PLATELETS are cytoplasmic fragments of megakaryocytes. They are formed by an elaborate membranous system known as the demarcation membrane system. In the course of maturation, megakaryocyte cytoplasm is demarcated by this system into platelet "zones" or "territories," each of which can then form a platelet, leaving the megakaryocyte nuclei behind to be phagocytized.

In recent years, new information has advanced our understanding of this process and as a result, some older concepts have changed. This review deals with the cell biology of platelet formation and release. Neither kinetics and labeling methodology nor the function and pathophysiologic considerations will be treated here. Other recent reviews are available on these subjects.1,2

TERMINOLOGY AND SOME PHYLOGENETIC CONSIDERATIONS

Platelets are non-nucleated cells and should be distinguished from the nucleated "thrombocytes" of lower vertebrates.3-6 However, in the literature pertaining to the mammalian system, the two terms are often interchangeably used, perhaps because Deckhuyse7,8 who first described the functional equivalence of platelet and thrombocyte (and coined the latter term), overemphasized their similarities.

In the course of evolution, the megakaryocyte-platelet system is limited to mammals. In lower vertebrates (pisces, amphibia, reptiles, birds, and even some mammalia of lower orders), the functional equivalent of the platelet is the nucleated thrombocyte.3-6 They are derived through successive division of their precursor cells, "thromboblasts," in a manner similar to the derivation of red cells from erythroblasts. In vertebrates, a specific cell type is not endowed with the platelet function; the hemocyte, otherwise equivalent to the leukocyte, carries out this function.

A simple computation indicates that the evolution of species has worked in an economic direction with regard to platelet formation: the majority of megakaryocytes have 8–16 nuclei with some even up to 64712 and were nuclear division associated with cytoplasmic division (as in thromboblast–thrombocyte system), at most 64 cells would have resulted, whereas a megakaryocyte, depending on its ploidy,13,14 can produce an average of 4000–8000 platelets.15-18

Phylogenetically, similarities exist between the platelet and the red cell. Both are nucleated cells in lower vertebrates and non-nucleated in mammalia. Both are produced intravascularly in lower vertebrates and extravascularly in mammalia. Both cell types are endowed with some transport function. Studies on the nucleated thrombocyte have contributed to the recognition of the significance of cell aggregation in thrombosis.

HISTORICAL BACKGROUND

Platelets were first recognized in 1842.19 During this year, four observers independently reported on the presence of small particles in the blood. During the
ensuing 40 yr an intense search was underway to elucidate the function of these particles. Several historical reviews have dealt with this tumultuous period, which culminated in establishing the identity of the platelet as a new blood element, in 1891, by Giulio Bizzozero. Bizzozero coined the term platelet (plättchen) and recognized that these, and not leukocytes, form the white thrombus. He differentiated between this initial stage of hemostasis and the subsequent coagulation. He also performed the first platelet kinetic study by removing the blood, separating its platelets, and reinfusing the platelet-poor blood. Platelets regenerated in 4–5 days, remarkably close to figures obtained through more elaborate techniques.

Megakaryocytes were probably first seen in the marrow as early as 1849 by Robin, who could not distinguish these cells from other large cells such as osteoclasts. Collectively, he called them “myeloplasxes.” Megakaryocytes, as an entity, were recognized in 1890 by Howell, who also introduced the term. It was Wright who, in 1906, discovered the megakaryocyte–platelet axis, and his discovery could not have come without the development of the staining method that carries his name.

Evidence for the Megakaryocyte–Platelet Axis

Wright’s evidence for the derivation of platelets from megakaryocytes was strictly morphological, and his view only slowly penetrated the thinking in hematology. In fact, argument is advanced against it as late as 1976. In addition to strong presumptive morphological evidence, the following findings indicate the presence of a megakaryocyte–platelet axis.

1) Experimentally induced thrombocytopenia is associated with compensatory increase in the size and the number of megakaryocytes. Conversely, thrombocytosis results in suppression of megakaryocytopoiesis.

2) Megakaryocytes and platelets share common antigens.

3) There are also numerous similarities between the chemical composition of megakaryocytes and platelets.

4) In the course of both phylogeny and ontogeny, the temporal appearance of the megakaryocyte and the platelet is simultaneous.

5) When megakaryocytes are labeled, the label subsequently appears in platelets.

