Some contributions of electron microscopy to knowledge of human platelets

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The years covered during publication of *Thrombosis and Haemostasis*, now celebrating its 50th anniversary, have witnessed the greatest surge of new knowledge about human platelets in the history of this area or interest. New findings regarding their biochemistry, physiology, structure, function and pathology have all made critical contributions to our current understanding of this cell. Examination of normal and abnormal platelets in the electron microscope has resulted in numerous findings which have provided a context for integrating new knowledge from many other fields into their cellular and sub-cellular structure. The present report will not attempt to review all of the contributions of electron microscopy to the platelet field. Instead it will focus on a few major observations provided by ultrastructural investigations over the past 50 years that have had an impact on our current state of knowledge.

Platelet shape

Improvements in light microscopes and techniques for studying platelets in the late 19th century had permitted identification of the resting, discoid form of these tiny cells. The basis for their characteristic lentiform shape, however, required another century of research. This was due in large measure to a failure to understand the influence of temperature and fixatives for electron microscopy on platelet shape and structure. In the early years of electron microscopy the standard procedure was to chill platelet-rich plasma (PRP) or washed platelets, sediment them to pellets in a cold centrifuge and fix them in chilled osmic acid. Cold dissolved structures important for maintaining platelet discoid shape and osmic acid alone destroyed them. Introduction of aldehyde fixations, particularly glutaraldehyde, in the 1960s, and realization of how important temperature was to preserving platelet lentiform appearance corrected the problem rapidly. Adding an equal volume of low concentration glutaraldehyde to the platelet suspension or PRP at room temperature for 15 minutes before sedimentation preserved platelet discoid shape supported by a circumferential coil of microtubules, and the subsequent steps of sedimentation, additional fixation in chilled glutaraldehyde and osmic acid did not destroy the coiled structure (Figs. 1, 2). Thus the major basis for the characteristic, discus-like form of resting platelets was recognized at last.

Platelet organelles

The improved light microscopes of the late 19th century were able to recognize organelles in the cytoplasm of platelets viewed on slides, but different types could not be distinguished. Even the markedly improved optics of light microscopes in the first half of the 20th century were unable to sort out the organelles as to their nature and structural differences. As a result, the organelles were treated collectively as the platelet granulomere.

Early years of platelet study in the electron microscope were not very helpful. Not until the improved methods for preparing platelets for ultra-structural study mentioned above and the application of cytochemical and ultrastructural immunocytochemical methods was it realized that platelets contained several different types of organelles involved in their physiology and pathology.

Alpha granules

Thin section of platelets prepared by better methods revealed a plethora of organelles including mitochondria, dense bodies and granules (Figs. 1, 2). At least two types of granules were present that could be distinguished by cytochemistry. Those containing acid hydrolases, including acid phosphatases and aryl sulfatase, were lysosomes. The great majority of granules contained a large variety of proteins synthesized by the megakaryocytes or taken up from plasma that could be distinguished immunocytochemically, such as fibrinogen or von Willebrand factor (vWF). These were the alpha-granules. Substructural organization of their contained proteins was suggested by the presence of a nucleoid more dense than the surrounding granule matrix, and the localization of vWF in the peripheral zone in the form of small tubules. However, human platelet alpha-granules appear significantly less

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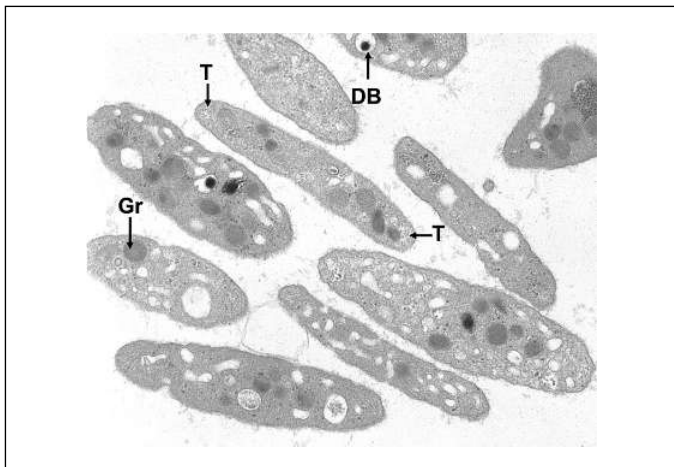E-mail: white003@umn.edu

