⁹ who can however carry the illness (only one chromosome being defective) without symptoms and transmit it through their genealogic tree. The important review paper [116] (Fig. 2.5), contains a large section on the “Royal hemophilia”. It is well known that Queen Victoria was a carrier of the disease and that members of many royal families got from her the defective chromosome (Fig. 2.6), a fact that was going to have important historical consequences (Fig. 2.7).¹⁰

⁸The fact that hemophilia is gender related was suggested by the American physician **John Conrad Otto** (1774–1844) as early as 1803.

⁹In numbers, 1 over 10,000 men is hemophilic. The probability that a woman is hemophilic is the square of that number. Cases of hemophilic women have been reported (see e.g. [90]).

¹⁰The only male child of Tsar Nicholas II (Aleksej Nikolaevič Romanov) got hemophilia through his mother Alice (third row in Fig. 2.5), who as a Tsarina took the name Aleksandra Fëdorovna Romanova. The Tsarina believed that Grigory Rasputin (a controversial character) was able to heal

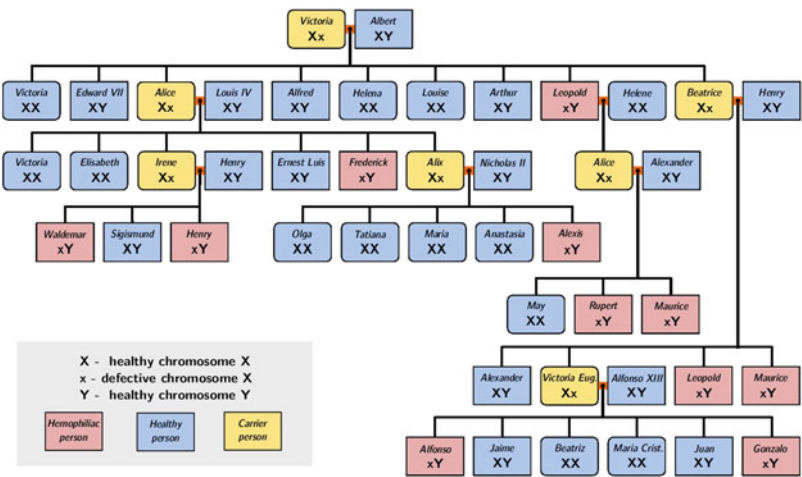


Fig. 2.6 Queen Victoria’s family tree, showing hemophiliac descendants [from: [116]]



Fig. 2.7 Aleksandra Fëdorovna Romanova and her hemophilic child Aleksej Nikolaevič Romanov [Romanov Collection, General Collection, Beinecke Rare Book and Manuscript Library, Yale University]

Hemophilia used to be a life threatening illness, since any minor injury could be fatal. Later on patients could benefit transfusions, but at the insurgence of HIV infection a great number of them died before adequate actions were taken.

Cancer may be a cause of hypercoagulability. Relationship between cancer and thromboembolism was recognized already in the 1860s by **Armand Trousseau**

the *tsarevich* with his prayers (he actually recommended not to credit the physicians) and in this way he acquired great power at court.

Fig. 2.8 Armand Trousseau

(1801–1867),¹¹ who had the chance to diagnose in that way his own fatal pancreatic cancer (Fig. 2.8).

The discovery of platelets marked the beginning of modern investigations on blood coagulation. Due to their smallness, their discovery was delayed until sufficiently powerful microscopes became available. In 1865 **Max Johann Sigismund Schultze** (1825–1874) made an accurate description of tiny cells that he recognized as a normal constituents of blood. However, there were previous observations: in 1836 by **Hermann Nasse**, in 1841 by **George Gulliver** (1804–1882) and in 1842 by the French **Alfred Donné** (1801–1878), generally referred to as the discoverer of platelets.¹² It was however **Giulio Bizzozero** (Fig. 2.9) (1846–1901) who understood their role in blood coagulation (1881).¹³ He coined the Italian name *Piastrine* and the German *Blutplättchen*.¹⁴ This was the starting point of

¹¹A distinguished French Medicine Professor, Trousseau related a state of hypercoagulability to the presence of some malignancies. He diagnosed his fatal illness having got a thrombophlebitis in his left upper arm. The presence of cancer induced thrombophlebitis has become known as Trousseau Syndrome.

¹²We will refer his contributions in the field of leukemia in the last chapter. The list of people who contributed to the early studies on platelets is actually longer (see the review paper [55]).

¹³In this connection the name of the eminent French hematologist **Georges Hayem** (1841–1933) has to be remembered as one of the founders of modern hematology. He performed the first count of platelets. In 1882 he illustrated the effects of *thrombocytopenia* (low platelets count).

¹⁴The eminent Canadian Physician **William Osler** (1849–1919) called them third corpuscles. The name platelets was introduced in 1910 by the American pathologist **James Wright** (1869–1928).

Fig. 2.9 Giulio Bizzozero

modern investigations on blood coagulation. The reader is referred to the papers [39, 86, 205].

The fascinating story of platelets is not finished, since, in spite of their smallness, they are a very complex universe still offering new surprises.

A side aspect of blood coagulation is the doubt about its reversibility, raised by the “liquefaction miracles” observed in old samples of blood, which was necessarily coagulated or coagulating when they were collected (see Chap. 1). It can be conjectured that the samples had lost all the fibrin because of natural degradation. Specific studies were quoted in Chap. 1.

2.3 Cells and Proteins Intervening in the Formation and Dissolution of Clots

Clotting and fibrinolysis proceed through sequences of chemical reactions of complicated nature (*cascades*), characterized by strong positive feedback. Platelets have a central role in initiating and regulating the chemistry involving a large number of chemicals (mainly proteins). Their interplay with the damaged and with the healthy endothelium is an important mechanism in the process. Such an interaction is mediated by various substances. Endothelial cells in the blood vessels (and in other tissues) are very active. We are going to list many of the chemicals they produce, having a pro- or an anti-coagulant effect. Though we will not mention it, the specialization of these cells may vary according to the nature and size of the vessel (see [4], which is a good review paper on thrombosis in general). In order to understand the general biological scheme of blood clotting it is necessary to describe

the various elements involved. Since they are very numerous we will group them in classes according to their role and nature. The exposition will be only moderately technical, since all we need to know is the role these elements have in the process, not for instance the details about their chemical structure, nor how the reactions actually take place.¹⁵ The reader may be discouraged by the length of this section, but at least a quick glance at it is necessary before to proceed. When the biological process will be described it will be very natural to come back to the present section in search of information. To the readers consolation we may say that, in spite of appearance, our exposition is actually synthetic and confined to the facts pertaining to coagulation.

We start by describing the cells which are suspended in the liquid component of blood (plasma).

2.3.1 Blood Cells and Coagulation

(A) Platelets

Platelets are cells with no nucleus produced in the bone marrow, having a diameter of $2\text{--}4\text{ }\mu\text{m}$ (volume $7\text{--}11\text{ fL}$)¹⁶ and a lifespan of $5\text{--}10$ days.¹⁷ Their average concentration in blood is $1.5\text{--}4.10^5/\text{mm}^3$. The process of platelets production is quite peculiar, since they come from giant parent cells (*megakaryocytes*), diam. up to $100\text{ }\mu\text{m}$, each delivering $1000\text{--}5000$ new platelets. Megakaryocytes nuclei also go into the bloodstream, eventually reaching lungs, becoming food for the alveolar macrophages. In the rest state platelets shape is discoid (thickness $\sim 0.5\text{ }\mu\text{m}$), but they have the ability of deforming as a response to various stimuli (chemical and mechanical). Deformations take place through a rearrangement of the cytoskeleton. We find *star shaped* platelets rolling over blood vessels wall (in this way they can inspect the vessel integrity). The most dramatic deformation occurs when platelets become *activated*, a fundamental step in the clotting process that will be described in the next section. Then their shape becomes very irregular, with the emission of *philopodia* (or *pseudopods*), allowing platelets to bind both among themselves (at distances of a few diameters) and (in the fastest stage of the coagulation process) to the growing *fibrin network* (Fig. 2.10).

Despite their smallness, platelets can perform an incredible number of actions, interacting with the environment by means of a rich array of *receptors*

¹⁵Large molecules like proteins have specific sites which are engaged in specific reactions.

¹⁶The volume unit *femtoliter* is often used for small particles. It corresponds to $1\text{ }\mu\text{m}^3 = 10^{-15}\text{ l}$.

¹⁷All data concerning human blood are subjected to large variations, according to sex, body weight, and health conditions. A parameter which instead is bound to stay in a narrow range is blood *pH* whose value must be between 7.35 and 7.45.

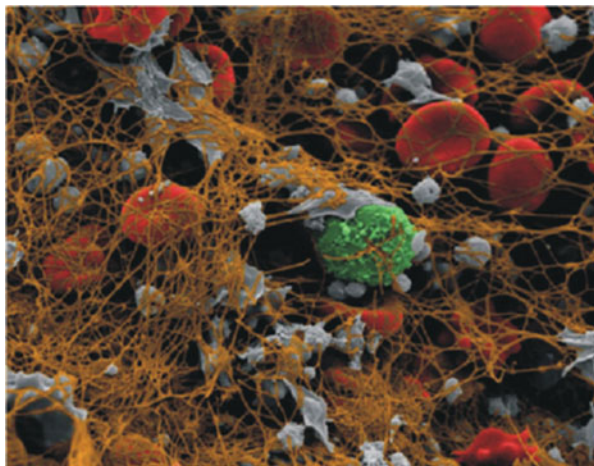


Fig. 2.10 Blood clot with the fibrin network and entrapped cells [with permission of Prof. John Weisel (Perelman School of Medicine, University of Pennsylvania)]

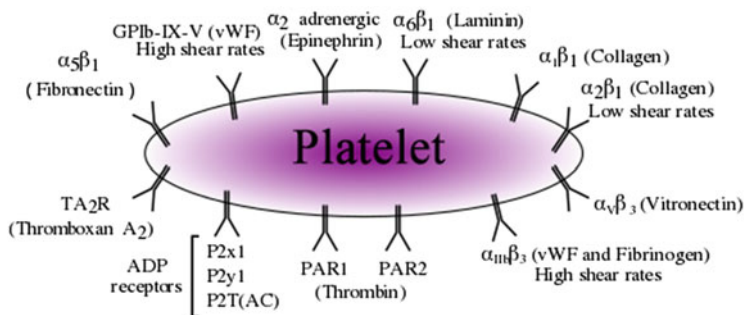


Fig. 2.11 Schematic representation of main platelets receptors (for a complete list see [121]) [based on Fig. 7.2 [35]]

on their membrane (Fig. 2.11).¹⁸ Concerning this subject we quote the papers [121, 209].

Figure 2.11 shows the specialization of each class of receptors, emphasizing the ability of platelets to interact with specific elements. Engaging receptors provides stimulus for platelet *activation*. Even though we have not yet introduced the proteins to be recognized by the receptors, it is useful to explain at least the meaning of the symbols. For instance the symbol vWF stands for *von Willebrand Factor* (see the subsection devoted to it). The $\alpha_{IIIb}\beta_3$ receptor binds to vWF and to *Fibrinogen* (one of the Coagulation Factors to be illustrated

¹⁸Most cells interact with the environment thanks to receptors able to recognize molecules carrying signals and inducing specific stimuli (*cytokines*).

later) and is important for interplatelets links, since these molecules can provide bridges among activated platelets. In the literature this glycoprotein is frequently indicated with the alternative symbol GPIIb/IIIa.¹⁹ bind to vWF. The indication *high stress* (common to $\alpha_{IIb}\beta_3$ and to GPIb) calls for the mediation of a sufficiently large mechanical stress, thus *providing a strict relationship with the fluid dynamics*, which is particularly important. The PAR 1 and PAR 4 receptors are sensitive to *Thrombin* (one of the principal Coagulation Factors: see the corresponding subsection). Gb IX-V is a complex receptor for vWF, thrombin and other coagulation factors. The $\alpha_1\beta_1$ and $\alpha_2\beta_1$ receptors are binding sites for *Collagen*,²⁰ possibly exposed by an injury of the vessel wall endothelium. In this way platelets can be immobilized at the wound site. The same kind of action is exerted by vWF, which is present both in blood (though normally in a non-active form), and in the endothelium. Binding of platelets to injured endothelium is a basic step of primary hemostasis, while mutual binding is important in all stages of the process. Sensitivity to *Epinephrine*²¹ results in an acceleration of the coagulation process for (moderate) increase of this hormone concentration in blood. Continuing the list of receptors we find the ones which bind to *Fibronectin*, *Vitronectin*, and *Laminin*, which are all very important proteins having a role in cell-cell and cell-ECM (Extra Cellular Matrix) adhesion. Then we come to the receptors for important platelet activators like *Thromboxane A2* (TXA2) and ADP (see next subsection).

It is appropriate to recall here that drugs have been produced preventing the activation of specific receptors. Such drugs are administered to prevent blood clotting, particularly after heart surgery. We devote a section to them, but it is worth to recall now the most known among a long list: *Plavix* (Clopidogrel), acting on the ADP and other receptors, and *Aspirin*, inhibiting the *Thromboxane A2* (TXA2) production. A natural platelet inhibitor, synthesized in the endothelium, is NO (*nitric oxide*).²² Platelets exhibit also an intense internal activity, since they possess corpuscles, called α *granules* and *dense (or δ) granules*. The α *granules* secrete, under activation, the binding factors vWF and *Fibrinogen* (see Coagulation Factors) in addition to *Platelet Factor 4* (another platelet activator) and other proteins having

¹⁹Gp recalls that these receptors are *glycoproteins receptors* denoted as GPIb (i.e. containing sugar chains).

²⁰A protein with elongated molecule (fibril) which comes in many different types and is abundant in many tissues, including blood vessels. It makes 1/4–1/3 of the protein content in the body.

²¹Commonly known as *Adrenaline*, it was the first hormone to be discovered (1900) though the term hormone was introduced in 1905 by **Ernest Starling** (see the next chapter), after the Greek word *ormè* (impetus), for the hormone he discovered (secretin).

²²In 1998 the Nobel Prize in Medicine was awarded to R.F. Furchgott, L.J. Ignarro, and F. Murad for their work on the signalling role of NO.

different functions²³; the δ granules produce platelet activators like the already mentioned *Thromboxane A2* and ADP, thus triggering positive feedback, but also *Serotonin* (5-hydroxytryptamine), a vasoconstrictor. Granules release their products through the so-called *Open Canalicular System* (OCS) (see the paper [274] for a description of their role). Activated platelets are also able to synthesize some of the coagulation factors.

An important role in platelets (and other cells activity) is played by the **membrane**. Not only through the receptors, but also by exposing negative charges to the exterior, which become sites of attraction of active coagulation factors. All cells membrane contain particular fat molecules (*phospholipids*). Platelets phospholipids can liberate an important substance, called *Arachidonic Acid* and involved in a chain of transformations, mediated by enzymes in the class *Cyclo-oxygenase* (COX 1-2-3) whose end product is TXA2, passing through *Prostaglandin H2*, with the final step performed by the enzyme *Thromboxane A-synthase*. Aspirin has the property of inhibiting the COX action.²⁴ We will return to this important subject in a later section.

Finally, we recall that in most cases the presence of the **ion** Ca^{++} is necessary for receptors to perform their action.²⁵ Such an ion is also contained in the δ -granules.

Literature on platelets is extremely large and includes several books (e.g. [75], and [160] whose foreword [53] contains a huge bibliography).

(B) White Blood Cells (WBCs)

Also called *Leukocytes*, all produced in the bone marrow, they circulate in blood for some hours and then distribute in the tissues to attack bacteria, destroy damaged cells, phagocyte antigen-antibody complexes, etc. They are distinguished in two main classes, with different size, shape of the nucleus and function:

- (I) **Granulocytes**, a group of three species: *neutrophils* (diam. 12–14 μ m, life span 5 and a half days in blood plus 1 or 2 days when they migrate to tissues), making 60–70% of the whole population; *eosinophils* (diam. 12–17 μ m, life span 8–12 h in circulation, but up to 12 days in tissues), are less numerous (1–6%); *basophils* (diam. 14–16 μ m, life span 3 days),²⁶ only 1% of WBCs or less. All cells in this group play a role

²³For instance *Thrombospondin 1*, a multifunctional protein (e.g. antiangiogenic), and *Nexin II*, whose role will be discussed later. Platelets also release *Growth Factors* which help repairing the damaged tissue.

²⁴Through the same property it exerts the anti-inflammatory and analgesic action. For more information on the transformation of arachidonic acid in platelets and the possible consequences of COX deficiency see [148].

²⁵Drugs containing citrates have the effects of lowering Calcium concentration, inducing some anticoagulant effect. Therefore their use must be avoided in while assuming potent anticoagulants.

²⁶*Mast Cells* are another group of granulocytes, affine to basophils, which reside in connective and mucosal tissues (as different species) and may live for weeks or months. They are part of

in controlling inflammations, neutrophils being particularly active. The names are justified by how they are stained by dyes: eosinophils stain red by the acid dye eosin, basophils stained blue by basic dyes, and neutrophils stain a pale pink.

(II) Agranulocytes comprising:

- (a) *Lymphocytes*. The name comes from the fact that they are found in lymph. They come in three types: *Natural Killer Cells* (cytotoxic), part of the *innate* immune systems, *B-cells*²⁷ (responsible for the production of antibodies in plasma), and *T-cells*²⁸ (both of similar size as RBCs) which mature in thymus (thymocytes),²⁹ attacking noxious cells, viruses and transplants; they make 20–50% of WBCs; B- and T-cells belong to the adaptive immune system.
- (b) *Monocytes*, the largest cells (diam. up to 20 μm), making 2–10% of WBCs, they differentiate in *Macrophages*, which together with monocytes, neutrophils, mast cells, and dendritic cells are phagocytes. Phagocytes are able to migrate chasing their prey using their chemical sensors, a mechanism known as chemotaxis, about which a large mathematical literature has been developed, starting from the celebrated Keller-Segel model [123].

The total concentration of WBCs in blood ranges between $4.5 \times 10^3/\text{mm}^3$ and $11 \times 10^3/\text{mm}^3$, making $\sim 1\%$ of blood volume, and they have generally short life (up to about a week, with the exception of Lymphocytes who can live some weeks). Their importance in the coagulation process consists in the ability of monocytes to produce *Tissue Factor* (defined below), which we will see to be a triggering factor for clotting. See e.g. [57].

(C) Red Blood Cells (RBCs)

Oxygen carriers in blood, thanks to the *hemoglobin* molecules³⁰ in their cytoplasm. They make $\sim 45\%$ of blood volume, with a concentration of $5 \div 6 \cdot 10^6/\text{mm}^3$, lifespan ~ 120 days. They have a diameter of $\sim 6\text{--}8 \mu\text{m}$ (volume $\sim 90 \text{ fL}$)³¹ and no nucleus. Their shape is discoid with a thickness of about $2 \mu\text{m}$

the **immune system**, which includes WBCs along with *dendritic cells* (present in blood in the immature stage and residing in various tissues: see the book [206]).