6) Finally, derivation of platelets from megakaryocytes can directly be observed by microcinematography.

MEGAKARYOCYTE MATURATION AND PLATELET FORMATION

In contrast to other hemopoietic cell lines, the megakaryocyte is a polyploid cell. It was once believed that the cytoplasmic maturation and nuclear divisions occur concurrently, as is the case with other hemopoietic cells. Hence, platelet formation should occur in the cell with high ploidy and in proportion to it. This is not the case, however. Before any evidence of cytoplasmic differentiation occurs, the cell synthesizes all the DNA that it ultimately will carry. During this period, the nuclear size increases without nuclear segmentation. Irrespective of the complement of DNA synthesized, the synthesis stops at the earliest sign of cytoplasmic differentiation. It has been postulated that the appearance, in the cytoplasm, of the contractile protein, thrombosthenin, may trigger a message to stop DNA synthesis.

At any rate, the cytoplasmic maturation and nuclear segmentation of megakaryocytes proceed only after DNA synthesis has stopped, irrespective of ploidy. As a result, platelets are formed from megakaryocytes of different ploidy; yet simply because the ploidy number correlates positively with the cytoplasmic size, the numbers of platelets formed per megakaryocyte correspond to the degree of ploidy. Furthermore, there is some evidence that cells with different ploidy number may respond differently to various stimuli and may form platelets of different size.

DEMARCATION MEMBRANE SYSTEM

The cytoplasmic maturation of the megakaryocyte is associated with the development of an extensive membranous system known as the demarcation membrane system. It has been postulated that in the process of megakaryocyte development, the demarcation membrane system is the first organelle to appear. This system demarcates platelet “zones” or “territories” by enclosing and defining parts of the megakaryocyte cytoplasm that would, in the end, be platelets. In this way, the demarcation membrane system forms the cell membrane of nascent platelets. By thin-sectioning electron microscopy, the demarcation system appears as profiles of round, oval, or elongated vesicles where the membrane encloses an empty core or cysterna (Fig. 1). These profiles may show extensive budding, coalescing, and branching. The demarcation system is readily distinguished from the rough endoplasmic reticulum because, unlike the latter, its cytoplasmic surface is not studded by ribosomes, nor is a proteinaceous substance seen within the cisternae. The demarcation system can also be differ-
MEGAKARYOCYTE-PLATELET AXIS

Fig. 1. Electron micrograph of a mature megakaryocyte in the rat. The nucleus (N) is seen on the left and the cell membrane on the right upper corner. Note the round, oval, or elongated vesicles of the demarcation system (DMS), distinguishable from profiles of endoplasmic reticulum (RER) by absence of ribosomes on the cytoplasmic surface. Within the rectangle a part of megakaryocyte cytoplasm is demarcated as platelet zone (×42,500).

entiated from the Golgi system by virtue of its distinct morphology and by its localization in the intermediate rather than the perinuclear zone of the cell. In well-developed megakaryocyte and in favorable sections, transverse sections of the demarcation system appear as circular vesicles that are arranged side by side, in a bead-like chain, thus forming the outline of a nascent platelet (platelet zone). In other areas, stacks of longitudinally sectioned profiles are seen to be oriented in parallel arrays. These have been called “dense compartment” by Behnke, who suggested they were foci of membrane assembly and proliferation. Hence, observations on thin sections suggest that the demarcation system consists of cylindrical-tubular structures whose appearance depends on the plane of section. Intense proliferation of the demarcation system, inordinate with other aspects of cellular maturation, is seen in state of active megakaryocytosis, such as in experimental thrombocytopenia and in preleukemia.

Origin of the Demarcation System

The origin of the demarcation system has variably been attributed to the megakaryocyte cell membrane, endoplasmic reticulum, Golgi system, and membranogenic areas of the cytoplasm. Current evidence indicates that none of the intracellular membrane systems are the origin of the demarcation system. On the other hand, there is abundant evidence to suggest that the demarcation membrane system is formed by invagination of the megakaryocyte cell membrane. This evidence is based on experiments with extracellular tracers that do not penetrate the intact biomembrane. Ferritin, horseradish peroxidase, ruthenium red, thorotrast, and lanthanum nitrate have been used. In all cases the tracers have readily penetrated the cisternae of the demarcation system but not other cytoplasmic organelles, indicating communication of the demarcation system with the extracellular space and arguing for its origin from the megakaryocyte cell membrane. By contrast, the absence of tracers in the Golgi system and endoplasmic reticulum indicates the lack of communication with the demarcation system and argues against its derivation from these intracellular membranous systems.

