# THE PLATELET AND ITS DISORDERS

Barry G Firkin

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# The Platelet and its Disorders

To A. E. McGuinness and C. R. B. Blackburn,
who introduced me to
clinical medicine and platelets.

# Preface

The aim of this book is to introduce the medical student, recent medical graduates involved in postgraduate training and physicians in practice to the role of the platelet in physiology and disease. It is not intended to be an encyclopaedic review of all the literature over the past few decades, but largely represents a personal account, and although resultant prejudices occur, an attempt has been made to emphasize to the reader areas of doubt, and point the way to other sources which may explore these questions more fully in selected references at the end of each chapter. I acknowledge the help of my secretaries, Ms Carolyn Harvey and Mrs Marjorie Brown without whom the book would never have been started, let alone completed. Some illustrations and figures were made by Mr W. Shepherd of Monash University and Ms A. Leaman of the Alfred Hospital. I am indebted to Mrs E. Hagon who read and corrected a number of the chapters, and for the constant help through discussion and debate with my two close collaborators in research, Dr Margaret Howard and Dr Sharron Pfueller. Dr Siew Choong, Mrs Maureen Broadway and Ms Ilona Lakatos documented the methods used in our laboratory to study platelets as outlined in the Appendix. I acknowledge research funding from the National Health and Medical Research Fund of Australia, the Life Assurance Fund of Australia, The Victorian Anti Cancer Council, The National Heart Foundation of Australia and the Alfred Hospital and Monash University Research Funds for support for my investigations over the past twelve years.

In conclusion I must thank my wife Ruth with whom I corrected the proofs.

*Barry G. Firkin*

# Contents

# 1
# Introduction

As multicellular organisms developed from their unicellular forebears in the seas and/or ponds on earth, an evolutionary train of events occurred to aid survival. This revolved around essential mechanisms of repair, nutrition and ultimately defence against foreign predators. Initial life forms consisted of a colony of similar cells bathed in their surrounding water milieu, from which they absorbed their nutrients. With time specialization occurred, so that some cells became capable of movement to sites where trauma had resulted in a fracture or tear of the multicellular colony, and these cells affected a reunion to reconstitute the integrity of the organism. Such cells probably developed humoral components in their cytoplasm, which on release following secretion or the cells' rupture could aid in such repairs. These cells were the forebears of the platelets and the fibroblasts. Increase in the size of multicellular organisms rapidly produced problems of nutrition which became difficult to sustain by the simple process of diffusion from the organism's gaseous and/or liquid environs. Specialized systems had to be developed to ingest and digest food, to enable movement to capture food, an internal circulation to distribute the gaseous and nutritional elements to all parts of the organism, and ultimately to ensure defence against physical injury or attack by other invading organisms.

The mammalian circulation is a system consisting of structural elements which not only act as conduits for blood, which contains the body's cellular and humoral elements concerned with nutrition, repair and defence, but is also specialized in some areas to enable gaseous exchange (the lungs) to eliminate waste products (the kidney) and to maintain circulatory flow (the heart). Socratic argument could reason that these vessels would not merely be passive, but would be able to actively contribute to these basic functions of the circulation, and this has been amply supported by work over the past years which has shown vessels to play an important role, both from a structural and metabolic view, to the maintenance of the integrity of the circulation to all parts of

the body. The two major areas which have so far been identified in this regard are firstly, the ability of the vasculature to set up collateral systems to bypass areas of block and to establish new supplies to endangered tissue, and secondly the properties of the endothelial cells lining the wall and their metabolic role in prevention of blockages occurring in the system which would result in the death of tissue supplied by the vessel.

The platelet is a cell evolved to maintain the integrity of the vascular compartment and, as would be expected, has an intimate association with other humoral and cellular elements in the blood concerned with repair, defence and nutrition, although its functional attributes and properties are more important for some aspects compared to others. Nonetheless, it should not be thought of as a cell in isolation, it has a particular affinity and integration with cellular elements of the vessel wall and humoral elements concerned with the maintenance, surveillance and repair of vascular integrity and more peripherally with the inflammatory response and the body's defence systems.