²⁷B-cells types: Plasmocytes, memory B-cells, B1- and B2-cells, marginal zone B-cells, Follicular B-cells, regulatory B-cells. The “B” comes from the name of the organ in which they mature in birds, *Bursa of Fabricius*, where B-cells were first discovered. It was then found that in humans B-cells mature in the *Gut Associated Lymphoid Tissue* (GALT), mainly located in the intestine.

²⁸T-cells types: helper, cytotoxic, memory, regulatory, natural killer (not the same as the ones already listed), mucosal associated invariant, gamma-delta.

²⁹From which the “T” derives.

³⁰Each RBC contains 270 million hemoglobin molecules.

³¹RBCs smaller than 80 fL are typical of *microcytic anemia*. When their volume exceeds 100 fL we have *macrocytic anemia*.

at the rim and about 1 μm at the center. They have no mitochondria either, so they do not consume the oxygen they transport. They are considered just as passive elements in blood coagulation, being trapped in the fibrin network and providing a large part of the clot volume. However the recent discovery that they can synthesize NO [127], which we know to influence platelets activity, may open new perspectives. More on the effect of RBCs in platelet aggregation can be found in [193].

2.3.2 Platelets Regulators

Here we list stimulators or inhibitors of platelets activity. Some of them have been already mentioned as a comment to Fig. 2.10.

(A) Activators

- TXA_2 (*Thromboxane A2*). It is produced by activated platelets, thus triggering a positive feedback. It favors platelets aggregation. It degrades to *Thromboxane B2*, eliminated through urine. We have already mentioned the steps leading to TXA_2 : arachidonic acid $\xrightarrow{\text{cox1}}$ prostaglandin H2 $\xrightarrow{\text{tromboxaneA-synthase}}$ TXA_2 .
- ADP (*Adenosine-Di-Phosphate*). A very important molecule,³² contained also in RBCs. For this reason hemolysis (RBC's disruption, which may occur under excessive mechanical stress³³) can liberate ADP in the blood-stream, activating platelets.
- *Serotonin* is a vasoconstrictor, thus favoring hemostasis.
- *Platelet Factor 4*.³⁴

(B) Inhibitors

- NO (*Nitric Oxide*), produced by endothelial cells, is a platelet inhibitor. Its main function in the body is vasodilation via relaxation of the vessel smooth muscle.

³²AMP, ADP, ATP contain 1 (Mono-), 2 (Di-), 3 (Tri-) atoms of phosphorus and they are obtained in that sequence by addition of a P atom (a process called *phosphorylation*). ATP has a vital importance in cells metabolism.

³³This condition can be produced by arterial stenosis, possibly as a consequence of clotting itself, or because of the mechanical action of implanted devices (rigid artificial heart valves).

³⁴Platelet Factors 1–3 actually regulate interactions with the Coagulation Factors IIa (thrombin), V, X.

- *Prostacyclin* (or *Prostaglandin I₂*, PGI₂),³⁵ also released by endothelial cells, prevents platelets adhesion and also counteracts the effects of TXA₂ binding.
- *Ecto-ADP-ase*, synthesized by the endothelium,³⁶ neutralizes ADP.

2.3.3 The Coagulation Factors

(A) Von Willebrand Factor (vWF)

The Finnish physician **Erik Adolf von Willebrand** (1870–1949) studied a severe, often fatal, bleeding disorder (which later took his name) affecting several individuals in a remote Finnish village, recognizing that it was different from hemophilia (for example individuals of both sexes were affected) (Fig. 2.12).

He published his studies in 1926, though he could not provide any precise explanation of the disease which he called pseudohemophilia. We know today that the cause of that disease is deficiency or dysfunction of the so-called von Willebrand Factor, which is a large multimeric molecule (molecular mass ~250 kDa) stored in cytoplasmic granules (*Weibel-Palade bodies*) of many

Fig. 2.12 E.A. von Willebrand [Helsinki University Museum]



³⁵Prostaglandins make a large family. The first prostaglandin was isolated in seminal liquid (1935) by the Swedish physiologist **Ulf von Euler** (1905–1983) and believed to be produced only in the prostate gland. Actually several other tissues are able to secrete prostaglandins. See the section on anticoagulants.

³⁶Ecto-enzymes act at the exterior of cells. For more details about this enzyme see [85].

cells (and in the platelet δ -granules, as we said), but also circulating in blood (see Subsection E below). vWF molecules are cleaved by the enzyme ADAMTS13 into molecules of smaller size. The fragmentation of the very large molecules is essential for vWF to work correctly. They can be unfolded by stress, thus exposing more binding sites to platelets. vWF stress activation is a property having a special influence on platelets adhesion to fibrin (see [26, 217]). The papers [216] and [214] are excellent sources of information about vWF. A specific analysis of the role of shear stress in platelets binding with vWF or to fibrinogen (see subsection C below) has been performed in [115]. In that paper it is shown that low shear stress (12 dyne/cm^2) favors fibrinogen mediated aggregation, which is however unstable and disappears when the stress is increased to 68 dyne/cm^2 , while at 80 dyne/cm^2 vWF takes over the binding role.

(B) Tissue Factor (TF)

Frequently called *Thromboplastin* and sometimes *Thrombokinas*.³⁷ TF has a fundamental role in the clotting process, since the chemical cascade leading to the final formation of the fibrin network is initiated by the direct exposition of endothelial TF to blood as a consequence of a lesion. The historical review paper [18] is very instructive. The fact that TF can be produced by monocytes, making blood born TF available was proved in [106], though there is some controversy about this fact (see the interesting review paper [47]). More recently evidence was provided that **platelets can synthesize TF** (see [61, 180]), and in special circumstances accumulate it in the open canalicular system and in the α -granules [146]. To our knowledge this fact has not yet taken into account in any mathematical model, but it may deeply modify the whole picture of the process.

(C) The numbered Factor pairs

The path which leads from TF exposure at the injury site to the formation of the fibrin network is basically a cascade of chemical reactions in which, at each step, a *zymogen* is modified to an *enzyme* (a *serine protease*),³⁸ which is said to be the active form of its precursor. In the cascade the enzyme will perform a similar operation on another zymogen (what is called an *enzymatic reaction*). Thus factors generally come in an activated and in a non-activated form. This is the basic structure of the biochemical model that will be illustrated in Sect. 2.4, though the actual cascade requires the intervention of platelets and of *complexes* (combinations of factors) and is also accompanied by antagonist reactions. Some factors once activated do not possess an enzymatic activity, but they bind in very active complex with another activated factor, to which, so to speak, they are subsidiary. For this reason they are called *cofactors*. In the 1950s the research on coagulation had an impressive acceleration and simultaneous

³⁷Though there is some disagreement on the exact meaning of this name, it is sometimes attributed to TF.

³⁸Serine proteases are a large class of enzymes. The list of serine proteases is impressively long. See [http://biochem.wustl.edu/\\$sim\\$protease/ser_pro_help.html](http://biochem.wustl.edu/simprotease/ser_pro_help.html).

and independent discoveries had produced a great confusion in nomenclature. For this reason in 1954 the International Committee for the Nomenclature of Blood Clotting Factors was created with the aim of standardizing the names of the *coagulation factors pairs* known at that time. The reader can find a short report of the activity of this Committee in [277]. The document is interesting because it lists the name of the members, including many fathers of modern hematology. In 1957 the Committee met in Rome, in 1959 in Montreux and then more times till 1963.

Roman numbers were attributed according to the chronological order of discovery, following the trend already established by Paul Morawitz (to whom we will return later) at the beginning of twentieth century (see Sect. 2.6), when only the first four were known thanks to the work of **Hermann Adolf Alexander Schmidt**³⁹ (1831–1894), and continued by the Norwegian **Paul Owren** (1905–1990), the discoverer of Factor V (1944)⁴⁰ [178]. The Committee produced a list which was later extended and is basically the one used today (though some of the former names are still in the literature). Here are the factors ordered from I to XIII (alternative names in *italic*):

- FI *Fibrinogen*,⁴¹ FIa *Fibrin*, resulting from polymerization of Fibrinogen
- FII *Prothrombin*, FIIa *Thrombin* (the main enzyme in the cascade, as its name indicates)
- FIII is nothing but *Tissue Factor*
- (FIV identifiable with Ca^{++})
- FV/ FVa [formerly *proaccelerin* and *accelerin*, resp.], FVa is cofactor of FXa
- FVI [later recognized to be identical to FVa]
- FVII/ FVIIa⁴² [formerly *pro-convertin*–*convertin*]
- FVIII/ FVIIIa⁴³ [formerly *anti-hemophilic globulin*, AHG], also known in the old literature as Platelet Cofactor I, FVIIIa is cofactor of FIXa
- FIX, or *Christmas Factor*⁴⁴/ FIXa

³⁹A distinguished physiologist born in today Estonia, Schmidt had the idea that fibrin had to come from fibrinogen as the result of an enzymatic reaction, whose agent he called thrombin, which in turn had a precursor, which he called prothrombin [220, 221].

⁴⁰Publication was delayed because of II World War.

⁴¹Fibrinogen was described as the precursor of Fibrin by **R. Virchow** in 1847 [252]. It has also other specific functions, illustrated in this chapter. It is also known to intervene in RBC's aggregation (forming the so-called *rouleaux*), a phenomenon of great importance in blood rheology.

⁴²Found by **B. Alexander** in 1949 [7] and isolated a few years later [131].

⁴³Isolated in 1937 by **A.J. Patek** and **F.H.L. Taylor** [185], following the experiments made a year earlier by Patek and **R.H. Stetson** [184].

⁴⁴Stephen Christmas was the first patient diagnosed with FIX deficiency (hemophilia B) (Oxford, 1952) at the age of five. He was a patient of **R. Biggs** and **R.G. Mcfarlane** [32]. He died in 1993 by AIDS. Many of transfusion dependent patients were infected by the HIV virus before blood screening became obligatory. A case which became emblematic was the one of Ryan Wayne White, affected by hemophilia A, who became discriminated when he was diagnosed with AIDS. He died still a teenager in 1990.

- FX, or *Stuart-Prower Factor*⁴⁵ /FXa
- FXI (formerly *Plasma Thromboplastin Antecedent*, PTA)⁴⁶ / FXIa
- FXII *Hageman Factor*⁴⁷ / FXIIa
- FXIII *Laki-Lorand Factor*⁴⁸ / FXIIIa

Factors VIII, IX, XI are also known as *antihemophilic factors* A, B, C respectively, since their deficiency causes hemophilia A, B, C (see the section about bleeding disorders). A review of the intense activity that led to the discovery of Coagulation Factors can be found in the papers [19, 210], and in the books [177, 237]. We specially recommend the historical review by Davie [63]. Not less interesting is the paper [88], containing the pictures of many of the patients who gave their name to the factors and of their doctors.

(D) Other proteins involved in blood coagulation

The numbered factors are not the only characters in the play, which is actually far more complicated. One more zymogen-serine protease pair is

- *Protein C*⁴⁹ (PC), produced in the liver, rarely called FXIV, with its *activated version* APC. Activation is performed by the *Thrombin-Thrombomodulin complex* (see below). It inactivates FVa and FVIIIa, thus contrasting coagulation.

The just mentioned action of APC is mediated by

- *Protein S* (PS)⁵⁰ (PS) as a cofactor.

A protein structurally related to serine proteases is

- *Protein Z* (PZ) that has a role in the degradation of FXa.

A crucial role in coagulation is played by a family of vitamins produced in the liver and collectively called

- *Vitamin K*.⁵¹ Actually, many coagulation factors (VII, IX, X) and the Proteins C, S, Z are **vitamin K-dependent** in the sense that their action is conditioned to the presence of vitamin K. Hence its fundamental importance.

⁴⁵Named after the patients Rufus Stuart and Audrey Prower and described in 1957 by C. Hougie and coworkers [109].

⁴⁶Identified in 1953 by R.L. Rosenthal and coworkers [212].

⁴⁷Named after Ratnoff's patient John Hageman (1955) [200, 201]. Oscar David Ratnoff (1916–2008) was one of the leading American scientists in hematology. See more in the section on anticoagulants.

⁴⁸K. Laki and L. Lorand suggested its existence in 1948 [136].

⁴⁹Discovered in 1960 by Walter H. Seegers.

⁵⁰A very important function of Protein S in the organism is to facilitate phagocytosis of apoptotic cells by macrophages. Discovered in 1979 (by E.W. Davie) in Seattle, takes its name after that city.

⁵¹Denominated after the German name *Koagulationvitamin*. Discovered in the 1930s, a Nobel prize was attributed in 1943 to H.K.P. Dam and to E.A. Doisy for their studies on it, though its real action in the coagulation process became clear only in the 1970s.

Drugs inhibiting vitamin K are widely used as anticoagulants. Since the most rapid action is on the anticoagulant proteins PC, PS, PZ, they first favor clotting, while the strong anticoagulant effect is seen with some delay. For this reason heparin (see next section) is simultaneously administered. Drugs neutralizing vitamin K are on the market with various trademarks. The most known are *Warfarin* (in the U.S.)⁵² and *Coumadin*.

Activated factors have their own *inhibitors*, that will be described below (Sect. 2.3.4).

(E) Complexes

The following *Factor Complexes* have an important role in the coagulation process:

- *Complex FVIII-vWF* is the main carrier of inactive FVIII in blood.⁵³ Under the action of FIIa it dissociates and FVIII is rapidly activated.
- *Complexes FVII-TF, FVIIa-TF* intervene in the initiation phase of the cascade.
- *Complex FVIIIa-FIXa* (+Ca++) is called **Tenase**⁵⁴ because it activates FX.
- *Complex FVa-FXa* (+Ca++) is called **Prothrombinase** because it promotes the transition from FII to FIIa. We will discuss its pivotal role in the cascade.
- *Complex Thrombin-Thrombomodulin*. Thrombomodulin is a protein expressed by endothelial cells. This complex induces the activation of Protein C and turns TAFI (see Sect. 2.3.4) into its active form, providing protection to Fibrin.

(F) More coagulation factors

According to the *Cell-Based Model* (Sect. 2.4), all the Factors listed above enter the chemical cascade leading to Fibrin production, **except FXII**. This is the main discrepancy with the *3-pathway Cascade Model* (Sect. 2.6), used until recently. In the latter, activation of FXII is the triggering event of the *intrinsic pathway* of coagulation (i.e. a process originated within blood, independently of exposure to TF, which is of *extrinsic* nature). Since there is evidence of clotting of intrinsic origin, and also of the fact that FXII can become activated when blood comes into contact with artificial materials, the intrinsic pathway, though not endorsed in the Cell-Based Model, still is worth being considered. It is also called *contact activation pathway*. Here we list the Factors which, in addition to FXII, take part in it.

⁵²See the section on Anticoagulants for some historical news on Warfarin.

⁵³Already in the 1950s it was known that deficiency of vWF was accompanied by a deficiency of FVIII.

⁵⁴The symbol X-ase is sometimes used.

- *Prekallikrein* (PK), also known as *Fletcher Factor*,⁵⁵ complexes with *High Molecular Weight Kininogen* (HMWK) by contact with collagen, in the presence of FXII.
- *High Molecular Weight Kininogen* (HMWK),⁵⁶ also known as *Fitzgerald Factor*.
- *Kallikrein*,⁵⁷ the active form of PK, following the formation of the complex PK-HMWK. In turn, Kallikrein is a fast activator of FXII. Another PK activator is the enzyme *prolyl-carboxypeptidase* (see [226]).

2.3.4 Fibrinolysis Factors

Fibrinolysis is the process eventually destroying the clot. It goes through positive feedback too, with the intervention of the following factors.

- *Plasminogen* (a zymogen).
- *Plasmin* (a serine protease), the active form of its precursor, attacks fibrin, gradually destroying the clot. Plasmin is also active on vWF and other proteins.
- *Tissue Plasminogen Activator (tPA)*, a serine protease catalyzing the transition from plasminogen to plasmin.
- *Urokinase (urokinase-type Plasminogen Activator: uPA)* (a serine protease), another activator of plasminogen.⁵⁸
- *Thrombin Activatable Fibrinolysis Inhibitor (Carboxypeptidase B2)*, or *TAFI* [172], when activated (by the thrombin-thrombomodulin complex) is an enzyme which protects fibrin from the action of plasmin by slightly modifying its structure. Therefore TAFI exerts a control action on fibrinolysis.

⁵⁵It was first described in 1965 by **W.E. Hathaway** [101] who investigated treats of abnormal coagulation in members of Fletcher family (Kentucky), not exhibiting bleeding disorders. Prekallikrein as a precursor of kallikrein was isolated in 1972 by **K.D. Wuepper** [280] who identified it with Fletcher factor one year later [279].

⁵⁶*Kininogens* are proteins which are precursors of kinins (see next footnote), such as *bradykinin* and *kallidin*, which are vasodilator. HMWK is also known as *Williams Factor* or *Flaujeac Factor*. The Flaujeac trait was described by **J.M. Lacombe** in 1975 [135]. The cause was soon identified with HMWK deficiency [281].

⁵⁷Here we refer to *Plasma Kallikrein*, distinct from the numerous group of *Tissue Kallikreins* which are enzymes performing various actions. It was named after the Greek words *kalli* (sweet, in this context) and *krein* (flesh) referring to pancreatic tissue. Plasma Kallikrein (like some of its tissue analogs) liberates kinins from the kininogens. The so-called *kinin-kallikrein system* has a role in regulating blood pressure, owing to its vasodilation action (see e.g. the book [69]). In 1909 **J.E. Abelous** and **E. Bardier** [1] observed that human urine injected in dogs caused a drop in blood pressure and conjectured that a substance produced in some organ and eliminated through urine had to be responsible. The (Kallikrein) substance was identified and named in 1930 [132], then extensively studied by **E.K. Frey**, **E. Werle** and others.

⁵⁸Also *Kallikrein* and FXIIa can activate plasminogen.

Besides tPA and uPA, plasminogen can be activated to plasmin by the Hageman factor FXIIa, a fact established long ago by Ratnoff himself and coworkers [94], and by Kallikrein. Another plasminogen activator is the enzyme *streptokinase*,⁵⁹ which is so effective to be frequently used as a fibrinolytic after myocardial infarction and pulmonary embolism.

2.3.5 Factors Inhibitors

Both coagulation and fibrinolysis factors have their inhibitors, as all proteases⁶⁰ do. The delicate game played in the body by proteases and their inhibitors tells us that indeed health requires the equilibrium of a huge numbers of substances in permanent mutual conflict. In a sense this fact expands to an unthinkable scale the naïve idea of the equilibrium among the four Hippocratic humors!

Serine Protease Inhibitors make a large group of proteins neutralizing specific serine proteases. Of course their list is as long as the one of serine proteases. The acronym **serpin** is frequently used to denote any of these proteins. For our purposes we are interested in the following ones.