Figure 1: Thin section of discoid human platelets. The improved methods of fixation have preserved the circumferential coil of micro-tubules (T) responsible for maintaining the lentiform appearance. The methods also maintain the random distribution of alpha granules (Gr) and protect platelet dense bodies (DB). Magnification X 17,000.
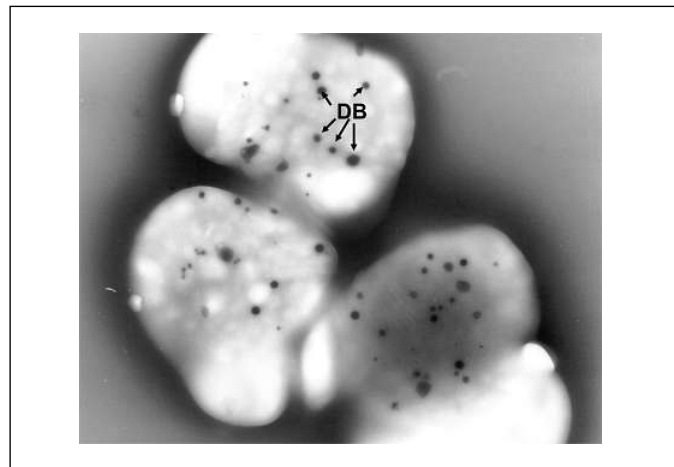

Figure 3: Whole mount preparation of normal human platelets. The cells were not fixed or stained, just placed on the surface of a plastic-coated grid and dried. Many inherently electron opaque dense bodies are evident in each platelet. Magnification X 8,000.
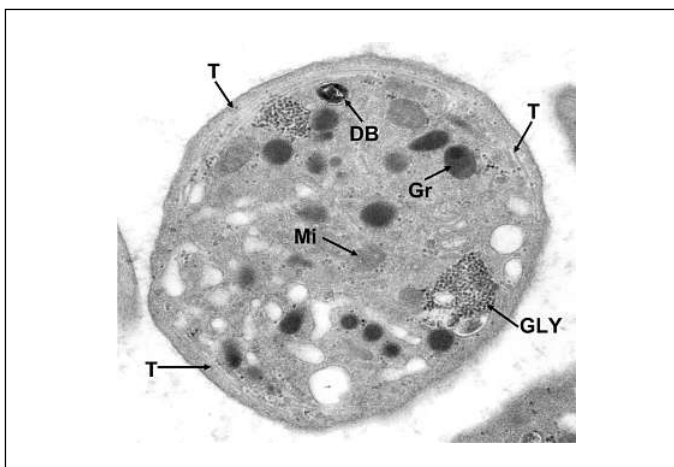

Figure 2: Discoid platelet sectioned in the equatorial plane. The discoid form is maintained by the circumferential coil of microtubules (T) lying just under the surface membrane. Many alpha granules (Gr), a few mitochondria (Mi) and occasional dense bodies (DB) are randomly dispersed in the cytoplasm. Particles and clumps of glycogen (Gly) are also preserved. Magnification X 27,000.
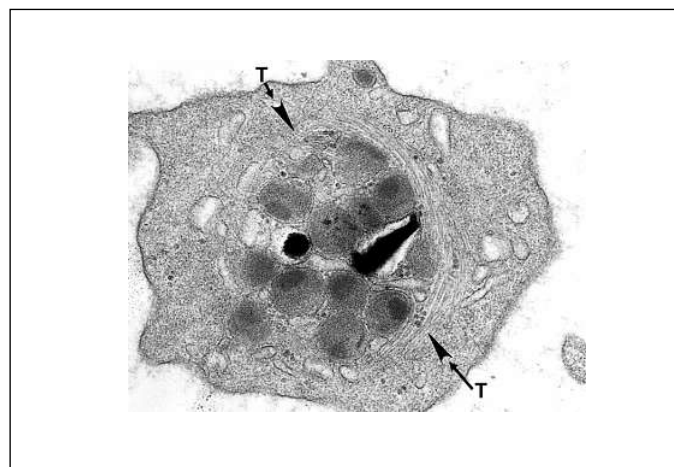