Three fluid phase systems have been identified in blood which appear intimately interrelated, and which have some striking similarities. One is that of blood coagulation wherein the platelet has a most important role and intimate connection, whilst the others concern inflammatory response and body defence mechanisms, namely the complement and kinin systems. All three systems rely on complicated interactions of enzyme precursors which are interlocked and perhaps interdependent for optimal efficiency. These fluid and cellular body defence mechanisms have been identified in most species studied. The degree to which they contribute to the body's defence seems to vary, but in all submammalian species there is a cell, which has a similar role to the platelet, called the thrombocyte. This cell is morphologically strikingly different to the platelet since it is nucleated, often has only a thin rim of cytoplasm, and is difficult to distinguish from a small lymphocyte, although in some species, e.g. the chick, it possesses a characteristic inclusion body. The basic properties of this cell and the platelets are similar, and since they exist from teleost to reptilia and aves, would seem to be fundamentally essential for this cell's function. These properties are:

(1) The ability to stick to foreign surfaces and traumatized vessels,
(2) The ability to stick to one another, i.e. aggregate,
(3) Their ability to take up certain foreign particles, e.g. virus or latex particles.

While the importance of the first two properties can be readily understood from current theories of haemostasis (*v. infra*), the role of the third in the function of the thrombocyte or platelet is still a mystery. In view of its universal presence in thrombocytes in all species so far examined, including mammalian platelets, it would seem likely that it has some major import or may be related in some inseparable way with one of the other two functions of the cell (*see* Glanzmann disease).

## ORIGIN OF THE PLATELET

In man the platelet is produced in the cytoplasm of a cell called the megakaryocyte, which is a large cell most commonly located in the haematopoietic tissue in the bone marrow. This cell may also be seen in capillaries in lung and in other sites where haematopoiesis occurs embryologically, such as the liver and spleen. In the latter instances, such a location is usually associated with some disease state such as myelofibrosis.

The megakaryocyte is an unusual cell because it is polyploid, and to achieve this undergoes a number of nuclear divisions without the cytoplasm of the cell dividing — termed *endomitosis*. Morphologically megakaryocytes are divided into three types which reflect the degree of polyploidy and maturity of the cell. Type I megakaryocyte or megakaryoblast is a cell 15 $\mu$m in diameter, and is largely constituted of a nucleus and a small rim of deep blue cytoplasm. The ploidy of the nucleus is usually between 4n and 8n. The Type II megakaryocyte or basophilic megakaryocyte is a larger cell, 20–30 $\mu$m in diameter, with a relatively higher cytoplasm to nuclear ratio (1:1), whose cytoplasm is usually blue with Romanowsky stains and whose nuclear ploidy ranges up to 64n. Type III megakaryocyte or granular megakaryocyte is the classical mature megakaryocyte whose cytoplasm to nuclear ratio is approximately 3:1, and which has an overall size of somewhere between 30 and 50 $\mu$m. The cytoplasm is pinker in colour, and in it can be seen the granules and inclusions which are characteristic of platelets. The nucleus has a ploidy which again ranges up to 64n. Both Type II and III megakaryocytes have a similar range of ploidy and both produce platelets (see Table 1.1). Morphological studies suggest that these platelets are produced by a cleaving of the cytoplasm of the cell, rather as one would tear a postage stamp from a stamp booklet, and arise from protuberances of the cell cytoplasm projecting into the venous sinuses of the bone marrow. One proposal is that this occurs along a membrane system which is found in the megakaryocytes' cytoplasm termed the demarcation membrane system, and this would account for the doubling or trebling of the surface membrane needed to produce the numbers of platelets from a single megakaryocyte. An alternative proposal is that the

**Table 1.1** (After Penington). Approximate distribution of ploidy of megakaryocytes *Type I, II and III in human bone marrow*

| *Ploidy* | *Percentage* | *Platelets produced* |
|---|---|---|
| 4n | 1% | *Dense* |
| 8n | 16% | Large platelet with high granule count and little intracellular membrane. |
| 16n | 66% | |
| 32n | 16% | *Light* |
| 64n | 1% | Small platelets with few granules and plentiful intracellular membrane synthesized during endomitosis. |