(A) Serpins neutralizing pro- or anti-coagulation factors

- *Antithrombin*. Most commonly referred to as *Antithrombin III (ATIII)*,⁶¹ it inhibits most of the activated coagulation factors (IXa, Xa, XIa, and IIa) and the FVIIa-TF complex. Its action is enormously enhanced by *Heparin*.⁶²
- *Tissue Factor Pathway Inhibitor (TFPI)* can inhibit thrombin (FIIa) and also FXa by forming a complex with it. In turn the latter complex can inhibit the FVIIa-TF complex (the initiator of coagulation). It is released by endothelial cells and also by platelets.
- *Alpha 1-antitrypsin*. This serpin interacts with many proteases and therefore is particularly important.⁶³ It is known to inhibit APC.
- *Protein C inhibitor* limits the expression of Protein C.
- *Protein Z-related protease inhibitor*. It neutralizes FXa in the presence of PZ. It also inhibits FXIa.
- *Kallistatin*, an inhibitor of Kallikrein.
- *Heparin cofactor II*, rapidly inhibits thrombin in the presence of heparin.

⁵⁹So-called because it is secreted by some bacteria in the streptococci family. Since it is not produced in the body it may trigger immune response and must be used with care.

⁶⁰Proteases are enzymes attacking proteins (proteolysis).

⁶¹AT I-IV are also found in the literature, with specific targets.

⁶²Discovered in 1918 [110], though isolated in 1916 in canine liver tissue [158]. See more in the section on Anticoagulants. Heparin can be secreted by basophils.

⁶³Its deficiency leads to degradation of tissues, particularly in the lungs, causing emphysema. Smoke is believed to inactivate this serpin, thus causing additional damage to lungs.

- *Nexin II*, secreted by α -granules of activated platelets, is an inhibitor of FXIa [285] and of FIXa [219].
- *C1-inhibitor*,⁶⁴ an important inhibitor of Kallikrein, FXIa, FXIIa.

(B) Serpins neutralizing pro- or anti-fibrinolytic factors.

- *Plasminogen activator inhibitor-1* (PAI-1) [172] and *Plasminogen activator inhibitor-2* (PAI-2)⁶⁵ inactivate both tPA and urokinase.
- *Neuroserpin* inhibits tPA and urokinase.
- *Alpha 2-antiplasmin* is a strong inhibitor of plasmin [172].
- *Alpha 2-Macroglobulin* is another multifunction serpin. It inhibits plasmin and Kallikrein.

We have listed most of the protagonists of the incredible show of coagulation. Now it's time to see them at work.

2.4 The Cell-Based Model for Secondary Hemostasis

In this section we will illustrate the model currently believed to be the correct biological interpretation of the blood coagulation process. Known as the *Cell-Based Model*, it has its roots in many papers (see [59, 68, 108, 150, 151, 175, 208, 231, 233] and the literature quoted therein). Its formulation is recent and must be considered a great achievement. We postpone the exposition of the model it has replaced (the three pathway cascade model) after the full explanation of the cell based model and the section about bleeding disorders. The reason not to respect the historical development is that the inadequacy of the old model can only be understood in the light of the difficulty of interpreting hemophilic disorders. Therefore we believe that it is much better to present the modern theory right away, rather than taking the reader through a devious path. The synthetic review [26] is interesting for its original approach.

The Cell-Based model has a four step structure: *initiation, amplification, propagation, termination*. Fibrinolysis goes in parallel and becomes visible over a longer timescale, according to a precise biological strategy. Indeed, while the clot has to be formed soon after the lesion, it has to dissolve slowly, allowing enough time for the wound to be repaired, and gradually, without breaking into pieces which would be dangerously released in the bloodstream with the risk of embolism.

We are going to examine the two processes of secondary hemostasis (at the light of the Cell-Based model) and of fibrinolysis separately. Before doing that, let us recall very shortly that **primary hemostasis** (the early stage of wound sealing) is

⁶⁴The main function of C1-inhibitor is to contrast some of the molecules in the *C1-complement* group part of the so called *complement system* having an adjuvant role in the immune system.

⁶⁵PAI-2 is detectable only in pregnant women, a fact that may justify the increased risk of thrombosis during pregnancy.

performed by platelets, see e.g. [270]. They quickly bound to the lesion site and among themselves forming the so called white thrombus, where they are highly concentrated. The ligands intervening in this process are vWF (stress activated) and FII, whose dimer structure and affinity with platelets receptors GPIb-IIIa makes it an ideal bridge between platelets. See the already quoted papers [214, 216], and [115].

2.4.1 Secondary Hemostasis

Stage 1: Initiation (Fig. 2.13)

Once tissue factor has become exposed to blood at the injury site, the complex FVII-TF is readily formed, and the tiny amount of FVIIa which normally circulates in blood (estimated as a 0.5% fraction) gives rise to the FVIIa-TF complex.⁶⁶ The latter activates FVII-TF and (at a low rate) FIX, FX. Now we have a small amount of FIXa, FXa (in turn able to activate more of the complex FVII-TF). In particular, at this stage a small quantity of FVa is produced as the result of the

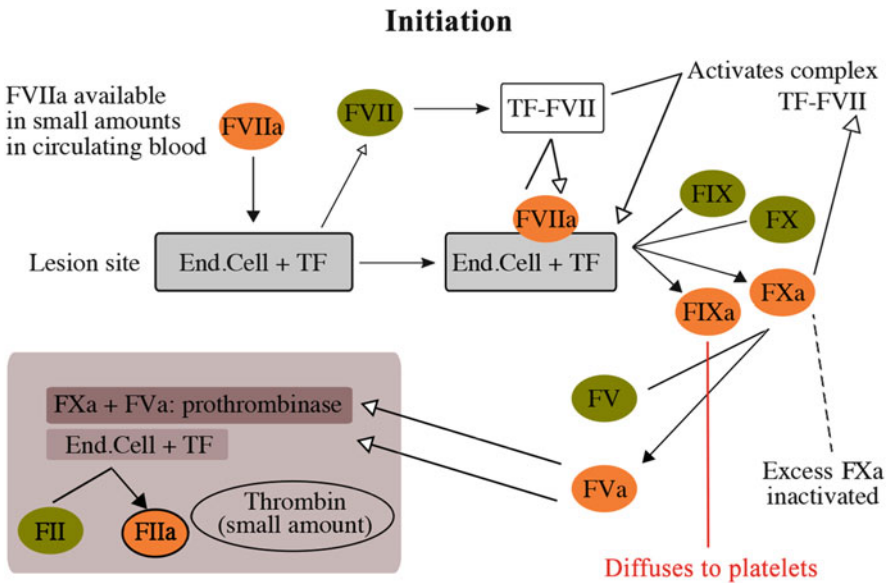


Fig. 2.13 Sketch of the initiation phase

⁶⁶Some FVIIa can reach TF in nonvascular tissues even in the absence of a lesion [289], thus making FIXa and FXa accidentally available. However, coagulation does not start because it requires for instance the intervention of platelets, which are not available out of the bloodstream.

action of FXa on FV. The ability of FXa to activate FV has been proved long ago (in [167], see also [82]). Though thrombin is by far the main activator of FV, while the activation rate by FXa is orders of magnitude less, the production of FVa even in very small quantity is absolutely crucial in order to give rise to complex FVa-FXa (prothrombinase) [149], which transforms FII (prothrombin) to FIIa (thrombin).⁶⁷ Thus the initiation stage has the fundamental task of making some thrombin available. We must not forget that in the meanwhile platelets keep accumulating at the lesion site.

It is now important to say that Fig. 2.13 tells only part of the story. Indeed two additional considerations are in order.

- FXa and thrombin leak from the clotting site and are carried by blood. However they are not going to trigger clotting downstream, because FXa is neutralized by ATIII (more rapidly than thrombin, and the same happens to FIXa) and by endothelium produced TFPI, while thrombin reaching endothelium combines with thrombomodulin (TM), losing its procoagulant activity. Moreover, the thrombin-thrombomodulin complex is an activator of Protein C, and now APC strongly inhibits FVa on the surface of endothelial cells, thus helping to keep the coagulation process confined close to the initiation site.
- In addition, the complex FXa-TFPI has an inhibiting action on the complex FVIIa-TF (see [160]). Therefore we can say that, due to the formation of FXa-TFPI and of APC, during this stage a regulatory mechanism is present, somehow delaying the exit to the next stage.⁶⁸

Stage 2: Amplification (Fig. 2.14)

Thrombin produced in the previous stage exerts many actions both at this and at later stages. We list them as (Thr. n):

- (Thr. 1). It dissociates the complex FVIII-vWF, at the same time activating FVIII,
- (Thr. 2). It activates FV, stimulating platelets to produce more of FV,
- (Thr. 3). It activates FXI. In turn FXIa activates FIX.

These actions have important consequences. FXIa is a fast activator of FIX, so that the amplification stage makes both FIXa and FVIIIa available. Moreover vWF can promote further platelets aggregation (if stress conditions are favorable). Finally, platelets become fully activated, releasing the granules content. We stress the fact that *thrombin is the only activator of FVIII*.

⁶⁷FXa itself is an extremely mild activator of prothrombin. The rate of thrombin generation by prothrombinase is 300,000 times larger the one by FXa alone [149]. The prothrombin to thrombin transition is a quite complicated process. Details can be found in the review paper [133].

⁶⁸The proteins responsible for this regulatory action have a fundamental role in eventually halting the clot growth. They are inevitably produced at this initial stage too, but it is known that free FVa inhibition by APC is far less efficient than on the surface of endothelial cells. One can wonder whether, besides the clotting confining action, the simultaneous slowing down of the initial process may have a precise aim, for instance letting the platelet plug become thicker.

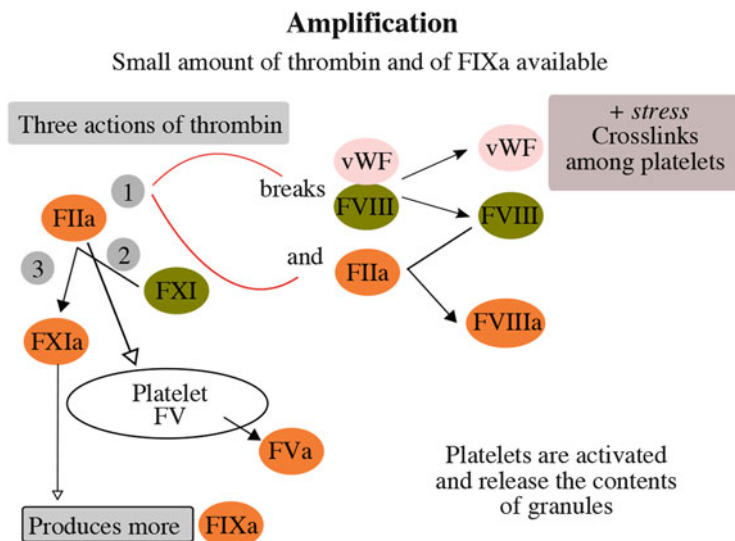


Fig. 2.14 Sketch of the amplification phase

Stage 3: Propagation (Fig. 2.15)

The coagulation machine is now ready to produce the **thrombin burst** (during which 95% of thrombin is produced [150]). The surface of activated platelets provides the ideal site for the combination of FVIIIa, FIXa into the complex tenase, which activates FX very rapidly. FXa combines with the available FVa (still on activated platelets surface) yielding the prothrombinase complex. From now on activation of prothrombin occurs at a large speed and the processes already described in the previous stage trigger an enormously effective positive feedback. The onset of the propagation phase is indeed recognizable by the sudden and marked increase of thrombin production rate. See Fig. 2.16.

Besides continuing actions (Thr. 1–3), thrombin performs two more important tasks:

- (Thr. 4) transition from FI (*fibrinogen*) to polymer FIa (*fibrin*),
- (Thr. 5) activation of FXIII.

Thrombin cleaves the so called *fibrinopeptides* from fibrinogen, thus transforming it into a *fibrin monomer* (see e.g. [215]). Polymerization occurs then at a high rate, so that fibrin appears soon after the formation of thrombin. The process continues with the fast aggregation of polymer chains into fibers [80]. From now on the fibrin network traps blood constituents and we may say that the clot progression is *fibrin dominated*. A statistical approach to the formation of fibrin has been developed in [165].

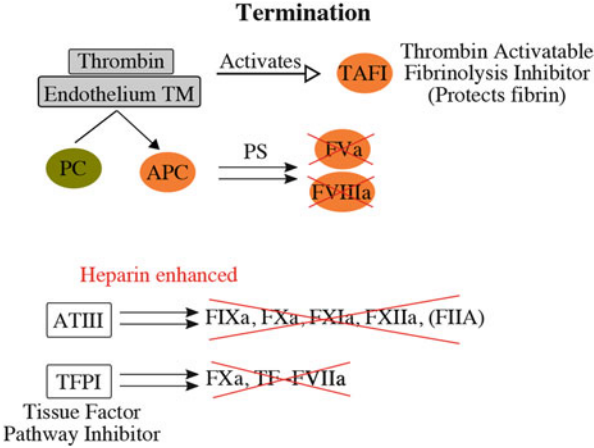


Fig. 2.17 Sketch of the termination phase

The role of FXIIIa is to consolidate the fibrin network by forming cross links among fibers. Clots lacking cross links are unstable, as proved by the fact that a nontrivial bleeding disorder is associated with deficiency of FXIII.

A further remark is about the fact that, while FVa is effectively inactivated by APC when not included in the prothrombinase complex, it appears to be protected from this action if combined with FXa. Protection of FVa from APC exerted by FXa was established in [173, 267].

Stage 4: Termination (Fig. 2.17)

As we have said several times, the biological processes of clot forming and dissolution are the result of an unbalance between contrasting elements which happens to be first in one direction and then in the opposite. If starting and accelerating the growth of a thrombus is important for arresting bleeding, terminating it is absolutely essential to prevent vessel occlusion. We have seen how thrombin helps in confining clotting. By means of the very same reaction it actually starts the termination mechanism:

- (Thr. 6) The thrombin-TM complex activates PC.

APC (with the cofactor PS) inhibits both cofactors FVa and FVIIIa,⁶⁹ thus switching off the production of tenase and of prothrombinase. Surviving FIXa, FXa, FXIa and thrombin are inactivated by ATIII (this action is greatly accelerated by heparin), and TFPI complexes with FXa, inhibiting FVIIa-TF at the same time.

⁶⁹FVa on platelets membrane is inactivated faster than the same factor complexed with FXa (see e.g. [173, 238]). Likewise FVIIIa is partially protected when it is complexed with FIXa. For more details and for the role of Protein S in these processes see [174].

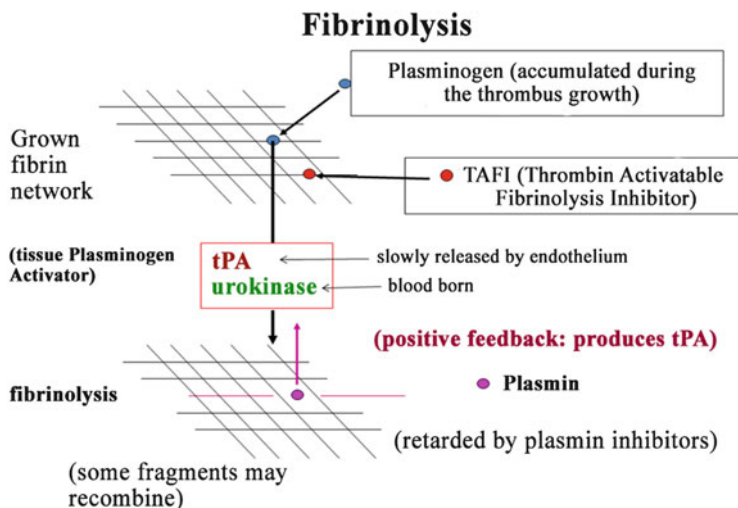


Fig. 2.18 Sketch of fibrinolysis pattern [from: Fig. 7.8 [35]]

The clot has grown to its maximum size and it is now time to take care of its gradual dissolution, which however needs to be delayed for the reasons we have said several times. Once more thrombin has a leading role:

- (Thr. 7) thrombin activates TAFI, which makes fibrin more resistant to the attack of plasmin.

2.4.2 Fibrinolysis

As we said, fibrinolysis (Fig. 2.18) goes in parallel to clotting, but it is regulated so that the fibrin network can develop sufficiently rapidly and that the clot dissolves slowly and gradually, without delivering dangerous fragments in the bloodstream. Since the clot is basically impervious to plasma and all diffusion processes (particularly of heavy molecules) are considerably slow in it, the fibrinolytic elements have to be stored within the clot during its growth. We have listed them in Sect. 2.3.

Plasminogen, synthesized by the liver, is available in circulating blood, so it is naturally trapped in the clot. We know that it is activated to Plasmin by the serine proteases tPA and urokinase (and also by Kallikrein and FXIIa). Secretion of additional tPA by the endothelium is stimulated by the clot itself. We have seen that TAFI, accumulated during clot formation, reduces plasmin action, retarding the process. The speed of the process is also controlled by the serpins inhibiting tPA and urokinase (mainly PAI-1) and plasmin (mainly α_2 -antiplasmin).

At the early stage of fibrinolysis fragments of the fibrin network may recombine.

A product of fibrin degradation is the so called *D-dimer*, whose presence in blood reveals ongoing fibrinolysis.

As a final remark, we mention the interesting point of view illustrated in the papers [50, 117], which stems from the experimental observation that fibrinopeptides are normally present in plasma in very low concentration, thus indicating that thrombin too has to circulate. Since fibrinopeptides have a lifetime of the order of few minutes and the same is for thrombin due to its inactivators (mainly ATIII), this requires continuous thrombin generation in blood. Then the question arises why clotting is not triggered. Paper [117] discusses how ATIII regulation of that “idling” clotting system can generate an activation threshold. The question of identifying a clotting threshold has attracted the attention of many mathematicians (see Sect. 2.8).

2.5 Bleeding Disorders

As we have seen, blood coagulation involves a very large numbers of elements of different nature and goes through numerous steps. Therefore there is plenty of room for something to go wrong. As a consequence, it is not surprising that the variety of bleeding disorders is likewise impressive. Bleeding disorders (or *coagulopathies*) can lead to ineffective coagulation (*hypocoagulability*, generally with *bleeding diathesis*, i.e. spontaneous bleeding), or, in the opposite case (*hypercoagulability* or *thrombophilia*), they can lead to unwanted clotting, i.e. thrombosis. Such phenomena can occur with different levels of intensity and consequently with a different impact on health. Understanding the cause of a bleeding disorder is clearly necessary for selecting a therapy, and we must not forget that it is precisely through the study of such disorders that much of the knowledge possessed today about blood coagulation has been built up. It is important to provide a sufficiently accurate sketch of bleeding disorders because this is largely an open field for mathematical modeling.⁷⁰

We can review bleeding disorders according to their origin. So a natural way of exposing this material is to follow the same articulation adopted in Sect. 2.3.