Figure 4: Thin section of a human platelet fixed after exposure to thrombin. The cell has lost its discoid form and developed lamella-podia. Organelles have been transported to the cell center where they are enclosed within a ring of microtubules (T) and microfilaments. Magnification X 50,000.

well organized than those present in bovine and equine platelets, and may vary from one another as to the nature of their contents.

Dense bodies

The earlier methods for preserving platelets for electron micro-scopy that destroyed the circumferential coil of microtubules also eliminated organelles rich in adenine nucleotides, serotonin and calcium. After better techniques were introduced dense bodies, also referred to as delta-granules, were visible, but appeared to be quite rare. This problem was overcome by replacing the usual buffers for glutaraldehyde and osmic acid with buffers containing higher concentrations of calcium. Then, instead of one dense body in a thousand platelet thin sections, as had been

reported in early publications, there were 1 to 1.5 dense bodies in each platelet section (Figs. 1, 2).

The dense appearance of delta-granules was considered by some workers to be due to osmophilia, but another ultrastructural technique showed this was not the case. Placing a small drop of PRP on a plastic-coated, 300-mesh grid, quickly washing with a few drops of distilled water, then drying with wedges of filter paper left many discoid platelets adherent to the surface. Fixatives and staining were not necessary. After drying, the grid could be inserted into the electron microscope. Platelets, due to their lipid membranes, were transparent to the electron beam, while delta-granules were inherently electron opaque (Fig. 3). They could be counted with ease and the number per 100 cells
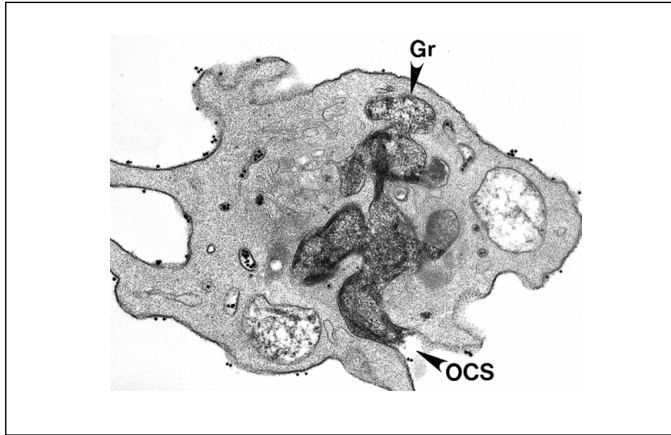
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Figure 5: Human platelet fixed after exposure to thrombin and stained with tannic acid. The reaction product of tannic acid and osmic acid, osmium black, stains fibrinogen/fibrin in alpha granules (Gr) which have fused with each other and with channels of the open canalicular system (OCS). This is the process of platelet secretion. Magnification X 37, 000.
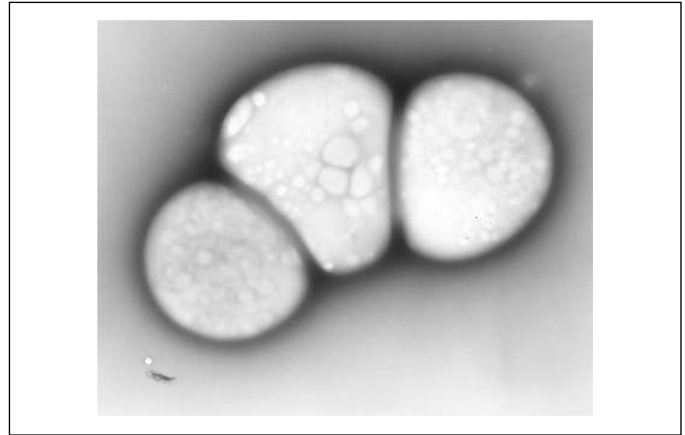