demarcation membrane system is in fact an invaginated membrane system, which in response to the appropriate stimuli becomes evaginated to form the long thin megakaryocyte cytoplasmic processes, these may protrude into the intravascular spaces by a mechanism similar in principle to platelet shape change (Figure 2.2). The open canalicular system in the platelet is perhaps a residue of the so-called demarcation membrane system. In some instances a larger portion of cytoplasm containing many unseparated platelets may be detached into the vascular stream, and the individual cells separated at distal sites, either in the bloodstream itself or in capillaries, lungs or spleen. In other instances the megakaryocyte may enter the circulation and lodge in the lungs where its cytoplasm may fragment to produce platelets. Some authorities estimate that between 20 and 50% of the circulatory platelet mass may be derived in this fashion. Following the platelets' entry into the circulation they remain there, like the red cell, until their death when they are thought to be removed by tissue macrophages in the reticulo-endothelial system. Since most studies put the average lifespan of a platelet in man as being between 8 and 10 days, each individual cell would be expected to traverse the circulatory system on many occasions, but how long cells spend in secluded areas of the circulation such as the sinusoidal system of the spleen, is not known. Microscopic observations in animals show that in blood flowing in the arterial vessels, platelets occupy a position between the centrally placed stream of red cells and the more peripheral white cells. The latter roll along the vessel wall like a number of cartwheels. Artificial model systems would suggest that vigorous jet streams, with or without eddy currents, around narrowings in vessels could result in the formation of platelet aggregates thereby precipitating thrombus formation in the arteries. Similarly, the arterial jet streaming could account for some of the dramatic clinical thrombotic problems which occur on the arterial side of the system, namely cerebral thrombosis and coronary occlusion.

Two major functions have been identified for the platelet – one is to support the normal integrity and physiology of the vessel wall; the other is to act as a police force to patrol the vasculature, and should any interruption or damage be located to pinpoint it by sticking to it and forming an aggregate around which coagulation occurs and repair is aided by such cells as the fibroblasts. In man the platelet count is maintained somewhere between 150 and $350 \times 10^9$/l. Taking the platelet lifespan as 10 days, and the platelet count to be $250 \times 10^9$/l with a blood volume of 5 litres, approximately $125 \times 10^9$ platelets are produced per day. The mechanisms by which the numbers of platelets produced by megakaryocytes are controlled remain uncertain. A postulated hormone thrombopoietin, is a poorly defined plasma protein which stimulates platelet production, and which is increased in activity when the platelet count is lowered by such methods as plateletpheresis or the injection of platelet antibodies. Artificially increasing the platelet count by platelet transfusion has also been shown to reduce the numbers of platelets produced, but again the

mechanism by which this is achieved is unknown. Although it has been felt for some time that platelets contribute to vascular integrity it has only been recently that apparently convincing evidence has been offered in support of this. This is based on animal studies in which the animal has been rendered thrombocytopenic either by irradiation or the use of platelet antibodies. Subsequent electron microscopic examination of the vasculature following exposure to agents such as thorotrast has shown that the thrombocytopenic animal's vessels are readily penetrated by thorotrast, and that the tight junctions between the endothelial cells become less evident. Such changes may be lessened by the exhibition of drugs such as prednisolone. Other workers have not been able to reproduce this work. The idea that platelets might in some way be continuously contributing to vascular integrity gave rise to the suggestion that platelets might be removed from the circulation in a random fashion rather than by senescence. Analysis of platelet survival data in the late 1950s and early 1960s led to a consensus view that the most important factor in the platelet's lifespan was age, and that random intravascular destruction of platelets was unlikely in physiological states. This is supported today by the lack of evidence of a free specific-platelet-protein in plasma such as $\beta$-thromboglobulin or platelet factor 4 (*v. infra*) which can be readily identified in situations where intravascular destruction of platelets has occurred, such as disseminated intravascular coagulation. In addition, there is no prolongation of platelet lifespan in people with normal platelet survival who are fully anticoagulated. The interpretation of lifespan data has usually been based on there being only one type of platelet population. In 1965 Webber and Firkin examined the effects of osmotic shocks on platelets, and found that two morphological types could be distinguished by electron microscopy following either hypo-osmotic or hyper-osmotic shock. One type was highly crenated in appearance, had a very well developed surface connecting system, and was darker than the second type which appeared blown up and light but still contained some granules. Serial section showed that these appearances were consistent in an individual cell, and it was therefore suggested that there might be two different types of platelets in the circulation which could be due to either:

(1) Ageing, or
(2) Different types of platelets with possibly different functional attributes.