(I) Platelet Related Bleeding Disorders

Since platelets are so complicated there are several types of dysfunctions that may produce bleeding disorders. Their number is astonishing, though most disorders are rare or very or even extremely rare. We decided to provide a reasonably complete list with the aim of showing how complicated the subject is and how difficult diagnosis can be.

- **Thrombocytopenia.** Literally it means scarcity of thrombocytes (i.e. platelets). It is diagnosed if the platelet count drops below 1/3 of the minimum (i.e. less than 50,000/mm³). There are very many possible causes of different gravity.

⁷⁰The book [154] collects and comments a number of historical papers on bleeding disorders and other blood diseases.

For instance the spleen may start storing too many platelets, sequestering them from the bloodstream, or the immune system may produce antibodies attacking platelets (*Immune Thrombocytopenia*), or it can be related to other pathological conditions. It can also be drug induced. A striking and puzzling example in the latter class is

- *Heparin Induced Thrombocytopenia (HIT)*. Thrombotic episodes (also of a massive character) were observed in patients under anticoagulant treatments with heparin. This counterintuitive phenomenon has been the object of many controversial studies. Today it is believed to result from an immune reaction triggered by heparin, which stimulates platelets activation and clustering. We recommend the reading of [124]. The recent report [156] points out that heparin antibodies are sometimes developed by patients after cardiosurgery with the consequent risk of HIT.

Thrombocytopenia is frequently accompanied by the appearance of bruises on the skin associated with subcutaneous bleeding (*purpura*).⁷¹ Two forms of the disorder are known:

- *Idiopathic⁷² Thrombocytopenic Purpura (ITP)*. It can sometimes be of immune (rather than unknown) origin (in that case the *I* in the acronym can stand for Immune. See the cornerstone paper [211]). It can be a very serious illness. Treatments include the surgical removal of spleen. The interesting paper [40] develops a mathematical model helping clinicians to decide whether or not the spleen removal can be advantageous, depending on the measurements of specific parameters.
- *Thrombotic Thrombocytopenic Purpura (TTP)*. This is in a sense the opposite, since it produces microscopic clots in the microcirculation. It is a rare disorder, actually only indirectly related to platelets, since it is caused by inhibition, or dysfunction or deficiency⁷³ of the enzyme ADAMTS13 (see the subsection on vWF, Sect. 2.3), which results in an excessive interaction between platelets and the uncleaved large vWF multimers. See [164] and [87].

• Other thrombocytopenia-related syndromes

- *Wiskott–Aldrich syndrome* [6, 276], originated by a mutation in the X chromosome (so typically affecting males, while females can be carriers), characterized by eczema, immunodeficiency, small platelets (*microthrombocytopenia*). It is one of the less rare in this group.
- *X-linked thrombocytopenia with dyserythropoiesis*, again an X chromosome anomaly, accompanied by defective maturation of RBCs.

⁷¹The Latin word for *purple*. *Purpura* denotes spots in the range 3–10 mm, smaller spots are called *petechiae*, and those more extended are called *ecchymoses*.

⁷²The Latin equivalent of the Greek derived word *idiopathic* is *sui generis*. In this context it means “of no specific origin”.

⁷³Congenital deficiency accounts for a small fraction of TTP cases and is known as *Upshaw-Schülman syndrome*.

- *Thrombocytopenia with Absent Radius (TAR syndrome)* is a rare genetic thrombocytopenia characterized by the absence of the radius bone in the forearm.
- *Amegakaryocytic thrombocytopenia with radioulnar synostosis*, i.e. scarcity of megakaryocytes in the bone marrow accompanied by the fusion of ulna and radius.
- *Congenital / Acquired amegakaryocytic thrombocytopenia.*
- *Congenital disorders with macrothrombocytopenia: MayHegglin anomaly* (not as rare as the others), *Sebastian syndrome*, *Fechtner syndrome*, *Epstein syndrome*. The first two are associated with granulocytes anomalies, and the others may be accompanied by nephritis and deafness.

- **Cancerous forms**

- *Cyclical thrombocytopenia*: platelets count cycling (10–25 days) with very low minima (it can be misdiagnosed as ITP). This is a cancerous disease (see Chap. 8).
- *Essential thrombocytemia* and *Acute megakaryoblastic leukemia* are other dysplastic forms leading to platelets overproduction (see more in Chap. 8).

- **Thrombocytosis** is the abnormally high platelet count (say, twice the maximum). It can be due to excessive production by the bone marrow, or to spleen dysfunction, or to medical treatments. It predisposes to thrombophilia.

- **Platelets receptors dysfunctions.** We have seen the crucial importance of the various platelets receptors both for primary and secondary hemostasis.

- *Glanzmann's thrombasthenia* [91] involves receptors GPIIb/IIIa, reducing the ability of binding with fibrinogen, vWF, etc.
- *Bernard–Soulier syndrome (Giant Platelets Syndrome)* [29], also relates to inability of binding to vWF because of defective GPIb receptors. It has an influence on platelet morphology and is accompanied by thrombocytopenia.

- **Platelets membrane dysfunctions**

- *Scott syndrome* is a defect in a delicate mechanism occurring on platelet membrane which prevents the formation of complexes tenase and prothrombinase, while the typical functions in primary hemostasis are not altered.
- *Stormorken syndrome*, still related to wrong membrane reactions, but with the opposite consequence of a self-activation of platelets, inducing thrombophilia.

- **α -Granules dysfunctions.** Also rare disorders. We just mention

- *Gray platelet syndrome* consists in α -granules absence with the consequent lack of the proteins there synthesized. Platelets appear grayish and larger.
- *Quebec Platelet Disorder*: the plasminogen stored in platelets is abnormally converted to plasmin, destroying some proteins in the α -granules and most noticeably FV (linked with urokinase excess), while FV level in plasma is normal.

- *Paris–Trousseau syndrome*. Platelets include a fraction of abnormally large α -granules. Thrombocytopenia is always present. Megakariocytes exhibit two populations. It may accompany a severe condition, known as *Jacobsen syndrome*, a congenital disorder affecting physical and mental evolution.
- *Arthrogryposis-renal dysfunction-cholestasis*. An extremely severe multisystem syndrome, which includes α -granules deficiency.

- **δ -Granules dysfunctions**

- *Hermansky–Pudlak syndrome* (extremely rare, except in Puerto Rico). It is associated with other disorders like albinism, neutrophils scarcity and immunodeficiency.
- *Chediak–Higashi syndrome*. Similar to the previous one, but more severe.
- *Idiopathic dense-granule disorders*. A not so well defined class of defective dense granules which may overlap with other platelet disorders.

- **Idiopathic α - and δ -granule disorders**. In very rare cases both granules are defective. Again this represents a class of disorders, not easy to identify.

- **More receptors disorders (rare)**. Diagnosis is generally uncertain.

- *Thromboxane A_2 receptor defect*. It results in a reduced platelet activation.
- *Platelet cyclo-oxygenase deficiency/Thromboxane A-synthase deficiency* have similar effects, since the platelet ability of producing TXA_2 is impaired.
- *ADP receptor defects*. Platelet activation by ADP is hindered.
- *Collagen receptor defects*. Defective receptors are GPVI or GPIa/IIa.
- *Adrenoceptor defects*. It is not clear to what extent a dysfunction of the adrenaline receptor α_2 contributes to bleeding.

Besides [52], good reviews are [96, 98] and [36], which is a systematic exposition rich in details (including incidence). Once more we quote [121] and [75].

(II) Disorders due to Coagulation Factors Deficiency

- **von Willebrand Disease (vWD)**

We have already illustrated the circumstances of its discovery in the mid 1920s. Many children in the Åland islands were affected by life threatening bleeding diathesis (consanguineous mating were an important factor, revealing the hereditary character of the disease). Both sexes were affected (girls could die by menorrhagia). Von Willebrand recognized it was not one of the disorders known at that time. Since vWF is known to be a carrier of FVIII in blood (as a complex), its deficiency can be accompanied by FVIII deficiency. We can distinguish three types of vWD:

- *vWD-1, partial vWF deficiency*,
- *vWD-2, partially defective vWF*, it contains more subclasses, depending on the type of defect (e.g. not forming the vWF-FVIII complex).
- *vWD-3, total vWF deficiency*.

We have already reported the hyperactivity of vWF connected with deficiency of the enzyme ADAMTS13, being responsible for thrombotic thrombocytopenic purpura. A recent study [204] indicates that vWF deficiency is accompanied by reduced platelets activation. We refer to [24] and once more the basic reviews [214, 216].

- **Hemophilia**

In the historical section we have already said much about hemophilia. There are three types⁷⁴:

- *Hemophilia A*, namely FVIII deficiency,
- *Hemophilia B*, or FIX deficiency,
- *Hemophilia C*, or FXI deficiency,⁷⁵

justifying the alternative names of anti-hemophilic factors A, B, C for FVIII, FIX, FXI, respectively. The first two types are ascribable to defective genes in an X chromosomal branch and are therefore extremely rare in women. Hemophilia C instead affects both sexes with equal probability and is a much less severe form. If one inspects the role of FXI in the cell based model, the latter statement may look very surprising, since FIX is activated by FXIa, suggesting that deficiency of FXI should prevent the activation of FIX, with effects completely similar to Hemophilia B. The explanation can be found in the ability of platelets to synthesize FXI, thus compensating the deficiency of the blood born factor (see [83, 195, 268]).

- **FV Leiden mutation**

First identified in the homonymous Dutch city (1994), it is probably the most frequent cause of thrombophilia. This mutated version of FV can do the same job of the normal protein, but the corresponding protease FVa is not attacked by Activated Protein C (see [31]). Other (rarer) mutations of FV (*FV Cambridge*, *FV Hong Kong*) have similar effects.

- **More disorders due to factors deficiency or malfunction**

They are rare or very rare.

- *FI deficiency*. There can be hypofibrinogenemia, afibrinogenemia, or dysfibrinogenemia.
- *FII deficiency*, hypoprothrombinemia (extremely rare). Of course it may have serious consequences.
- *FII G20210A mutation*. It may cause hyperprothrombinemia.
- *FV deficiency*. *Parahemophilia* was the original name given by P. Owren [179] who came to the discovery of FV in 1944 studying the bleeding disorder of a patient (known as “Mary”).
- *Combined FV-FVIII deficiency* (see [235]).

⁷⁴In 1944 an Argentinean doctor (**Alfredo Pavlosky**) had the idea of mixing two blood samples from hemophilic patients and observed that the mixture had acquired the ability to clot. He concluded that there must have been different types of the illness.

⁷⁵More common among Ashkenazi Jews.

- *FVII deficiency* may have important consequences.
- *FX deficiency*, very serious in the severe form.
- *FXII deficiency* is not a significant cause of bleeding⁷⁶: *we shall see that this was a crucial argument to reject the 3-pathway cascade model.*
- *FXIII deficiency* can cause important bleeding. An appropriate reference is [111].
- *Tissue Factor deficiency* has obvious consequences on secondary hemostasis, but it may also interfere with other biological processes.
- *Prekallikrein deficiency*, and *High Molecular Weight Kininogen deficiency* are of little importance if not accompanied by more serious conditions.⁷⁷

(III) Disorders due to Proteins C, S, Z or to Vitamin K Deficiency

- **Protein C deficiency**
- **Protein S deficiency**
- **Protein Z deficiency**

Due to the anticoagulant actions of PC, PS, PZ, deficiency in each of these proteins predisposes to thrombophilia. They are rare. We quote [60].

- **Vitamin K deficiency**

Induced by warfarin treatment or (rarely) occurring naturally. We have already discussed its consequences.

(IV) Disorders due to Fibrinolysis Factors Deficiency

- **Plasminogen deficiency** It is frequently associated with inflammation of mucous membranes (*ligneous*—i.e. rich in fibrin—conjunctivitis or gingivitis).
- **Tissue Plasminogen Activator deficiency** impairs fibrinolysis, but can also be responsible for other disorders connected to fibrosis.
- **Urokinase deficiency** may have consequences on the cardiovascular system.
- **TAFI deficiency** induced in mice seems to have no consequences.

(V) Serpins Deficiencies

The lack of a specific serpin produces a disorder corresponding to a hyperactivity of the enzymes which fail to be inhibited. They are all rare disorders. For example **the deficiency of α_2 -antiplasmin** produces bleeding since the absence of a contrast to plasmin strongly accelerates fibrinolysis [49].

⁷⁶Mr. **John Hageman** died in 1968, quite unexpectedly, by pulmonary embolism following a hip fracture, a fact that was puzzling for Ratnoff himself [203]. It was conjectured that FXII may actually protect against thrombosis. Such a behavior has been explained considering that FXII is a plasminogen activator (see Sect. 2.3, ref. [94]). Correlations of FXII deficiency with miscarriage, coronary infarct, etc. have been reported, but the interpretation of such findings is not unanimous (see [236]). An interesting review paper on FXII is [48].

⁷⁷As we said, the *kallikrein-kinin* system is particularly important in the regulation of blood pressure. Therefore any dysfunction of it can cause hypertension (see [194]).

(VI) Deep Vein (or Venous) Thrombosis (DVT)

Blood stasis or significantly reduced circulation in limbs is known to be responsible of clot formation in deep veins, particularly in the large veins in the legs (femoral, popliteal, saphenous, etc.).⁷⁸ It can also affect deep veins of the pelvis and more rarely in the arms (succlavian or axillary veins).⁷⁹ It causes swelling and it may cause pulmonary embolism. For that reason it has to be treated as soon as possible with strong anticoagulants.⁸⁰ A typical consequence is a permanent damage of vein valves.⁸¹ Altered circulation can produce conditions favorable to clotting particularly in the valves pocket.

Clot formation in the absence of lesions and in an environment of reduced stress is difficult to explain on the basis of the Cell-Based model, since the process has a clear intrinsic origin. It is out of question that fibrin production requires the prothrombinase complex, thus the activation of both FV and FX.⁸² Rather than appealing to the contact activation pathway, involving FXII (see next section), the modern view is to identify TF delivery as a triggering cause.⁸³ An interesting discussion about DVT in different veins can be found in [4], where some hypotheses are presented for the TF source. It can be secreted by the endothelium (particularly the endothelial cells lining the valves) into the blood under abnormal condition, or it can be attributed to leucocytes (which can even happen to remain trapped in the valves). Hypoxia⁸⁴ is also an element to be considered as a possible stimulus for TF release. In addition we know that platelets (that can suffer hypoxia and can also be trapped in the valves) are able to produce TF. To these considerations we may add that RBCs in stagnating blood could release ADP, activating platelets.

DVT can also be induced by other pathological conditions. For instance it was noted long ago [248] that DVT occurrence was more frequent in patients with tumors. Today such a phenomenon is attributed to an overexpression of TF [207].⁸⁵

⁷⁸Since immobilization is a frequent cause, DVT is also called the economy class syndrome, because many cases have been reported in passengers after long flights.

⁷⁹As in the *Paget-Schrötter disease*. We also recall the tumor related Trusseau syndrome (see the historical section).

⁸⁰Not with fibrinolytic proteins (like tPA or urokinase), because they could fragment rather than gradually dissolve the clot. Fibrinolytic therapies are instead used to attack arterial thrombosis in the heart or the brain.

⁸¹Major veins are provided with valves preventing flux inversion, thus helping circulation in the presence of reduced pressure gradients (see more on valves in Chap. 1).

⁸²Some hemophilia therapies actually bypass the intervention of FVIII and FIX. We will return to this question in Sect. 2.7.

⁸³Nevertheless, in the paper [50] experiments were performed emphasizing the importance of the Hageman Factor FXII in resting blood.

⁸⁴Reduced oxygen concentration is more marked in valves, since, differently from veins and other blood vessel, they do not possess their own vessels (*vasa vasorum*).

⁸⁵This paper is an extensive study on the role of TF and of thrombin in promoting angiogenesis and contains a large bibliography. Excessive TF production may be accompanied by upregulated

(VII) Heart Arrhythmia and Thromboembolism

In the family of disorders related with altered blood flow we must include the possible formation of clots caused by atrial fibrillation.⁸⁶ Since clots can then reach the brain (causing a stroke) or the kidneys, patients suffering from atrial fibrillation are frequently given an anticoagulant therapy. See more on heart arrhythmias in Chap. 7.

(VIII) Coagulation on Artificial Surfaces

This is a very important subject, because the implant of artificial bodies (heart valves, stents, joints, etc.) is likely to be followed by blood coagulation.⁸⁷ Clotting can be caused by high shear stress in blood (also possibly causing hemolysis). Some materials have affinity to fibrinogen, which can produce platelet aggregation on the body surface (similarly to what vWF does in primary hemostasis), and/or trigger immune reaction, by aggregating leukocytes (see http://courses.washington.edu/overney/NME498_Material/NME498_Lectures/Reading_on_Adsorption_Kinetics.pdf). Cell-born TF does the rest. Hence the particular attention in designing anticoagulant coatings. Traditionally this kind of coagulation used to be attributed to *self-activation* of FXII on artificial surfaces (a fact established by the discoverer of FXII, O.D. Ratnoff [201]), initiating the so-called intrinsic pathway (see next section). This can be a concomitant process, but, as we shall see, the intervention of TF is anyway required.

(IX) Thromboangiitis Obliterans, or B rger Disease

It is an inflammation of small and medium blood vessels in the limbs, accompanied by clotting and occlusion. The cause is unknown but a strong correlation with smoking is sure. There is no real cure, but it is treated with vasodilators (*prostaglandins*).

(X) Disseminated Intravascular Coagulation (DIC)

This disorder consists in a deep dysregulation of the entire coagulation-fibrinolysis system, which may be caused by other pathologies (sepsi, tumors, etc.). It occurs when abnormal, critical conditions stimulate TF secretion in blood e.g. by leukocytes, resulting in the production of circulating thrombin. As a consequence microthrombi are diffusely generated, sequestering platelets from blood. Therefore, paradoxically, hypocoagulability sets in, with resultant bleeding in various parts of the body. Blood perfusion of vital organs is altered which may induce failure and death.

expression of VEGF (the angiogenic factor) and downregulated expression of thrombospondin 1 (see Sect.2.3).

⁸⁶Clots are mostly originated in the left atrium and more precisely in an area called left *atrial appendage*.

⁸⁷For this reason patients receiving artificial mechanical heart valves are permanently treated with anticoagulants. Valves taken from pigs do not have this inconvenience, but have a limited duration (~15 years).

(XI) Ischemic Episodes Related to Sickle-Cell Anemia (SCA)

SCA (or sickle-cell disease) is a genetic disorder due to a mutation. It affects hemoglobin molecules causing a strong tendency to form fibrous aggregate after deoxygenation.⁸⁸ As a consequence the hosting cell deforms acquiring the shape of a sickle and losing membrane elasticity. The cells are not able to recover the normal shape and they eventually obstruct capillaries producing ischemic disorders and other serious and painful syndromes. Sick cells die at a high rate (lifetime 10–20 days) and anemia sets in.