Other evidence in favor of the cell membrane derivation of the demarcation system is summarized here:

1. In murine leukemia, budding of viruses occurs not only on the cell surface but also on the cisternal surface of the demarcation system suggesting a unity. In other infected cells, budding occurs only on the cell membrane.

2. Freeze-fracture studies indicate an orderly progression, with respect to size and density of intramembranous particles, from the megakaryocyte cell membrane to the demarcation system to the platelet membrane, suggesting a direction in which the membrane differentiates. No such similarities are seen between the demarcation system and other intracellular membrane systems.

3. Acetylcholinesterase, a lipid-dependent enzyme, present on the outer layer of the red cell membrane and used as a marker, is also present in the megakaryocyte cell membrane in several species. Cytochemically, this enzyme has also been localized to the demarcation system.

4. Platelets display a surface coat of acid mucosubstance that reacts with cationic dyes, ruthenium red, Alcian blue, lanthanum salts, and colloidal iron. This coat appears as a fuzz on the platelet surface. A coat with similar reactivity is also found in the megakaryocyte cell membrane and its demarcation system.

A Theoretical Dilemma

Although the consensus of opinion now favors the cell membrane origin of the demarcation system, until recently, it was not clear how the process of platelet demarcation came about. For derivation of the demarcation system from the cell membrane, the latter must invaginate deeply into the cytoplasm. The invagination can give rise to the formation in the cytoplasm of
cylindrical-tubular structures, the cysternae of which are in communication with the extracellular space. This view is consistent with observations on random sections and tracer experiments.

Here a theoretical dilemma exists: to enclose a part of the cytoplasm and to demarcate platelet zones, a flat sheet rather than tubular membrane is called for. It was MacPherson who appreciated the problem of transition from cylindrical-tubular membrane into a sheet of flat membrane capable of demarcating platelet zones. In search for a solution he advanced “the single site theory,” which maintains that the initiation of the demarcation system formation occurs at a single localized linear area and proceeds by concentric infolding of the cell membrane. Thus, he attempts to resolve the theoretical problem by postulating that the system is a flat sheet from the beginning. Because the random sections could not resolve the dilemma, this was left to the application of freeze-fracture technique. By contrast to sections, freeze-fracture technique permits, depending on the fracture path, a three-dimensional view of the inner parts of the cell and both its intracellular and cell membranes. In freeze-fracture, when the fracture line exposes the cell membrane, scattered invaginations are seen that are continuous with the demarcation system. This does not support the single site theory of MacPherson. When the system is fractured tangentially (Fig. 2), it appears as a fenestrated sheet, the fenestrations having a parallel alignment. When the fracture plane is transverse, however, it appears as a canal system, the basic element of which is membrane-wrapped tubules. These tubules may be cylindrical, like a pipe, or somewhat flattened, like an empty toothpaste tube. In favorable preparations, bead-like chains of these tubules, lying side-by-side (comparable to the profiles seen in thin sections), are seen connected by membranous bridges which, in oblique fracture planes, appear to be the result of focal fusion of membranes of two parallel tubules.

**Fusion–Fission Reorganization of the Demarcation System**

Based on these observations, it is now possible to reconstruct a developing membrane model whereby platelets are formed from megakaryocytes. This is shown schematically in Fig. 3. Through the invagination of the cell membrane, tubules are formed within the cytoplasm (left upper part of Fig. 3). These tubules are membrane-wrapped and their cysternae are in communication with extracellular space. When these structures are oriented parallel in their long axes, their membranes undergo point fusion in the direction A. These point fusions become extensive, yet leaving unfused areas in between. These appear as fenestration in a tangentially fractured demarcation system. Separation or “fission” of these structures in direction B (perpendicular to A) yields two sheets of scalloped membrane. Thus, the process of fusion–fission has reorganized the tubular membrane into two flat sheets. Upon the completion of fission, the two sheets of membrane form cell membranes of two adjacent nascent platelets. The open canalicular system of platelets may then be interpreted as unused demarcation membrane. The process is a dynamic, asynchronous, and three-dimensional.
It is to be remembered that fusion–fission reorganization of membrane is not limited to the megakaryocyte–platelet system. It is one of the most ubiquitous events in cell biology, mediating such diverse biologic phenomena as fertilization, cytokinesis, neuromuscular transmission, endocytosis, exocytosis, formation of secondary lysosomes, and the release of enveloped viruses.85,86