When platelet sizing techniques became available it was appreciated that there could be considerable differences in platelet sizes, and that there were some genetic characteristics, i.e. people from the Mediterranean area usually seemed to have bigger platelets than Caucasians. The introduction of density gradient techniques by Karpatkin (1969) demonstrated differing populations of platelets which he believed to result primarily from an ageing process, the lighter platelets being the younger and the cells becoming more dense as they grew older. He supported this contention by pulse labelling experiments using $^{75}Se$ (selenomethionine). This was supported by another group

employing radioactive $^{51}Cr$ and another with $^{35}S$. It was pointed out that density is related to the presence of dense granules, and after the release reaction (*v. infra*) platelets become lighter and their density may reflect previous platelet activation, perhaps, therefore, one might expect old platelets to be less dense, i.e. the reverse of the above. Penington *et al.* (1976) believes a more likely explanation of the differing subsets of platelets is that populations of platelets are derived from megakaryocytes of varying ploidy, those with the lower ploidy producing the larger dense platelets containing the most granules and the higher ploidy producing the smaller and lighter platelets (see Table 1.1). This would envisage a whole family of circulating platelets of differing size with similar lifespans. The megakaryocyte does not divide, but the membrane produced during nuclear division is increased intracellularly to produce an extensive and highly ramified surface connecting system. The less dense platelets contain fewer granules, have a much higher content of membrane and a more extensive surface connecting system. They, therefore, may be more important for adhesion or in providing fibrillar bayonet type platelets important in the interaction with other cells and fibrin (*v. infra*). These platelets are derived from megakaryocytes with higher ploidy, 32 n–64 n (Table 1.1). It would be interesting to know whether the distribution of ploidy in megakaryocytes was different in the Mediterranean races who produce larger platelets, but so far this information is not available.

The role of the spleen in the physiology of the platelet has been much debated over the years. At one stage it was proposed that it might secrete hormones which were important in the stimulation or inhibition of platelet production. The present view is that the spleen acts as a filter, perhaps retaining the stickier and larger of the platelets to form a 'pool' of between 10 and 20% of the total platelet population in the vascular system. The presence of this pool was postulated because it was noted that there was a difference in the percentage of labelled platelets recovered after infusion in patients with splenomegaly secondary to portal hypertension compared to normal people. It was further found that such patients with splenomegaly had a yield following infusion of radioactive platelets of only 4% which following splenectomy rose to 50% (Firkin, 1963). This was confirmed by more definitive studies showing that the platelets in the spleen were available to the circulation (Aster, 1966) and that platelets could be recovered from spleens removed following splenectomy by direct perfusion (Penny *et al.*, 1966). Under pathological conditions of splenic enlargement, the pool may be dramatically increased, so that in some instances the spleen may contain seven times the number of platelets circulating in the blood. This is most classically seen in congestive splenomegaly secondary to portal hypertension, the peripheral blood platelet count will appear to be reduced to approximately $\frac{1}{2}$ to $\frac{1}{3}$ normal but the overall mass of platelets in the body, because of the accumulation in the spleen, remains the same.

The spleen in some instances may act as a safety mechanism to prevent

active or sticky platelets entering the circulation; there is an increased incidence of thromboses reported after splenectomies in numerous clinical situations, especially in patients who have had splenectomy for hereditary haemolytic situations which are not completely remedied. In these circumstances it may be as much a result of an overall increase in platelet count rather than a circulation of platelets which are 'stickier than normal'.

## References

ASTER, R. H. (1966). Pooling of platelets in the spleen: role in pathogenesis of 'hypersplenic' thrombocytopenia. *J. Clin. Invest.*, **45**, 645

FIRKIN, B. G. (1963). The platelet. *Aust. Ann. Med.*, **12**, 3

KARPATKIN, S. (1969). Heterogeneity of human platelets. I. Metabolic and kinetic evidence suggestive of young and old platelets. *J. Clin. Invest.*, **48**, 1073

PENINGTON, D. G., STREATFIELD, K. and ROXBURGH, A. E. (1976). Megakaryocytes and the heterogeneity of circulating platelets. *Br. J. Haematol.*, **34**, 639

PENNY, R., ROZENBERG, M. C. and FIRKIN, B. G. (1966). The splenic platelet pool. *Blood*, **27**, 1

RADLEY, J. M. and HALLER, C. J. (1982). The demarcation membrane system of the megakaryocyte: A misnomer. *Blood*, **60**, 213

WEBBER, A. J. and FIRKIN, B. G. (1965). Two populations of platelets. *Nature*, **205**, 1332