We do not continue this long list, but it is clear that there are disorders caused by an excess of coagulation factors, with a tendency to thrombophilia, and there are acquired bleeding disorders due to other diseases or to pharmaceutical treatments, traumas, etc.

2.6 The 3-Pathway Cascade Model

The first steps towards the interpretation of blood coagulation as the result of a chemical cascade were made by the German physician **Paul Oskar Morawitz** (St. Petersburg 1879–Leipzig 1936). A short cascade, though, since the “factors” preceding fibrin known at his time were only four: fibrinogen/fibrin, prothrombin/thrombin, thrombokinase (TF), and calcium ion, i.e. the ones described by Alexander Schmidt in his already quoted papers (1861, 1862). His seminal paper [169], in which he reported his experiments on the isolation of thrombokinase (TF), opened the way to modern research, wiping out the riddle of assumptions circulating about blood coagulation at the beginning of twentieth century. He was only 26 years old. Then for long time the actual mechanism interposed between the action of thrombokinase and the activation of prothrombin has remained ignored. As we have seen, the discovery of FV was unveiled only in 1947, starting a restless chase for new factors. The picture which matured over the years lead to the *Cascade* or *Waterfall* model, proposed in 1964 independently by Earl W. Davie (b. 1927), Oscar D. Ratnoff (1916–2008) [64] and by Robert Gwyn Macfarlane [147]. Such a model has been the undisputed biological basis of clotting until very recently and it represented a decisive leap forward in understanding the actual mechanisms of blood coagulation, though the necessity of some correction emerged at the beginning of the present century. Once more we quote the remarkable historical note [63]. We believe that it is important to discuss the cascade model not only for historical reasons, but also because, for instance, it elucidates the role of FXII, which is ignored in the cell based model, though it can contribute to the process.

⁸⁸It was the American chemist **Linus Carl Pauling** (1901–1994), Nobel laureate (1954), to describe the causes of the disease in 1949 [187]. This was an early success of molecular biology, of which Pauling was one of the founders.

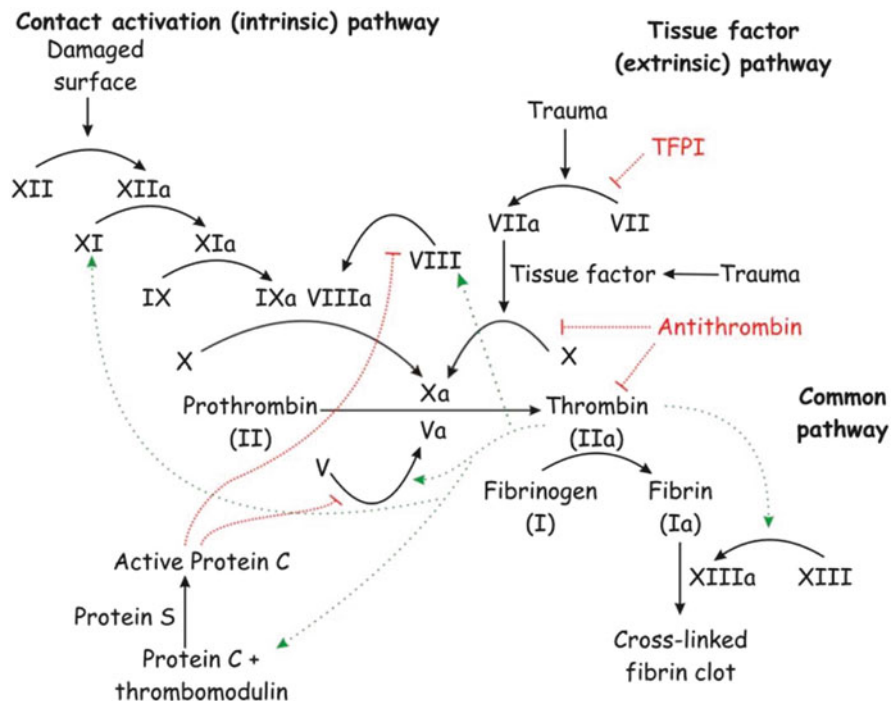


Fig. 2.19 Modern scheme of the 3-pathway cascade model (note that thrombin is “exported” to intrinsic from extrinsic pathway, so to activate FVIII) [from: Fig. 7.9. [35]]

The cascade model is characterized by the presence of three pathways: *intrinsic*, *extrinsic*, and *common* pathway, whose modern approach, compatible with the cell based model, is synthetically illustrated in Fig. 2.19. An alternative name for the intrinsic pathway is *contact activation* pathway. Extrinsic pathway is also called *TF-pathway*.

The model offers two ways of clotting initiation. The extrinsic origin starts with the formation of the FVIIa-TF complex, exactly like in the cell-based model. The intrinsic origin requires the activation of the Hageman factor FXII and, according to the setting of Fig. 2.19, it needs the intervention of thrombin, which can only be provided via the alternative path. Thus we stress that Fig. 2.19 illustrates a real biological process and it is a correction of the old scheme, which we are going to discuss in order to explain its troubles.

The original scheme was conceived so to provide two independent ways leading to the production of FVa-FXa complex. A simple illustration of its deficiency can be found e.g. in [208]. We are going to discuss this question here. Let us first deal with the extrinsic pathway, which was designed so to lead directly from the formation of complex FVIIa-TF to the activation of FX. Then, entering the common pathway, the subsequent production of prothrombinase was taking place via the activation of FV and its complexing with FXa and Ca^{++} . Thus the extrinsic pathway was sufficient to eventually produce thrombin and fibrin.

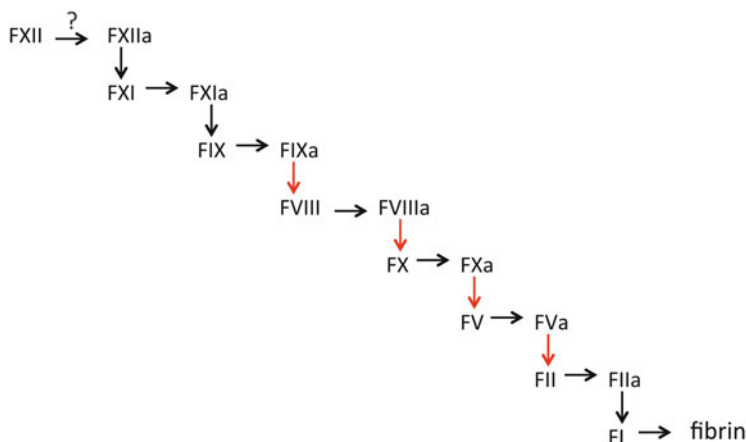


Fig. 2.20 Scheme of the *waterfall* model. This is a translation to modern notation from the original one (after [63]), which included the question mark about FXII activation. *Red arrows* emphasize discordances with the cell-based model

The intrinsic pathway is triggered by the activation of FXII. Mild activators of FXII are negatively charged surfaces, like collagen or the membrane of activated platelets [20] (but also e.g. of foreign bacteria). This phenomenon is mediated by HMWK and induces the Prekallikrein to Kallikrein conversion. Kallikrein is now a fast activator of FXII, so that a positive feedback sets in. Then, FXIIa activates FXI [202], and FXIa activates FIX. At this point let us look at the former scheme by Davie and Ratnoff [64].

From the appearance of FIXa onward the sequence in the cascade in Fig. 2.20 is rather different from the one we know (in which FVIII is activated by thrombin, FXa is a product of the tenase complex, and thrombin is generated by prothrombinase) and in this form it is autonomous from the extrinsic pathway.

The sketch of Fig. 2.19 has instead the correct chemical correlations, letting products from extrinsic pathway (thrombin, FXa) enter the intrinsic pathway, which in this way becomes so to speak subsidiary. The purely intrinsic pathway from the original scheme was instead based on the assumption that FVIII is directly activated by FIXa, which would make it really independent of the parallel extrinsic pathway.

Two main facts clash with the former 3-pathway cascade model:

- The TF-pathway in the original scheme *bypasses FVIII*, but is nevertheless sufficient to produce clotting,
- FXII deficiency, as we have seen in the previous section, is experimentally known to produce no significant bleeding disorder.⁸⁹

⁸⁹Veterinarians know that some mammals, like whales and dolphins, do not even possess FXII.

So the troubles about the 3-pathway cascade model come from the position occupied by FVIII, whose deficiency we know to be associated with Hemophilia A. If the TF-pathway alone would be enough for clotting, FVIII deficiency would be inessential. Moreover, FXII deficiency would exclude FVIII activation from the process, causing the same effects as FVIII deficiency. None of this facts is true, hence the necessity of a model, like the cell-based model, which on one side can make without FXII and on the other recognizes the crucial role of FVIII. Once more we stress the fact that in the cell based model *the only activator of FVIII is thrombin*.

Nevertheless, it would be wrong to dismiss the role of FXII altogether. Its ability of becoming activated on artificial surfaces can enhance clotting on implanted bodies, which is a very serious problem for instance in the design of artificial hearts or artificial valves. A paper recently devoted to FXII and its possible alternative functions is [218].

Laboratory tests to check the efficacy of the extrinsic and of the intrinsic pathway, respectively, are the PT-test (Prothrombin Time) and the PTT (or APTT)-test (Partial Thromboplastin Time, or Activated PTT).

2.7 Anticoagulant Drugs, Thrombolytic Drugs, Hemophilia Therapies

This is a very large, extremely important, and continuously evolving subject, that can effectively be incorporated in a mathematical model for blood coagulation.⁹⁰ Some of such drugs have been already mentioned in Sect. 2.3. In order to introduce them in a mathematical model it is important to know how they work.

2.7.1 Anticoagulant Drugs

A large number of anticoagulant drugs have been produced. They can be classified according to their target:

- I Drugs targeting platelets.
- II Vitamin K antagonists.
- III Adjuvant drugs to inhibitors of activated factors.
- IV Direct inhibitors of activated factors.

⁹⁰For the sake of brevity we omit to list hemostatic drugs. Some of them act on platelets stimulating their adhesion (*microfibrillar collagen hemostat*, *chitosan*), some force strong vasoconstriction (anhydrous aluminum phosphate). *Batroxobin* (or *reptilase*) acts in a way similar to thrombin. It is contained in the venom of a group of Southern American vipers. Venoms of many snakes exert a strong coagulant action in various ways (including direct activation of Factor X).

[I] Drugs targeting platelets

Antiplatelets drugs can act in various ways. This is actually a large class. We list just a few of them.

- *Acetylsalicylic Acid (ASA)*,⁹¹ with the famous trademark Aspirin, inhibits the production of Thromboxane A₂.
- *Thromboxane A₂ receptors inhibitors* and *Thromboxane A-synthase inhibitors*. Drugs in this class (*ifetroban*, *picotamide*, *terutroban*, etc.) have the effect of either neutralizing TXA₂ or preventing its production.
- *ADP receptors inhibitors*. The most known drug in this class is Clopidogrel (trademark Plavix), but there are many others.
- *Glycoprotein IIB/IIIA (or $\alpha_{IIb}\beta_3$) inhibitors* (*abciximab*, *eptifibatide*, *tirofiban*, with various trade names) prevent binding of platelets with fibrinogen.

The action of Aspirin is indirect. It irreversibly inactivates enzymes of the COX family (cyclooxygenase) having a primary role in the production of *prostaglandin H₂*, further transformed into TXA₂ by the enzyme *Thromboxane A-synthase*.

Clopidogrel and ASA are often used in combination (*Dual Antiplatelet Treatment*). The long term effect of daily low dose of aspirin in the prevention of thrombotic events, which saved many lives, was proved long ago by **Carlo Patrono** and his coworkers [186].

[II] Vitamin K antagonists

As we said, vitamin K has an important role in the activation of the so-called vitamin K dependent factors (see Sect. 2.3.3). Drugs in this group do not prevent the production of the vitamin, but rather inhibit its transition to the active form (performed by the enzyme vitamin K epoxide reductase), thus producing the same effect as vitamin K deficiency.

- *Coumarins* is the name often used for a family of drugs which include Warfarin (also known with other brand names, among which *Coumadin* is widely used). *Acenocoumarol* is another drug in the same family. This class of drugs derives its name from a natural substance (*Coumarin*), found in high concentrations in the tonka bean and many other plants, characterized by sweet smell. It is a precursor of 4-hydroxycoumarin, a fungal metabolite, further transformed into *dicoumarol*, which is the actual anticoagulant. Such a natural process was discovered in the fermentation

⁹¹The history of Aspirin is controversial. The synthesis of pure ASA (1897) has been credited to the young Bayer chemist **Felix Hoffmann** (impure ASA had been previously produced by the French chemist **Charles Frédéric Gerhardt** in 1857). Many years later **Arthur Eichengrün**, another German chemist employed at Bayer at the time of Aspirin discovery, claimed that Hoffmann had just followed his plans. Though recent studies have confirmed that Eichengrün's thesis was credible, Bayer officially dismissed his claims. The analgesic and anti-inflammatory properties of the willow (latin *salix*) leaf have been known since antiquity. It has been documented in a Sumer tablet and in the Ebers Papyrus. Hippocrates mentioned it and its effects were well known in western and also Arab civilizations well before ASA chemical synthesis.

Fig. 2.21 Sweet clover

of sweet clover, responsible for the *sweet clover disease*, a hemorrhagic illness affecting cattle fed with that kind of fermented feed. Such a disease was studied intensively in the 1920s, particularly in Wisconsin but only in 1940 the anticoagulant agent, later named dicoumarol, was isolated, under the guidance of the chemist **Karl Paul Link**, 1901–1978 of Wisconsin University (Fig. 2.21).⁹²

The next year dicoumarin was patented and the research started for a more effective drug, which was eventually patented in 1948, as a rodent poison, with the name of Warfarin, based on the acronym WARF (Wisconsin Alumni Research Foundation) with *-arin* reminding the origin from coumarin. The step to the application to humans as an anticoagulant drug took some years and was approved in 1954. One of the first recipients was the U.S. President Dwight Eisenhower, who was treated with it after suffering a heart attack in 1955. This was not the only episode in which Warfarin surfaced in history. Indeed it has been suggested by some historians (in 2003) that Stalin may have been poisoned with Warfarin in a plot orchestrated by a group including Lavrenty Beria and Nikita Khrushchev.⁹³ Read more about the history of anticoagulants in [269].

Warfarin acts more rapidly on proteins C, S thus inducing first a pro-coagulant effect. For this reason heparin must be administered during the first few days of the treatment [122]. Drugs in this class have a long half-life and their use require frequent monitoring, due to the variability of the response.

⁹²Farming, and especially milk production, was the backbone of Wisconsin economy (“the Dairy State”). The disease, described in 1924 by the veterinarian **Frank Shofield**, was brought to Link’s attention by a Wisconsin farmer in 1933.

⁹³This theory may have some ground, since Stalin was going to launch another purge and Beria would have been one of the first victims. The symptoms produced by warfarin poisoning would have been compatible with the brain hemorrhage that actually killed Stalin and with his clinical picture. Others claim that Stalin’s murder was ordered by Tito.

- *1–3 Indandiones: Phenindione, Diphenadione, etc.* The use of some of them is limited to rat killing because of the occurrence of undesirable collateral effects.

[III] Adjuvant drugs to inhibitors of activated factors

- *Heparin.* Heparin (a polysaccharide, molecular weight 5–40 kDa or more, average ~15 kDa) exerts its action by binding to ATIII in a way that increases its rate of inactivation of thrombin and factor Xa by up to three orders of magnitude. There has been some controversy about heparin discovery (see [269] and [152]). It is a polymer, also naturally produced by endothelial cells (as *heparan sulfate*). A side effect can be a strong reduction of platelets count (*Heparin Induced Thrombocytopenia, HIT*), see [124]. HIT can be sometimes observed in patients undergoing hemodialysis, during which heparin is supplied to prevent clotting (after passing through the dialyzer and before being returned to the patient, *protamine sulfate* is sometimes added, which neutralizes heparins action). Platelet Factor 4 contrasts the action of heparin on platelets. Heparin is naturally produced also by basophils and by mast cells.
- *Low Molecular Weight Heparins (LMWHs)* are obtained by partial depolymerization of heparin (there are eight types with average molecular weight ranging from 3.6 to 6.5 kDa). Compared to unfractionated heparin, they have less effect on thrombin but similar efficacy on Xa, the risk of heparin induced thrombocytopenia is reduced, and, contrary to heparin, their action is only slightly neutralized by protamine sulfate. There are several LMWHs (*ardeparin, certoparin, enoxaparin, etc.*), each with its respective trade name (*Normiflo, Sandoparin, Clexane, etc.*), obtained with different depolymerization techniques. Fondaparinux (Arixtra) is another drug with an action similar to heparin, but less side effects. ATIII stimulated by Fondaparinux inhibits FXa, but not thrombin.

[IV] Direct inhibitors of activated factors

This is the most recent class of anticoagulant drugs, generally considered of simpler use, because they are taken orally, their action is more predictable and they are less interactive with other drugs. They are known with the acronym NOACS (Novel Oral Anticoagulants). See the review papers [99, 246].

- **Direct Thrombin Inhibitors (DTIs).** This class includes
 - The so-called *Hirudins* (*hirudin, lepidurin, etc.*), derived from the natural secretion of *Hirudo medicinalis* (the leech we have dealt with when discussing bloodletting), also known as *Bivalent DTIs*. Another natural product with anticoagulant properties is *Hementin*, secreted by the giant Amazon Leech (*Haementeria ghilianii*), which strictly speaking is not in this class, since it acts on fibrinogen, preventing its transition to fibrin.
 - *The Univalent DTIs: Dabigatran* (the most used in this class), *Argatroban* (used to treat heparin induced thrombocytopenia), etc. The

performance of Dabigatran vs. traditional treatments has been tested in a series of clinical trials (see e.g. [222]).

- **Direct Xa inhibitors**

Very recently these drugs (whose names terminate in—xaban: *Apixaban*, *Rivaroxaban*, *Edoxaban*, *Darexaban*, etc.) have become available, derived from products isolated from the secretion of the Mexican leech (*Haementeria officinalis*) and of some species of ticks (*Ornithodoros moubata*). Extensive trials have been performed to evaluate the advantages of Rivaroxaban (see e.g. [3, 137, 241]) and Apixaban [92, 95, 138]. More recent papers on the subject are [246, 247].

2.7.2 Thrombolytic Drugs

In Sect. 2.3 we have mentioned three strong fibrinolytic agents: tPA, streptokinase, and urokinase. Thrombolytic drugs with various trade names used in the clinical practice are based on those principles: Alteplase, Retaplase, Tenecteplase are in the tPA family, etc. Urokinase (preferably used for pulmonary embolism) has a tendency of producing *fibrinogenolysis* with elevated risk of bleeding. All fibrinolytic drugs have to be administered with great caution. They are used to resolve thrombotic events in the heart, brain and lungs. A detailed review on thrombolytic drugs and on the clinical procedures applied for thrombi removal is [129]. Recent studies show that tPA action can be enhanced when the factor is loaded into *echogenic liposomes* (sub-micron sized phospholipid bilayer vesicles) and released upon ultrasounds applications. This technique allows to reduce the tPA dose, lowering the risk of hemorrhages. We refer to the paper [228], illustrating the underlying physics and the related literature. We will mention stroke treatments with High Intensity Focused Ultrasound (HIFU) in the HIFU dedicated section of Chap. 6.