PLATELET RELEASE

In Wright-Giemsa-stained smears of aspirated marrow, mature megakaryocytes appear as if they were being fragmented into individual platelets. Indeed, this was the appearance that led Wright25,26 to formulate his concept of megakaryocyte–platelet axis. He postulated that cytoplasmic parts of the megakaryocyte projected through the neighboring vessel wall and were pinched off, as platelets, into the circulation. This concept still dominates the thinking of most practitioners of hematology. Moreover, Pisciotta et al.87 studied megakaryocytes by phase-contrast microscopy and reported that the cell membrane ruptured and platelets were extruded into the medium, but this observation has not been corroborated.66 As Crosby15 has pointed out, the appearance may be misleading, and the megakaryocyte in smears of marrow resembles the in vivo megakaryocyte as a fried egg resembles the egg inside the hen. Platelet release should be studied in marrow megakaryocyte and with its anatomical relations intact.

Recent electron microscopic studies using in situ fixed marrow tissue,88 have indicated that megakaryocytes are located in the subendothelial region of vascular sinuses.68,69,88 Statistical analysis of Lichtman et al.89 indicates that this arrangement is not a chance occurrence, and it may be a determinant of platelet release.69,89

In this subendothelial location, the cytoplasm of the megakaryocyte penetrates the endothelium to reach inside the lumen. This penetration is through the endothelium (transendothelial) and not between two endothelial cells (interendothelial), a phenomenon that may have a regulatory function in platelet release.16,69 In this manner, large segments of the cytoplasm enter the lumen. These are known as “proplatelets.” Proplatelets are elongated structures of about 2.5 x 120 µ, and it has been computed that a mature megakaryocyte can produce about 6 proplatelets.16 Considering that a megakaryocyte might produce 8000 platelets,15 a proplatelet might be expected to give rise to some 1200 platelets. It is not known if platelets are immediate progeny of proplatelets or if there are more intermediate steps. The concept of proplatelet may explain the size heterogeneity of circulating platelets, with younger forms being the larger ones.90 It is probably outside the marrow that proplatelets are further fragmented into platelets. This may occur within the general circulation or within the pulmonary circulation where proplatelets could be trapped. The finding that more platelets leave the lung than enter it (see below) support the latter possibility.

Proplatelets were first observed in vitro by Bessis and his colleagues using microcinematography.50,51 They observed projections that progressively became elongated, giving the megakaryocytes an octopus-like appearance. Subsequent cinematographic and electron microscopic studies have confirmed this observation.16,52–55,66,78

EXTRAMEDULLARY DISTRIBUTION OF MEGAKARYOCYTES

Not only the cytoplasmic pieces of megakaryocytes, the proplatelets, but also the whole cell can pass the marrow–blood barrier and move into the circulation. This passage has actually been documented in the elegant studies of Kinosita and Ohno.35 During their study on the living microscopy of the rabbit bone marrow, these authors produced a microcinematographic film. In one sequence, a megakaryocyte is shown attached to the wall of a sinus. Within a few seconds, the cell tumbles into the sinus and off the screen, carried away by the bloodstream. They suggested that if these megakaryocytes do not complete further development in the marrow or within
the circulation, they may be trapped in the pulmonary circulation, completing their development in alveolar capillaries.

Moreover, the presence of megakaryocytes in the circulation has been amply documented, and the contention that these cells may reflect a serious disorder of the marrow has not been validated. It is now evident that megakaryocytes are normal constituents of blood and that they are derived from the marrow. Their reported concentration varies, depending on the technique and on the state of health or disease. It may be said that in healthy adult humans, 10 megakaryocytes/ml of peripheral blood is a reliable figure. Their concentration is increased after surgery and in cancer and inflammatory diseases. In cancer, they can be misinterpreted as circulating cancer cells. Cytochemistry and differential lysis of cancer cells have been helpful differentiating them from circulating cancer cells.

Megakaryocytes are also found in other tissues, including the lung, spleen, kidney, liver, and heart. Here again, their number is increased and their distribution is wider in disease states than in health, and this has been considered a sign of "stress."