# 2
# Morphology of the platelet

When examining peripheral blood, either with phase-contrast illumination or a fixed film and a Romanowsky stain, the smallest elements seen are platelets. These cells usually range from 1 to 3 $\mu$m in diameter, although an occasional larger cell up to 5 $\mu$m may be seen. With higher magnification a central area is seen, termed the granulomere because it contains granules, and a peripheral clearer area called the hyalomere. When wet preparations are examined by phase-contrast the granules move within the cell. If a fixed film has been obtained from a finger prick, rather than from venous anticoagulated blood, clumps of platelets are observed due to the triggering of platelet aggregation following the tissue trauma and glass contact. Artifacts may occur in the platelets' appearance if the blood is collected into anticoagulants and allowed to stand for any length of time. This is particularly so when the anticoagulant used is ethylene diamine tetra-acetic acid (EDTA) in which the platelets swell on incubation. Studies of recalcification of platelet-rich plasma using phase-contrast microscopy showed a rapid change in shape from disc to a spiny sphere and the early appearance of long thin filaments with aggregation into masses of cells which coalesce and lose the granules, and from these radiate strands of fibrin. This phenomenon was called viscous metamorphosis, but examination by electron microscopy shows that each individual cell retains its integrity, and that the changes observed by the light microscope reflect both the release reaction (*v. infra*) and the redistribution of the cells' organelles during platelet aggregation as well as changes in the platelets' shape.

Figure 2.1 is a diagrammatic representation of the platelet viewed in coronal and longitudinal section by transmission electron microscopy. The cell is bound by a typical unit membrane consisting of a phospholipid bilayer, on the external surface of which is glycoprotein and protein components with the internal surface lined by protein components alone. In this trilaminar structure the glycoprotein surface is quite dense when it is viewed with stains such as

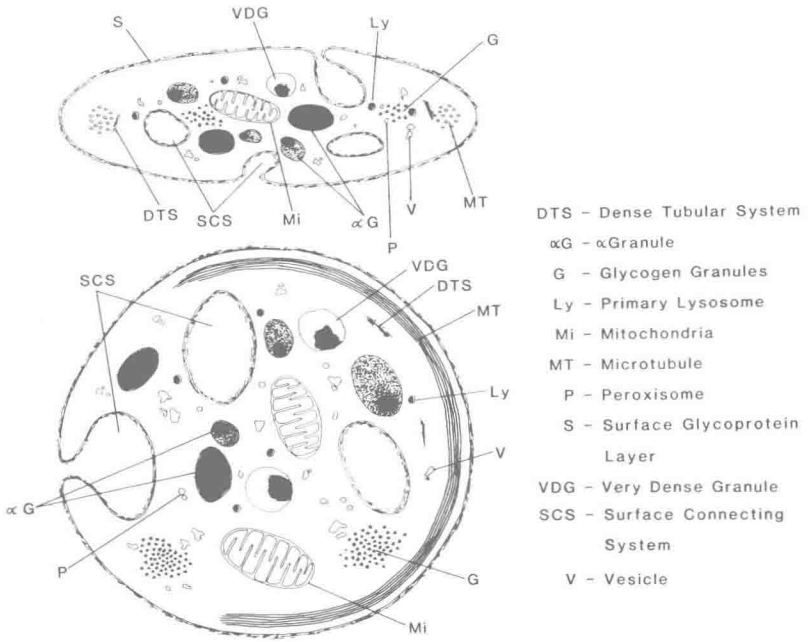


**Figure 2.1** Diagrammatic representation of longitudinal (above) and coronal (below) section of the platelet seen by transmission electron microscopy

ruthenium red, giving the platelet an atmosphere of some 20–40 nm. There are lake-like areas in the cell's interior which are also lined by glycoprotein, and which are believed to connect to the surface through a continuous canalicular system called the surface connecting system (see Figure 2.1). The electron micrographs of cells which have been either incubated in EDTA or with a proteolytic enzyme such as trypsin demonstrate a complex canalicular system which interlinks the lakes of the cell with each other and with other sectors of the surface connecting system (SCS), and in some instances platelet granules (*v. infra*) are contained within this system. How much of the SCS is freely connected to plasma and therefore part of the cell's surface is conjectural, but inert particles such as thorotrast are able to pass along it to finally lodge in the $\alpha$-granules (*v. infra*) of the cell. Immediately subjacent to the unit membrane fibrillar components of the cell can be recognized, which range from 5 to 6 nm in width. In disrupted platelets two types of filaments have been distinguished, one 6 nm in width and resembling actin filaments varying in length up to 1–2 $\mu$m. These were found to react with high molecular weight myosin to form arrow head complexes, supporting the contention that these were actin filaments. The other filaments have a width which varies in the central part from 8 to 18 nm and these correspond to myosin. These filaments were often closely associated in disrupted platelets. Actin comprises 10% of the platelet protein, whereas only 1% is myosin. It is likely that the filamentous structures