2.7.3 Hemophilia Therapies

Hemophilia A and B are normally treated supplying the corresponding deficient factors (FVIII, FIX), which can be derived from the blood of healthy individuals or artificially synthesized (*recombinant factors*). However, some patients (mainly with hemophilia A) develop antibodies (inhibitors) attacking the injected factors. Such an immune reaction can arise spontaneously in adult age and is called *acquired hemophilia* (see [89]). While a frequent symptom of congenital hemophilia is *hemarthrosis* (bleeding into the joints), the main manifestation of acquired hemophilia is a form of extensive *purpura* (bleeding into the skin). Acquired hemophilia can be treated either by administering a mix of prothrombin, FX and FVIIa (a drug called *FEIBA*), or a suppressor of the auto-antibody production (*rituximab*), or with high doses of recombinant FVIIa (rFVIIa). The success of the

latter therapy raises interesting questions about the actual role of FVIIa and TF in the whole coagulation process, stimulating updating in mathematical models. How can coagulation bypass FVIII or FIX deficiency? We know that thrombin can be produced at the initiation stage without the intervention of those factors, but in very small quantities. The way FVIIa (in pharmacological doses), phospholipidic surfaces, TF, and platelets interact to produce sufficient quantities of thrombin is discussed in various papers (including the already quoted [146]). Particularly important is the paper [232], presenting a series of interesting experiments and numerical simulations, and the related comments of [168]. We quote also the earlier papers [44, 45] in which the combined role of the various mentioned elements is highlighted on an experimental basis and with the definition of a *kinetic efficiency* of thrombin generation. Recombinant FVIIa treatment is compared with FEIBA treatment in the short note [102]. Hemophilia therapies based on TF and rFVIIa have been reviewed in [103].

Recombinant FVIIa has found application also as a treatment of congenital FVII deficiency, and Glanzmann's thromboasthenia.

2.8 Mathematical Models for Blood Coagulation

Mathematical models for blood clotting have evolved in parallel to the biological interpretation of the process, starting from the early times of the 3-pathway cascade model and eventually targeting the new structure of the cell based model. Owing to the enormous complexity of the problem many authors have adopted more or less severe simplifications, trying to emphasize specific properties. Models of increasing complexity have been proposed over the years, incorporating more and more details, though, in our opinion, not only *“the”* blood coagulation model has never been formulated, but the idea of assembling a huge number of equations, containing an exorbitant number of parameters, many of which are hardly measurable, looks discouraging. Indeed, such a “complete” model would actually be of little use. Approaching phenomena involving, mechanics, chemistry and biology, coupled through complicated mechanisms, requires much caution and the awareness that the comprehensive mathematical description of the process, from primary hemostasis to fibrinolysis is excessively ambitious. The more so if one wants to go deep into the analysis of proteins reactions at the molecular scale, a field that we have avoided in the biological introduction of coagulation (proteins folding-unfolding, sites of the molecules intervening in specific reactions) (see [250, 251]). The latter observation points out that any mathematical model should be proportionate to the scope it wants to achieve, and that the selection of the space scale is one of the most critical choices. The critical review paper [104] warns about the superficial use of models, even if they manage to fit experimental data.

In this section we will review some mathematical models which are representative of classes of models, with the caveat that the relevant literature is much larger.

We quote the review papers [51, 258, 259] in addition to the already mentioned [35, 71]. It is quite hard to group the many models proposed in homogeneous categories and we must say that the classes we are going to define may overlap in many ways, so that a model may belong to more than one. We will distinguish two broad classes: models based on ODE's or on PDE's systems. The ones in the first group focus on the local evolution of the biochemistry and (possibly) of platelets and have mainly the scope of emphasizing some qualitative property (like the existence of a threshold for clotting initiation). The second class includes spatial inhomogeneity, blood flow, clot mechanics, etc.

Let us add a few general remarks about the notation and the reactions kinetics that will be generally used in this section.

The concentration of a substance C will be denoted by $[C]$. Reaction kinetics found in the literature are usually of one of the following three types:

$$k[C], \quad k[C_i][C_j], \quad k \frac{[C_i][C_j]}{k' + [C_j]}$$

i.e. first order, second order, and Michaelis-Menten kinetics. k is the rate constant (dimensionally sec^{-1}) and, in the last case, k' is the Michaelis-Menten constant (dimensionally a concentration).

2.8.1 ODE's Models

The pioneer paper [142] appeared in 1966, soon after the formulation of the cascade model, providing a mathematical scheme for a reaction cascade of the type zymogen-enzyme consisting in the following ODEs system:

$$\begin{aligned} \frac{dy_{1a}}{dt} &= k_1 [U(t) - U(t-a)] - H_1 y_{1a} \\ \frac{dy_{2a}}{dt} &= k_2 y_{2a} y_{1a} - H_2 y_{2a} \\ &\vdots \\ \frac{dy_{Na}}{dt} &= k_N y_N y_{(N-1)a} - H_N y_{Na}, \end{aligned} \tag{2.1}$$

where y_i , y_{ia} , $i = 1, \dots, N$, are the concentrations of the i -th zymogen-enzyme pair, H_i are decay rates, and $U(t)$ is a unit step function, so that the difference $U(t) - U(t-a)$ is a unit pulse of duration a , the activation time. Of course the quantities y_i obey a similar differential system describing their consumption. Specifying the initial data, an explicit solution is found and its behavior as a function of the activation time is studied.

ODE's models typically focus on the chemistry. Further generalization were [155], including thrombin absorption by fibrin and fibrinolysis, [223], and [171], where the activation time was taken as a random variable.

A substantial step forward came with the paper [126] in 1989, which modeled the TF-pathway taking into account nonlinear dependence of the reaction terms on the concentrations. The system reads as follows

$$\begin{cases} \frac{d[VIIa]}{dt} = \alpha K_1 - H_1[VIIa] \\ \frac{d[Xa]}{dt} = K_2[VIIa] - H_2[Xa] \\ \frac{d[Va]}{dt} = K_3[IIa] - H_3[Va] \\ \frac{d[IIIa]}{dt} = \frac{K_4[Xa][Va]}{K_a + [Va]} - H_4[IIIa], \end{cases} \quad (2.2)$$

where α is the stimulation coefficient (in short, the concentration of the FVII activator). The system is largely simplified not only because it considers a small number of factors, but also because it takes a constant production rate of FVIIa (we have seen that the real process is much more complicated). Moreover it only considers the activated factors, (tacitly) meaning that the inactivated ones are considered so abundant that their concentrations stay practically constant. Nevertheless, the qualitative analysis of system (2.2) performed in [126] helps to identify a particular value α_0 for α which can be considered an activation threshold. The starting point is the observation that at the initiation phase $[FVa]$ is small enough to apply the Tikhonov reduction theorem [240], then a linear stability analysis leads to the following conclusions: if $\alpha > \alpha_0$ the equilibrium solution is stable. Hence the threshold character of α_0 whose value is

$$\alpha_0 = K_a \frac{H_1 H_2 H_3 H_4}{K_1 K_2 K_3 K_4}.$$

This formula agrees with the intuition that increasing any of the reaction rates favors coagulation (by decreasing α_0) and the opposite happens if any of the decay rates is increased (since α_0 increases). It is convenient at this point to jump to the much later paper [254] (2002) which generalizes the work just described in the

same spirit. The differential system is now more complicated

$$\begin{cases}
 \frac{d[VIIa - TF]}{dt} = [VIIa - TF]_0 - h'_1[VIIa - TF][TFPI][X_a]k_{14} \\
 \frac{d[X_a]}{dt} = k_2[VIIa - TF] + k_{21}[IXa] \left(\frac{VIII_a}{d_2 + VIII_a} \right) \left(c_0 + \frac{f([IIa])}{1 + f([IIa])} \right) \\
 \quad - h'_{21}[TFPI][X_a] - h'_{22}[ATIII][X_a] \\
 \frac{d[IXa]}{dt} = k_3[VIIa - TF] - h'_3[ATIII][IXa] \\
 \frac{d[IIa]}{dt} = k_4[Xa] \left(\frac{[Va]}{d_1 + [Va]} \right) \left(c_0 + \frac{f([IIa])}{1 + f([IIa])} \right) - h'_4[ATIII][IIa] \\
 \frac{d[Va]}{dt} = k_5[IIa][V] - h_5[Va] \\
 \frac{d[VIIIa]}{dt} = k'_6[IIa][VIII] - h_6[VIIIa]
 \end{cases} \quad (2.3)$$

and involves also the dynamics of the complex FVIIa-TF, and of factors FIXa, FVIIIa. Once more the concentrations of inactivated factors are not among the unknowns, with the important exception of FVIII. The evolution of complex FVIIa-TF is governed by some constant input and moderated by the inhibiting action of TFPI (complexed with FXa: see Sect. 2.3). FIXa and FXa are produced (actually at small rates) by complex FVIIa-TF (from the respective zymogens) and inhibited by ATIII. Moreover, FXa is inhibited by TFPI. The second term in the equation for FXa expresses the activation by the tenase complex, here taken directly proportional to the production rate of the complex itself. The factor

$$c = c_0 + \frac{f([IIa])}{1 + f([IIa])}, \quad (2.4)$$

where f is an increasing twice differentiable function, represents the concentration of activated platelets, with a saturable dependence on thrombin concentration. A similar structure can be seen in the evolution equation of thrombin: inactivation rate by ATIII and production rate regulated by prothrombinase (taken proportional to the production rate of the same complex) and by c . The system is completed by the dynamics of FVa, FVIIIa. Following the idea of [126] and with similar techniques a linear stability analysis is performed, pointing out the existence of a threshold value for the intensity of a suitably defined “coagulation stimulus”. Since [FVIII] enters the dynamics, it was possible to highlight the consequences of FVIII deficiency, simulating the corresponding bleeding disorder.

The papers [126] and [254] are two among many others addressing the question of identifying a threshold phenomenon in the triggering of a cascade of enzymatic reactions characterized by positive feedback and balanced by inhibitors. We quote [27] and [120]. The latter paper opens a new perspective, since it includes the effect

of diffusion (though not yet the interaction with the flow), involving the Damköhler number (the ratio between the diffusion time scale and the reaction time scale). Thus it brings us to the domain of PDE's model.

A model of similar complexity (with more nonlinearities), concerned with the TF-pathway, was [275], which produced some comparison with experimental data. Not surprisingly, models with an increasing number of differential equations have then been proposed for the same purpose. In [118] a system of 20 ODE's was presented, and the model of [141] included as many as 36 ODE's.

A model with 9 ODE's for the contact pathway was formulated and discussed in [196]. In the same framework we quote the paper [130] in which a model with 35 ODE's was used to predict the outcome of the APTT-test (see Sect. 2.6). Contact pathway is also the subject of the paper [227], modeling Hageman factor activation in the context of a particularly slow clotting process which has been ascertained to be triggered by FXIIa. In this model two equations stand out (and actually are the only ones reported from the intrinsic pathway cascade):

$$\begin{aligned}\frac{d[XIIa]}{dt} &= k_1 \frac{[XIIa][XII]}{K_1 + [XII]} + k_2 \frac{[K][XII]}{K_2 + [XII]} - h_1[XIIa][C1_{inh}] \\ \frac{d[K]}{dt} &= k_3 \frac{[XIIa][PK]}{K_3 + [PK]} + k_4 \frac{[PRCP][PK]}{K_4 + [PK]} - h_2[PK][C1_{inh}],\end{aligned}$$

where $[K]$, $[PK]$ are the concentrations of Kallikrein and Prekallikrein, $[C1_{inh}]$ is the concentration of inhibitor of complement C1 (Sect. 2.3.5), and $[PRCP]$ is the concentration of prolyl-carboxypeptidase, an enzyme here considered along with XIIa as a Prekallikrein activator.

Continuing the analysis of this very rich class of models, we mention the massive 34 ODE's model (with 42 rate constants) of [107] (known as the Hockin-Mann model) and the papers [41, 42, 46] among the many that have used it. In the recent paper [161] the same model has been utilized to study the effects of recombinant FVIIa in the therapy of hemophilia (see Sect. 2.7). The same authors have used the model of [107] to simulate treatments of patients who have got massive transfusion which led to dilution in blood of the coagulating factors [162, 163]. For the sake of completeness we report the list of reactions in the Hockin-Mann model from the seminal paper [107], since it has been used as a benchmark by many authors and also because it emphasizes some usually omitted details:

- (1) $TF+VII \leftrightarrow TF-VII$
- (2) $TF+VIIa \leftrightarrow TF-VIIa$
- (3) $TF-VIIa+VII \rightarrow TF-VIIa+VIIa$
- (4) $Xa+VII \rightarrow Xa+VIIa$
- (5) $IIa+VII \rightarrow IIa+VIIa$
- (6) $TF-VIIa+X \leftrightarrow TF-VIIa-X \rightarrow TF-VIIa-Xa$
- (7) $TF-VIIa+Xa \leftrightarrow TF-VIIa-Xa$

- (8) $\text{TF-VIIa+IX} \leftrightarrow \text{TF-VIIa-X} \rightarrow \text{TF-VIIa+IXa}$
- (9) $\text{Xa+II} \rightarrow \text{Xa+IIa}$
- (10) $\text{IIa+VIII} \rightarrow \text{IIa+VIIIa}$
- (11) $\text{VIIIa+IXa} \leftrightarrow \text{IXa-VIIIa (tenase)}$
- (12) $\text{IXa-VIIIa+X} \leftrightarrow \text{IXa-VIIIa-X} \rightarrow \text{IXa-VIIIa+Xa}$
- (13) $\text{VIIIa} \leftrightarrow \text{VIIIa1-L} + \text{VIIIa2}^{94}$
- (14) $\text{IXa-VIIIa-X} \rightarrow \text{VIIIa1-L+VIIIa2+X+IXa}$
- (15) $\text{IXa-VIIIa} \rightarrow \text{VIIIa1-L+VIIIa2+IXa}$
- (16) $\text{IIa+V} \rightarrow \text{IIa+Va}$
- (17) $\text{Xa+Va} \leftrightarrow \text{Xa-Va (prothrombinase)}$
- (18) $\text{Xa-Va+II} \leftrightarrow \text{Xa-Va-II} \rightarrow \text{Xa-Va+mIIa}^{95}$
- (19) $\text{mIIa+Xa-Va} \rightarrow \text{IIa+Xa-Va}$
- (20) $\text{Xa+TFPI} \leftrightarrow \text{Xa-TFPI}$
- (21) $\text{TF-VIIa-Xa+TFPI} \leftrightarrow \text{TF-VIIa-Xa-TFPI}$
- (22) $\text{TF-VIIa+Xa-TFPI} \rightarrow \text{TF-VIIa-Xa-TFPI}$
- (23) $\text{Xa+ATIII} \rightarrow \text{Xa-ATIII}$
- (24) $\text{mIIa+ATIII} \rightarrow \text{mIIa-ATIII}$
- (25) $\text{IXa+ATIII} \rightarrow \text{IXa-ATIII}$
- (26) $\text{IIa+ATIII} \rightarrow \text{IIa-ATIII}$
- (27) $\text{TF-VIIa+ATIII} \rightarrow \text{TF-VIIa-ATIII}$

The system emphasizes that inactivations occurs by a complexing mechanisms.

Finally, we quote the paper [54], just appeared, which presents a mathematical model for the mechanical activation and subsequent aggregation of platelets in conditions of high shear stress, like the one produced by vessel stenosis. The paper is interesting because it is based on sophisticated experiments in which blood is exposed to shear stress in the platelet activating range ($120\text{--}2400\text{ s}^{-1}$, source [65]). An inhibitor of the most common blood born platelet activators (ADP, TXA_2 , thrombin) is added so that the shear effect can stand out. Introducing the quantities $P(t)$ (global platelet density), $c(t)$ (the enhancing aggregation response), $u(t)$ (shear rate), and $y(t)$ (thrombus size measured by its area), the model is expressed as follows

$$\tau \frac{dy}{dt} + y = c(t)u(t), \quad c(t) = \frac{c_0}{1 + e^{-c_1 P(t)}}.$$

⁹⁴Equation (13) (as well as (14) and (15)) shows the phenomenon of dissociation of FVIIa in subunits. This factor has a very complicated structure. For an illustration see e.g. [73].

⁹⁵An intermediate step in prothrombin activation.

2.8.2 PDE's Models

Here there is a considerable variety of approaches. A very detailed description, covering a large area of modeling procedures can be found in [35], which follows a classification according to scales, which is the same we are going to adopt here.

(a) Nanoscale models (sub-cellular)

The main class is based on the study of *Molecular Dynamics* (MD), i.e. following the motion of interacting molecules. Papers [250], [251] have been already quoted in this context. There are variants like the *Steered MD*, in which an external stimulus is used to analyze the material response. This has been applied to the clot mechanics in [143].

(b) Microscale models (cellular)

Typically cells motion and actions are studied at this level with various techniques generally employed to model multiphase microfluid mechanics. Methods in this class are variants of the *Euler-Lagrange Particle Tracking* (ELPT) method, in which the fluid motion is studied with an Eulerian approach and the Lagrangian tracking is adopted for the particles. In the basic form the particles trajectories are determined through the fluid velocity field, but the action of fluid on solid particles can be expressed via a drag coefficient (depending in a complicated way on the Reynolds number) and buoyancy (see e.g. [170, 261]). However, the influence of the solid on the liquid component is neglected. In the *Immersed Boundary* (IB) method an interaction force between the two components is introduced. A lattice Boltzmann implementation of the IB method was used in [278, 286]. A further variant is the *Immersed Finite Element* (IFE) method. A rather different class of methods (*Discrete Particles*) is the one in which the whole flow is described as an interaction between discrete particles (real or fictitious clusters of liquid molecules). Actually, it comprises a variety of different methods:

Dissipative Particle Dynamics (DPD), sets of mesoscopic particles with mutual binary interactions;

Fluid Particle Model (FPM), interaction forces have finite range;

Moving Particles Semi-Implicit Method (MPS), a body force term is introduced in the Navier-Stokes equation expressing the influence of cells; the differential operators acting on quantities associated to particles are suitably discretized.

We do not go into more details not to make our exposition too technical. The interested reader is referred to [35]. Clearly, in this context the coupling of blood flow with the cellular activity and with the whole chemistry is an essential feature. These techniques are particularly useful in the study of particles transport in blood and deposition on blood vessel walls, like in the case of atherosclerosis (see e.g. [128]), the motion of leukocytes [182, 183] and of erythrocytes [21, 144, 159, 260]. Applications specifically oriented towards the formation of thrombi are found in many papers (e.g. [25, 38, 67, 170, 244, 261, 265]).