**Pulmonary Megakaryocytes**

Megakaryocytes are particularly abundant in the lung and the pulmonary circulation. Aschoff, who first observed intravascular pulmonary megakaryocytes, proposed that they originated from the marrow and were embolized into the lung. Kaufman et al. rearranged the pulmonary vessels in dog so that blood from the right heart perfused first the right lung and then went through the left lung. Many megakaryocytes were seen in the right lung but few in the left lung, suggesting that circulating megakaryocytes are filtered out by the lung. This is in complete agreement with the postulate of Aschoff and of Kinosita and Ohno as described above. It has also been demonstrated that the platelet count is higher in the pulmonary vein than in the pulmonary artery, inevitably suggesting that pulmonary megakaryocytes contribute to the platelet production.

It is now generally accepted that these pulmonary megakaryocytes originate from the marrow, and because of their size, most are trapped in the pulmonary circulation where they release platelets. It has been estimated that 40,000 megakaryocytes are delivered in this manner to the lung every minute. Agreement, however, is lacking on the extent of their contribution to the total platelet production. Values as varied as 7% to almost 100% have been reported. These values are invariably derived at by computations based on either differential platelet counts in pulmonary artery and vein or the estimation of the number of megakaryocytes that may reach the lung and the number of platelets that each can produce. The data base is subject to too wide a variation to permit reliable information. Labeling kinetic studies may help to clarify this point.

**Megakaryocyte–Endothelial Relation**

Localization of megakaryocytes in the subendothelial region of marrow sinuses has been implicated as a determinant of platelet release. The arrangement may be of more functional significance. In this location, megakaryocytes subserve an adventitial function, since adventitial cells are absent where megakaryocytes are present. In this respect, they are similar to marrow fat cells, also located in the subendothelial region. Fat cells, however, are believed to derive from adventitial cells, and megakaryocytes are not.

Moreover, in this location, megakaryocytes project multiple small processes through the endothelium into the lumen. These processes may contain cytoplasmic organelles, similar to those of platelets, and their luminal penetration may be a part of proplatelet formation and platelet release. Often, however, they are remarkably free of organelles and their endothelial penetration may simply serve as a mechanism to "monitor" the circulation and to receive information as to the requirement of body for platelet formation. A similar arrangement has been reported for perisinal macrophages in the rabbit marrow where macrophages penetrate the endothelium, reaching the lumen and phagocytizing red cells. The nature of the regulatory information that may be so received by the megakaryocyte is not clear, although it is believed that the oxygen tension may have of a regulatory function, either directly or through thrombopoietin, on platelet formation and release. Information is scant in these areas that deserve further exploration.

**CONCLUSION**

Megakaryocyte–platelet axis is limited to mammals. In lower species, a nucleated cell—thrombocyte—is endowed with most of the mammalian platelet function and is derived from thromboblast in a manner analogous to submammalian erythropoiesis.
through an elaborate membrane system known as the demarcation membrane system, which originates from the invagination of megakaryocyte cell membrane. The system consists first of tubular structures, but cytoplasms penetrate the marrow sinus wall, projecting and demarcating and forming the cell membrane of that each megakaryocyte can produce 6 proplatelets, enclose pieces of the megakaryocyte cytoplasm, through a developmental process, using fusion-fission the invagination of megakaryocyte cell membrane. The latter enclose pieces of the megakaryocyte cytoplasm, demarcating and forming the cell membrane of nascent platelets.

During the last developmental stages, megakaryocytes penetrate the marrow sinus wall, projecting and releasing elongated structures (2.5 x 120μ) into the lumen. These are known as proplatelets. It is estimated that each megakaryocyte can produce 6 proplatelets, each giving rise to some 1200 platelets. The actual platelet formation from proplatelets probably occurs outside the marrow and within the pulmonary circulation where proplatelets are trapped. Megakaryocytes can also move, in toto, into the circulation from whence they are carried out by the blood stream into the pulmonary circulation, where they are fragmented into platelets. The contribution of these megakaryocytes to the platelet production is uncertain.

The penetration of sinus endothelium by the megakaryocytes may serve proplatelet release but may also serve as an “anchor” to stabilize the cell in this location. This arrangement can also be a means of receiving information as to the requirement of body for platelet formation.

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Megakaryocyte--platelet axis and the process of platelet formation and release

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