Among the authors who have studied platelets activation and aggregation by means of particles tracking methods one of the most prolific is A.L. Fogelson [58, 76–79, 81, 134, 225]. In the same framework we quote also [243]. The paper [134] is particularly important as one of the most serious attempts to model the coupling of blood flow, platelet activation and tissue response. It has been considerably extended in [140], where platelets have been divided in four classes: mobile unactivated ($P^{m,u}$), mobile activated ($P^{m,a}$), platelet-bound activated ($P^{b,a}$), and subendothelium-bound activated ($P^{se,a}$). It is interesting to report from [140] the system describing the evolution of the respective families:

$$\begin{aligned}
 \frac{\partial P^{m,u}}{\partial t} &= - \underbrace{\nabla \cdot \{W(\phi^T) (\mathbf{u}P^{m,u} - D\nabla P^{m,u})\}}_{\text{Transport by advection and "diffusion"}} \\
 &\quad - \underbrace{k_{\text{adh}}(\mathbf{x}) \{P_{\text{max}} - P^{\text{se},a}\} P^{m,u}}_{\text{Adhesion to subendothelium}} - \underbrace{\{A_1(e_2) + A_2([ADP])\} P^{m,u}}_{\text{Activation by thrombin or ADP}}, \\
 \frac{\partial P^{m,a}}{\partial t} &= - \nabla \cdot \{W(\phi^T) (\mathbf{u}P^{m,a} - D\nabla P^{m,a})\} - k_{\text{adh}}(\mathbf{x}) \{P_{\text{max}} - P^{\text{se},a}\} P^{m,a} \\
 &\quad + \{A_1(e_2) + A_2([ADP])\} P^{m,a} - \underbrace{k_{\text{coh}}g(\eta)P_{\text{max}}P^{m,a}}_{\text{Cohesion to bound platelets}}, \\
 \frac{\partial P^{b,a}}{\partial t} &= - k_{\text{adh}}(\mathbf{x}) (P_{\text{max}} - P^{\text{se},a}) P^{b,a} + k_{\text{coh}}g(\eta)P_{\text{max}}P^{m,a}, \\
 \frac{\partial P^{\text{se},a}}{\partial t} &= - k_{\text{adh}}(\mathbf{x}) (P_{\text{max}} - P^{\text{se},a}) (P^{m,a} + P^{m,u} + P^{b,a}).
 \end{aligned}$$

As a comment to this system we just say that $e_2 = [IIa]$, $g(\eta)$ is a binding affinity function, expressing the influence of bound platelets in attracting $P^{m,a}$, where η is a quantity obeying an advection-diffusion equation, expressed by bound platelets, A_1 , A_2 are Michaelis-Menten type functions, and $W(\phi^T)$ is a function decreasing from 1 to 0 when ϕ^T (the ratio between the total to the maximal platelet concentration) goes from 0 to 1. The blood velocity field \mathbf{u} obeys a Navier Stokes equation (with constant viscosity), modified by the addition of a Brinkman-like correction,⁹⁶ expressing friction with bound platelets.

A particular place in the category of models focusing on particles is occupied by the *Cellular Potts Model*,⁹⁷ based on a cell-lattice structure with energy constraints. Papers in which this method has been applied for thrombus growth are e.g. [256, 257].

⁹⁶It consists in a term proportional to the product of viscosity and velocity, typical of the equation describing the flow of a Newtonian fluid through coarse porous media.

⁹⁷Named after the Australian mathematician **Renfrey Potts** [197].

(c) Mesoscale models (statistical methods)

This class of models is somehow intermediate between particle tracking and continuum models. Here the approach is more in the spirit of statistical mechanics, where particles are not treated individually but by means of probability densities. The *Lattice Boltzmann Method (LBM)* (see e.g. [198]), which is used as a discrete approximation of Boltzmann's equation, consists in describing the probability density evolution at the nodes of a discrete lattice, where only discrete velocities \mathbf{v}_i are allowed. If $f(\mathbf{x}, \mathbf{v}_i, t)$ is the distribution function defined on the lattice, the macroscopic density $\rho(\mathbf{x}, t)$ and velocity field $\mathbf{u}(\mathbf{x}, t)$ are obtained as

$$\rho(\mathbf{x}, t) = \sum_i f(\mathbf{x}, \mathbf{v}_i, t), \quad \rho(\mathbf{x}, t)\mathbf{u}(\mathbf{x}, t) = \sum_i f(\mathbf{x}, \mathbf{v}_i, t)\mathbf{v}_i.$$

The variation of the distribution function in a discrete time step δt is determined by its deviation from the equilibrium distribution:

$$f(\mathbf{x} + \delta t\mathbf{v}_i, \mathbf{v}_i, t + \delta t) - f(\mathbf{x}, \mathbf{v}_i, t) = \frac{1}{\tau} [f^{eq}(\rho, \mathbf{u}) - f(\mathbf{x}, \mathbf{v}_i, t)] \quad (2.5)$$

where τ is a relaxation time. Applications of LBM to blood coagulation are found in [30, 100, 176].

The class of stochastic models for coagulation includes also [139, 145].

A statistical approach to the problem of fibrin polymerization has been used in [165].

It is well known that statistical methods have been applied extensively to model aggregation processes (a particularly rich literature exists both in the field of polymerization⁹⁸ and in the dynamics of dispersions⁹⁹), for which a basis is provided by the celebrated *Smoluchowski equation* [234]:

$$\frac{\partial f(a, t)}{\partial t} = \frac{1}{2} \int_0^a K(a-b, b) f(a-b, t) f(b, t) db - f(a, t) \int_0^\infty K(a, b) f(b, t) db, \quad (2.6)$$

where a, b are particle volumes, f is the size distribution function, and K is the aggregation (or coalescence) kernel. The two integrals in (2.6) describe the rate of formation of particles of volume a by binary aggregation of smaller particles and their rate of disappearance due to collision with other particles, respectively.

⁹⁸See e.g. [84].

⁹⁹See e.g. [5, 66].

It is quite natural that this kind of approach has found application in blood coagulation models too. In [28] it has been used to model platelet aggregation under the action of a shear rate G , borrowing from liquid dispersions dynamics the kernel

$$K(a, b) = \frac{G}{\pi} [a^{1/3} + b^{1/3}]^3.$$

A model more complete than (2.6) includes a spontaneous fragmentation dynamics generating the terms

$$\int_0^\infty H(a, b)f(a + b)db - f(a, t) \int_0^a H(a - b, b)db \quad (2.7)$$

(to be added in Eq. (2.6)), where $H(a - b, b)$ is the fragmentation kernel expressing the probability of the decay of a particle of volume a into a pair $(a - b, b)$.¹⁰⁰ A model in this framework has been used in [112–114] to study platelet aggregation and disaggregation.

Models of the form (2.6)–(2.7) have their discrete analogs, which have been used extensively, particularly in polymer science, generating a large literature in that area, but also with reference to platelets dynamics [139].

(d) Macroscale models (continuum mechanics)

These models consist of systems of PDE's describing the blood flow, possibly including the interaction with the blood vessel wall, the reaction-advection-diffusion equations governing the thrombus formation and dissolution, and phenomena concerning cells behavior described by means of macroscopic quantities. Distinction between this and the previous classes is not so sharp. The typical structure of a reaction-advection-diffusion system in the blood velocity field \mathbf{u} , involving N chemical species is

$$\frac{\partial C_i}{\partial t} + \nabla \cdot (\mathbf{u} C_i - D_{C_i} \nabla C_i) = R_{C_i}(C_1, C_2, \dots, C_N) \quad (2.8)$$

where R_{C_i} describes how the species of concentration C_i reacts with all other species.

Thus the core of the model is the specification of the reaction rates (as for the spatially homogeneous models), but the presence of the diffusivities D_{C_i} calls for the specification of boundary conditions and makes integration far more difficult. The determination of the diffusivity for large molecules like proteins is by no means a trivial question. In [264] the following structure has been proposed (D in cm^2/s):

$$D = 8.34 \cdot 10^{-8} \frac{T}{\mu M^{1/3}}, \quad (2.9)$$

¹⁰⁰The case in which a finite bound is imposed to particles volume requires a different setting, with the introduction of a *volume scattering* term (see [70]).

where T is the absolute temperature, M is the molecular weight, and μ is the dynamic viscosity. More techniques of predicting proteins diffusivity are exposed in [43]. The coupling with blood dynamics is of paramount importance, since clotting develops very differently in static and in dynamic conditions. Such a concept has been particularly emphasized in [229, 230] and is of central interest in many other papers.

One of the earliest model in this class is found in [282, 283], whose analysis was later performed in [284]. The model includes the concentration of the fundamental complexes tenase $[Z]$ and prothrombinase $[W]$:

$$[Z] = k_{8,9} \frac{[VIIIa][IXa]}{K_{8,9} + k_a[APC]}, \quad [W] = k_{5,10} \frac{[Va][Xa]}{K_{5,10} + k_a[APC]}, \quad (2.10)$$

(with the inhibiting action of Activated Protein C). The reaction rates of the factors considered in the model are

$$R_{IXa} = k_9[XIa] - K_9[IXa], \quad (2.11)$$

$$R_{Xa} = k_{10}[IXa] - K_{10}[Xa] - \bar{k}_{10}[Z], \quad (2.12)$$

$$R_{IIa} = k_2[Xa][II] - K_2[II] + \bar{k}_2 \frac{[W][II]}{k_{2m} + [II]}, \quad (2.13)$$

$$R_{II} = -k_2[Xa][II] - \bar{k}_2 \frac{[W][II]}{k_{2m} + [II]}, \quad (2.14)$$

$$R_{VIIIa} = k_8[IIa] - K_8[VIIIa] - k_a[APC] ([VIIIa] + [Z]), \quad (2.15)$$

$$R_{Va} = k_5[IIa] - K_5[Va] - k_a[APC] ([Va] + [W]) \quad (2.16)$$

$$R_{APC} = k_{APC}[IIa] - K_{APC}[APC], \quad (2.17)$$

$$R_{Ia} = k_1[IIa], \quad (2.18)$$

$$R_{XIIa} = k_{11}. \quad (2.19)$$

Note that the last equation provides the stimulus for the cascade, namely the activation of FXI, triggering the cascade (2.11)–(2.18). For all diffusing factors initial and boundary conditions must be specified. For FIXa a flux condition is given exhibiting an additional activation parameter A on the clotting surface: $(\nabla[IXa]) \cdot n = A$, and zero flux is imposed elsewhere).

Such a model contains many of the numerous components intervening in a clotting process, recognizing the importance of the quantities Z , W , APC . Again, most of the inactivated factors are implicitly supposed to have constant concentration.

The aim of including more and more elements has encouraged various authors to formulate macroscale models of increasing complexity. In an important series of papers M. Anand, K.R. Rajagopal et al. [8–12] made quite a substantial step

forward. The reaction rates are far more complicated than the ones we have just seen, since the model incorporates fibrinolysis too. We take them from [12], where the following equations are considered:

$$R_{XIa} = \frac{k_{11}[IIa][XI]}{K_{11} + [XI]} - h_{11}^{A3}[XIa][ATIII] - h_{11}^{L1}[XIa][\alpha_1AT], \quad (2.20)$$

$$R_{XI} = -\frac{k_{11}[IIa][XI]}{K_{11} + [XI]}, \quad (2.21)$$

$$R_{IXa} = \frac{k_9[XIa][IX]}{K_9 + [IX]} - h_9[IXa][ATIII], \quad (2.22)$$

$$R_{IX} = -\frac{k_9[XIa][IX]}{K_9 + [IX]}, \quad (2.23)$$

$$R_{Xa} = \frac{k_{10}[Z][X]}{K_{10} + [X]} - h_{10}[Xa][ATIII] - h_{TFPI}[TFPI][Xa], \quad (2.24)$$

$$R_X = -\frac{k_{10}[Z][X]}{K_{10} + [X]}, \quad (2.25)$$

$$R_{VIIIa} = \frac{k_8[IIa][VIII]}{K_8 + [VIII]} - h_8[VIIIa] - h_{C8} \frac{[APC][VIIIa]}{H_{C8} + [VIIIa]}, \quad (2.26)$$

$$R_{VIII} = -\frac{k_8[IIa][VIII]}{K_8 + [VIII]}, \quad (2.27)$$

$$R_{Va} = \frac{k_5[IIa][V]}{K_{5M} + [V]} - h_5[Va] - h_{C5} \frac{[APC][Va]}{H_{C5} + [Va]}, \quad (2.28)$$

$$R_V = -\frac{k_5[IIa][V]}{K_5 + [V]}, \quad (2.29)$$

$$[Z] = \frac{[VIIIa][IXa]}{K_{dZ}}, \quad (2.30)$$

$$[W] = \frac{[Va][Xa]}{K_{dW}}, \quad (2.31)$$

$$R_{IIa} = \frac{k_2[W][II]}{K_2 + [II]} - h_2[IIa][ATIII], \quad (2.32)$$

$$R_{II} = -\frac{k_2[W][II]}{K_2 + [II]}, \quad (2.33)$$

$$R_{Ia} = \frac{k_1[IIa][I]}{K_1 + [I]} - \frac{h_1[PLA][Ia]}{H_1 + [Ia]}, \quad (2.34)$$

$$R_I = -\frac{k_1[IIa][I]}{K_1 + [I]}, \quad (2.35)$$

$$R_{ATIII} = -h_9[IXa][ATIII] - h_{10}[Xa][ATIII] - h_2[IIa][ATIII] - h_{11}^{A3}[XIa][ATIII], \quad (2.36)$$

$$R_{APC} = \frac{k_{PC}[IIa][PC]}{K_{PC} + [PC]} - h_{PC}[APC][\alpha_1AT], \quad (2.37)$$

$$R_{PC} = -\frac{k_{PC}[IIa][PC]}{K_{PC} + [PC]}, \quad (2.38)$$

$$R_{TFPI} = -h_{TFPI}[TFPI][Xa], \quad (2.39)$$

$$R_{tPA} = 0, \quad (2.40)$$

$$R_{PLA} = \frac{k_{PLA}[tPA][PLS]}{K_{PLA} + [PLS]} - h_{PLA}[PLA][\alpha_2AP], \quad (2.41)$$

$$R_{PLS} = -\frac{k_{PLA}[tPA][PLS]}{K_{PLA} + [PLS]}, \quad (2.42)$$

$$R_{\alpha_2AP} = -h_{PLA}[PLA][\alpha_2AP]. \quad (2.43)$$

Actually we have here 22 differential equations plus (2.30) and (2.31), defining pointwise the concentrations of tenase and prothrombinase, owing to the fact that the reactions producing them are very fast. They need no explanation. We recall that (A)PC is the (Activated) Protein C, TFPI is the tissue factor pathway inhibitor, tPA is the tissue Plasmin Activator, PLA is plasmin, PLS plasminogen, α_1AT is Alpha 1-antitrypsin, and α_2AP is Alpha 2-antiplasmin. Though not complete, this system contains most of the main protagonists of the process of coagulation and of fibrinolysis. The system above extends the biochemical model of the previous paper [9], which on the other hand addressed for the first time the very difficult question of modeling platelets activation by stress. The main idea (already announced in [8]) was to introduce a phenomenological activation number, quantifying platelets shear stress exposure. Such a coefficient is defined as follows:

$$A(t) = A(0) + \frac{1}{A_0} \int_0^t \exp \left[k \left(\frac{\tau}{\tau_{thr}} - 1 \right) \right] H(\tau - \tau_{thr}) dt', \quad (2.44)$$

where τ is (in short) the shear stress at the platelet location, τ_{thr} is a threshold value, and k, A_0 are constants. $A(0)$ is the initial value (null for platelets never exposed to a

stress beyond threshold, i.e. resting platelets). H is the Heaviside function, ensuring that the stress action takes place only beyond threshold. Formula (2.44) defines implicitly the *activation time* t_{act} as the time at which $A(t)$ reaches the threshold value for activation A_{thr} . The authors consider also a second critical value A_{damage} beyond which platelet lysis takes place. Numerical simulations for a 3-D case have been performed in the papers [33, 34].

This concept has been generalized in the paper [37], where the r.h.s of (2.11) was multiplied by one more Heaviside function $H([Ia] - [Ia]_{thr})$, meaning that enough fibrin has to be present for clotting (with reference to secondary hemostasis). Of course the computation of the activation number requires the knowledge of the blood flow field, which involves blood rheology. In [9] a shear-thinning viscoelastic model was assumed for blood flow, following [199]. In [37] a simpler generalized Newtonian rheological model with shear dependent viscosity was adopted, and the concept of activation time was used to describe a clotting process proceeding in an axisymmetric way around a (not too) small blood vessel because of an inflammatory state of the wall. Though the situation is somehow extreme, that approach was useful to point out that accepting the existence of an activation time implies the onset of a platelet activation front and also of a pre-activation front (the one at which the stress attains the threshold value), preceding the clotting front. Thus the approach includes *multiple free boundaries*. Moreover it was emphasized that prothrombinase has a pivotal role in the cascade, in the sense that if $[W]$ happens to be a known function, then only the shorter terminal segment of the cascade remains to be solved.

The concept of activation time is alternative to the one of delay activation time, put forward in [192], where its effect on ADP-activated platelets has been investigated. In that case the choice of delay was random and based on the existence of an activation distance.

Another important contribution, based on the blood coagulation model developed by Anand et al. [12], has been recently given by Yazdani et al. [263]. Here platelet motion within blood flow and adhesion to the damaged endothelial wall are solved by using an Eulerian-Lagrangian approach. A spectral/hp element method [119] is used to solve the blood flow equations and the biochemical equations of the coagulation cascade on a fixed Eulerian grid; the platelet-wall and platelet-platelet mechanisms are tracked using a Lagrangian framework. A force coupling method [157] is introduced to couple blood flow with platelet motion. Simulation results have been tested against in vivo and in vitro experiments, demonstrating the efficacy of the proposed model for a wide range of shear rates.

The issue of platelet mechanical activation has received considerable attention in the literature. We have already discussed this question when dealing with von Willebrand Factor (Sect. 2.3). The influence of stress in the activation-adhesion of platelets was also pointed out in [270] and further developed in [62], where the deposition rate was assumed to depend linearly on the shear stress at the wall

(an idea borrowed from [249]). The deposition model in [62]¹⁰¹ includes platelet diffusion-advection with platelet diffusivity selected on the basis of Stokes-Einstein equation¹⁰²

$$D_{pl} = \frac{k_B T}{6\pi\mu r_{pl}}$$

(k_B Boltzmann constant, namely $1.38064852 \cdot 10^{-23} \text{ m}^2 \text{ kg s}^{-2} \text{ K}^{-1}$, T absolute temperature, μ plasma viscosity, r_{pl} platelet (equivalent) radius), which yields $D_{pl} \sim 1.7 \cdot 10^{-13} \text{ m}^2/\text{s}$. The same order of magnitude was already suggested in the much older paper [93].

Among the authors who have worked more in the direction of increasing the model complexity we mention F. Ataullakhanov and V.I. Zarnitsina (and coworkers), that we have already quoted many times. Papers [14–17] incorporate important biological data that have been used very frequently in the literature. The model in [181] contains about 30 differential equations. It has been used in [242] to study contact pathway coagulation and hemophilia B. An even larger model [231] includes the coupling with blood flow with the aim of emphasizing how it controls the onset of coagulation. The model is fully illustrated in the “Additional file 3” attached to the paper, where a number of physical parameters (including factors diffusivities) are listed with the respective sources. Just to give an idea of the complexity of the model we report the balance equation for [Xa]:

$$\begin{aligned} \frac{\partial[Xa]}{\partial t} = & D_{Xa} \left(\frac{\partial^2[Xa^F]}{\partial x^2} + \frac{\partial^2[Xa^F]}{\partial y^2} \right) - V_x \frac{\partial[Xa]}{\partial x} - V_y \frac{\partial[Xa]}{\partial y} \\ & + \frac{k_{cat}^{X,VIIa-TF} \cdot [X] \cdot [VIIa - TF^F]}{K_M^{X,VIIa-TF}} \\ & + k_{eff}^{X,VIIa} \cdot N_m \cdot [X] \cdot [VIIa] + \frac{k_{cat}^{X,IXa \text{ local}} \cdot [IXa^{B^F}][X^B]}{(N_l + N_m) \cdot K_M^{X,IXa \text{ local}}/k} \\ & + \frac{k_{cat}^{X,IXa-VIIIa \text{ local}} \cdot [IXa^{B^F}][VIIIa^{B^F}][X^B]}{(N_l + N_m) \cdot K_d^{IXa-VIIIa \text{ local}}/k \cdot (N_l + N_m) \cdot K_M^{X,IXa \text{ local}}/k} \\ & - (k_a^{Xa,AT-III} \cdot [ATIII] + k_a^{Xa,\alpha_2 M} \cdot [\alpha_2 M]_0 \\ & + k_a^{Xa,\alpha_1 AT} \cdot [\alpha_1 AT]_0 + k_a^{Xa,PCI} \cdot [PCI]_0) \cdot [Xa^F] \\ & - k_a^{Xa-Va^B,AT-III} \cdot [ATIII] \cdot [Xa - Va^B] \end{aligned}$$

¹⁰¹ See the experimental literature quoted therein.

¹⁰² Actually applicable to spheres, it practically coincides with (2.9).

$$- \left(k_a^{Xa,TFPI} \cdot [Xa^F] [TFPI] - k_d^{Xa-TFPI} \cdot [Xa - TFPI] \right).$$

Besides diffusion and advection, it contains: FX activation by the FVIIa-TF complex, direct activation by FVIIa and by FIXa, activation by tenase, and the action of various inhibitors. Note that factors may appear in different forms (with superscripts F , B) meaning that they are free or bound to platelets, respectively. Thus the model has a very fine structure, taking into account many details: all factors, both in the activated and inactivated form (and in different states, as we have seen), all reactions in the cascade, including (in a way more complicated than (2.24)) the dynamics of TFPI (not always considered elsewhere: other exceptions are [140, 255], etc.). As it can be seen clearly from the above equation, the model is formulated in two spatial dimensions, a limitation due to the great computational difficulties. Apart from the latter consideration, it is to be remarked that not even such a huge model is really complete. For instance the dynamics of platelets activation is absent and fibrinolysis is not included. Neither of these aspects is a minor detail, as we know, since platelets have a fundamental role and even though fibrinolysis develops over a longer time scale, its roots are already in the forming clot. Not to mention von Willebrand Factor, which, to our knowledge, has never been considered in any mathematical model. In any case the model just discussed remains a basic reference for two main reasons: the extremely detailed description of the cascade and the great amount of experimental data provided.

The question remains of how to formulate a fully comprehensive mathematical model for blood coagulation. In our opinion this is actually a too ambitious target and it is instead more reasonable to ask how it could be possible to produce models which are substantially meaningful for specific purposes, but constructed using less elements.

Leaner, but still significant models have been used to produce 3D simulations. In [224] simulations take into account the stenosis of the interested blood vessel. The same paper studies the stability of clotting in quiescent plasma where the adopted model has a continuum of equilibria.

Due to the complex multiscale and multiphysics nature of the clotting process, involving fluid mechanics, cell mechanics and biochemistry, diverse studies based on multiscale models and computational methods have been successfully used to model platelet adhesion and thrombus formation.

An interesting multiscale algorithm, starting from a sub-microscale molecular dynamics model with microscale dissipative particle dynamics and macroscopic Navier-Stokes models was presented in [74]. (see also e.g., [97, 256, 257, 259, 262, 287] for other multiscale models and algorithms). A detailed overview of these studies will not be presented here.

(e) More free boundary models

We have already discussed the generation of free boundaries in the presence of deterministic assumptions as the one of platelet activation time, based on (2.44). Now we review other clotting models involving free boundaries.

(e1) A model including blood slip

In the paper [72] the clotting front was defined as the level set $[Ia] = [Ia]^*$ (the minimal fibrin concentration immobilizing RBCs) and the process was studied from the beginning of the so called propagation phase.

Various new elements were introduced:

- (i) a shortcut in writing the equations for the cascade,
- (ii) a slip condition for blood at the vessel wall,
- (iii) the direct role of activated platelets in the cascade.

Any choice of shortening the cascade system is inevitably accompanied by some degree of arbitrariness. It is a common procedure in chemical engineering to lump groups of equations in fewer equations giving an equivalent output. In the context of the coagulation cascade the proposal of [72] was to exploit the special position occupied in the system by prothrombinase $[W]$, as pointed out in [37], to write down a virtual equation for it, with a reaction term of the form

$$R_W = k_W \hat{C}_P [IIa] \left(1 - \frac{[IIa]}{[IIa]_M} \right) - (h_{1W}[APC] + h_{2W}[ATIII]). \quad (2.45)$$

It is important to stress that none of the indicated reactions actually takes place. The first term synthesizes the positive feedback loop prothrombinase-thrombin-prothrombinase, which in reality goes through the sequence of reactions leading to the activation of factors V, VIII, IX, X, XI and the production of tenase (thus involving many more equations). Likewise, the negative term tries to reproduce the inhibiting action of APC and of ATIII which is not exerted directly on prothrombinase, but on the activated factors leading to its production. The factor $\left(1 - \frac{[IIa]}{[IIa]_M} \right)$ has the effect of preventing unbounded growth ($[IIa]_M$ is the maximum expected thrombin concentration), and the dimensionless coefficient \hat{C}_P brings in the system the contribution of activated platelets, through which the action of blood slip is expressed in the way we are going to see. Of course, owing to the theoretical nature of (2.45), the rate coefficients k_W , h_{1W} , h_{2W} have to be chosen so that the outcome is the one expected, e.g. by comparison for instance with the results obtained in [9]. Equation (2.45) takes the place of equations (2.20)–(2.31) and can be associated with the rest of that system, i.e. (2.32)–(2.43).

Before we go to the description of the influence of blood slip, some further comments about (2.45) are in order. The underlying strategy is to reduce the number of equations with a twofold scope: making numerical simulations easier (a very important target, particularly when dealing with full 3D geometry), and to introduce a smaller number of parameters (always a source of uncertainty in this domain). One more remark is concerned with the aim one wants to reach: if one wants to describe the effects of deficiency of one of the factors or the action of a drug on a specific factor (e.g. a direct Xa inhibitor: see Sect. 2.7), then it is quite obvious that it is necessary to employ a model which either includes the whole cascade, or a different synthesis which makes that specific factor appear. In general we can say

that, when dealing with a phenomenon so complicated, it is important to choose the simplifications which allow to reach the specific target one has in mind. Therefore we stress once more that (2.45) can be good for some purposes and not for others. Moreover, having ignored all the key factors in the initiation phase (FVII, FIX, FX, TFPI), the model cannot describe that stage of the process and is dependent on other models for the initial conditions.

Let us proceed to the analysis of (ii) and (iii), which are not subordinate to (i), but are strongly mutually related.

Concerning slip condition at the wall, we believe that its importance for blood flow in vessels of the size of small arteries, down to arteriolas, has not been fully understood. This question has been discussed in [72] and can be summarized as follows. Treating blood as a homogeneous fluid makes sense as long as space scales smaller than or comparable to RBCs size are not considered. The shear thinning property of blood, which has been mentioned several times, is a good example of how phenomena involving the cells may influence the rheology. However it has nothing to do with the boundary conditions for the flow. Here the thin cell free plasma layer at the vessel wall, having a lubricating effect, plays the main role. If we look at blood as a mixture, we then realize that cells (making almost half of its volume) have a non-zero speed even very close to the wall. Going back to the homogeneous picture, the latter phenomenon should be interpreted as a slip. The possible occurrence of slip, which is mostly neglected in the recent literature, was instead studied with some care years ago. A remarkable example is [105], a study conducted also on experimental basis.¹⁰³ To slip we may attribute also a flattening of blood velocity profile (see the interesting paper [23]), frequently interpreted in terms of yield stress, attributing to blood a viscoplastic behavior. Concerning this fact we observe that yield stress is normally measured starting from static conditions, i.e. when RBCs aggregation is strong, but it should be not given for granted that it should actually be considered during physiological flow (see [266]). The presence of slip is of great relevance to clot growth, since it may increase the supply rate of activated platelets to the clotting site, thus accelerating the process. Since slip is of much lesser importance in veins, it may be one of the causes of the structural difference between arterial and venous clots.

With the perspective of emphasizing the role of slip, the factor \hat{C}_P appearing in (2.45) is split into the sum of two terms:

$$\hat{C}_P = \hat{C}_P^0 + \hat{C}_P^{slip}, \quad (2.46)$$

where the first term expresses the platelets recruitment rate simply due to the advancement of the clotting front, and the second term is the slip contribution. The concentrations in (2.46) are dimensionless: they are the actual concentrations divided by the normal platelet concentration ρ_P .

¹⁰³From that paper it can be concluded that slip up to 20% of blood maximal velocity in the vessel is possible.

Preliminary assumptions are:

- (a) the clot base Ω remains confined within a known boundary, its area is σ , the projection of its diameter in the direction orthogonal to the blood flow velocity is L_Ω .
- (b) activated platelets flowing over the clotting front in a layer of thickness h can attach among themselves and to fibrin.

The definition of h is empirical and it can be estimated on the basis of the following consideration. If ρ_P is the platelets density in mm^{-3} and if they are uniformly distributed, then $1/\rho_P$ is the volume of blood associated to one platelet. If we imagine platelets placed in a cubic arrangement, the side of each cube, expressed in mm, will be $(\rho_P)^{-1/3}$. Thus, if e.g. $\rho_P = 3 \cdot 10^5 \text{ mm}^{-3}$, the mutual average distance among platelets will be about $15 \mu\text{m}$. Since platelets can considerably deform when activated, this distance must be compatible with clotting in situations of slow flow or stasis. We conclude that activated platelets can interact at a distance of 5 diameters or more, thanks to their deformability. We recall that bridges among platelets are provided by fibrinogen and by vWF. Therefore it seems reasonable to set $h \sim 10 \mu\text{m}$.

Deducing \hat{C}_P^0 is simple: $\hat{C}_P^0 = 1$ if all intercepted platelets are captured, otherwise, it is set equal to a monotone decreasing function $\varphi(\tau_W)$ of the shear stress at the interface, such that $\varphi(0) = 1$, to account for stress competition to capture.

The expression suggested for \hat{C}_P^{slip} in the flow field surrounding the clot is

$$\hat{C}_P^{\text{slip}} = \chi(\tau_W)B, \quad B = \frac{1}{2}A_P \frac{u}{V_n} \frac{h}{H} \frac{[Ia]}{[Ia]^*}, \quad (2.47)$$

where $\chi(\tau_W)$ is a function similar to $\varphi(\tau_W)$ and with the same meaning, u is blood velocity (not less than the slip velocity u_s), H is a length defined as $\frac{\sigma}{L_\Omega}$ (expressing the importance of the aspect ratio of the domain Ω , seen from the flow direction),¹⁰⁴ A_P is the fraction of activated platelets, and the factor $1/2$ is motivated by the fact that slip is likely to take place only on the clot surface facing upstream, while downstream a region of slow flow is present (see [72] for suggestions on how to define boundary conditions in the vicinity of the clot). For A_P the following empirical expression is adopted:

$$A_P = \min\left(\frac{V_n}{V_n^*}, 1\right)$$

to signify that $A_P = 1$ (all platelets activated) when the front speed V_n exceeds a characteristic value V_n^* , and goes to zero when V_n goes to zero. This is a way to circumvent the dynamics of platelet activation in an already complicated model.

¹⁰⁴If Ω is a circle of radius R , then $H = \pi R/2$.

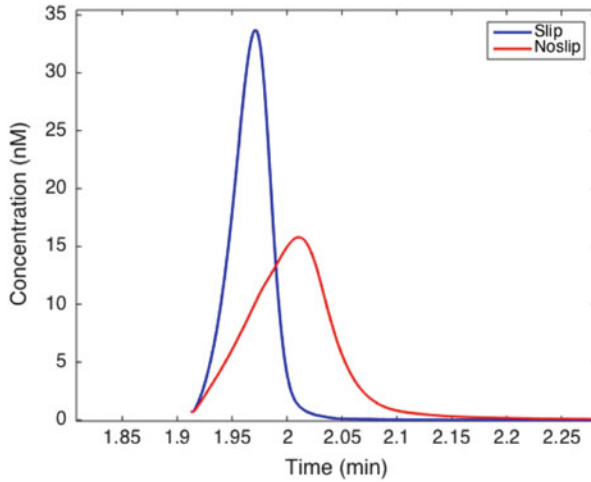


Fig. 2.22 Slip vs. no-slip comparison for thrombin production (2-D simulation from [189])

Since V_n is generally of the order of $1 \mu\text{m/s}$, $\frac{h}{H} \approx 0.1$, taking for u_s a modest 0.1 mm/s , it turns out that at the clot boundary B is 50 times A_P , showing that \hat{C}_P^{slip} can be much larger than 1.

It has also to be said that, on the contrary, slip can slow down thrombin production by sweeping away the activated factors. Thus there is some compensation between the two phenomena.

Numerical simulations have been performed in [188–190]. We just show the 2-D simulation in [189] comparing thrombin production in the propagation phase with or without the slip condition (Fig. 2.22), and the 3-D simulation showing cases of hypocoagulability due to ATIII excess (Fig. 2.23) or to platelet deficiency (Fig. 2.24). For the details about the numerical values used for the various parameters and for the geometry see the respective papers.

(e2) A model of clot accretion by platelets deposition

Platelet deposition on subendothelium is a phenomenon typical of primary hemostasis. Besides [62] and the already quoted papers by Fogelson et al., there is a quite substantial literature on the subject, which received a particular impulse by Affeld et al. [2], where an old experimental technique (the stagnation point flow chamber [191]) was resumed to point out the prevailing role of diffusion in platelet deposition in a platelet rich plasma. A more sophisticated approach to modeling platelet adhesion was proposed in [271], where the process was coupled with the blood flow (considered just Newtonian) and influenced by the concentration ψ of platelets already captured by the binding surface. The concentration w of free platelets obeys an advection diffusion equation and the deposition law is modeled as

$$-D\partial_n w = k(\psi, \tau)w, \quad (2.48)$$

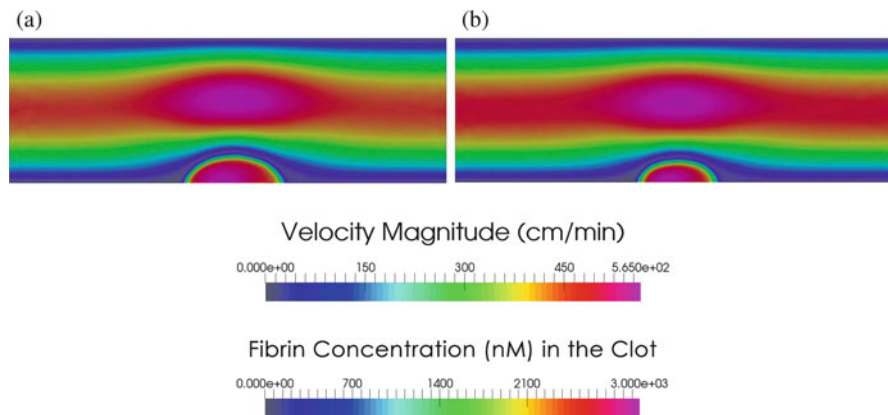


Fig. 2.23 3-D simulation at the peak of clot formation in normal case and with ATIII concentration exceeding normal concentration by 30% (from [190]). **(a)** Normal case. **(b)** ATIII surplus

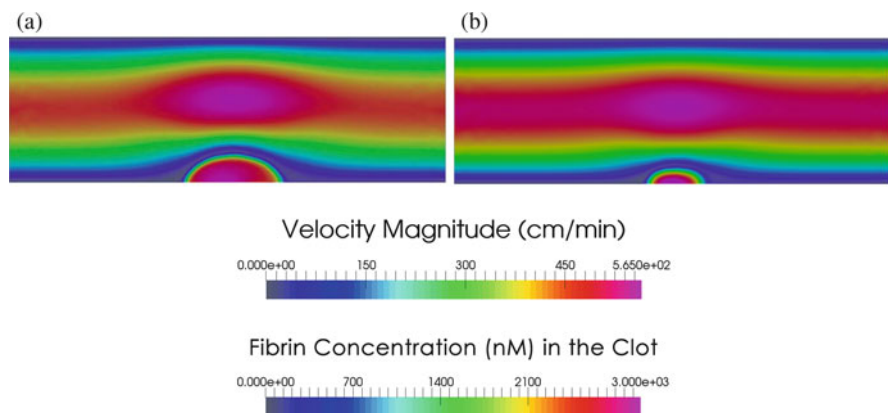


Fig. 2.24 Result of a 10% platelet reduction. 3-D simulation from [190]. **(a)** Normal case. **(b)** Platelets deficiency

(τ is the shear stress) while the corresponding increase of ψ is described by

$$\partial_t \psi = k(\psi, \tau)w. \quad (2.49)$$

The adhesion rate function $k(\psi, \tau)$ is chosen as

$$k(\psi, \tau) = (\lambda_1 + \lambda_2 \tau) \left(1 - \frac{\psi}{\Psi} \right) + (k_1 + k_2 \tau) \frac{\psi}{\Psi} \quad (2.50)$$

saying that the original surface is reactive until ψ reaches the limit value Ψ , with the second term expressing the binding action by the already deposited platelets.

In the model the flow domain neglected the presence of the thrombus. It was extended in [272] to account for the presence of such a free boundary. To the previous equations the free boundary condition

$$v = \alpha \nabla w \quad (2.51)$$

was added to describe the motion of the deposition surface. The solution was computed using the level set method.

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