Figure 6: Whole mount preparation of platelets from a patient with the Hermansky-Pudlak syndrome (HPS). Dense bodies are absent from patient platelets. (See Fig. 3). Together with albinism the absence of platelet dense bodies is diagnostic of the HPS. Magnification X 10,000.

determined. Other cytoplasmic structures, including electron dense chains, clusters and "fuzzy balls" were also present, but easily distinguished from the sharply rounded delta-granules. Many delta-granules had long, whip-like tails, some more than one, extending for long distances in the cytoplasm. Their origin remains obscure, but the tails are a further aid to identify dense bodies.

Platelet physiology

The electron microscope has played an important role in establishing the nature of platelets as muscle cells. Actin, myosin and other proteins involved in contractile function are the major constituents of resting platelet cytoplasm, and their assembly following activation drive the processes of shape change, pseudopod extension and internal transformation. Platelets contain a calcium binding reservoir, the dense tubular system, very similar to the sarcoplasmic reticulum of embryonic muscle cells. Even when external calcium ions are bound by chelating agents, the calcium ions released from the dense tubular system following activation are sufficient to trigger the process of internal contraction and secretion. The events of platelet internal contraction and involvement in clot retraction have been beautifully demonstrated by electron microscopy (Fig. 4).

Electron microscopy showed that, following exposure of platelets to activating agents, alpha-granules fused with channels of the surface connected open canalicular system (OCS) and extruded their contents to plasma or damaged vascular surfaces without simultaneous loss of cytoplasmic chemical substances (Fig. 5). Recognition of these events was made possible by advanced techniques of electron microscopy.

Platelet pathology

The electron microscope has played a significant role in defining the pathology of human platelets.

Hermansky-Pudlak syndrome and storage pool deficiency disorders

The Hermansky-Pudlak Syndrome (HPS), consisting of oculocutaneous albinism and abnormal haemostasis due to a platelet abnormality, was recognized long before the structural basis for the platelet defect was defined. Improvements in techniques for preserving platelets for study in the electron microscope described above quickly corrected that gap in our knowledge. Dense bodies readily visible in whole mounts of normal platelets and properly prepared thin sections were shown to be rich in adenine nucleotides, serotonin and calcium. These chemical substances were deficient in HPS platelets. It was not surprising, therefore, that HPS platelets were found to be devoid of dense bodies (Fig. 6). Thus electron microscopy has become the gold standard for the diagnosis of HPS, and helpful for recognition of the storage pool disorders in patients whose platelets have decreased numbers of dense bodies and/or alpha-granules.

Giant platelet disorders

Light microscopy recognized giant platelet syndromes and the paucity of organelles in some of them, but could not identify the nature of missing organelles it could not resolve.

Gray platelet syndrome

A classis example is the gray platelet syndrome (GPS). The virtual absence of organelles in gray platelets was visible in the light microscope, but it took the electron microscope to demonstrate alpha granules were missing. Further electron microscopy demonstrated the organelles could form in patient megakaryocytes, but failed to retain products concentrated within them (Fig. 7). In time the defect in structure-linked latency responsible for the GPS will be defined by ultrastructural and biochemical methods.

White platelet syndrome

The white platelet syndrome (WPS) is another classis example of a giant hypogranular disorder that required electron microscopy
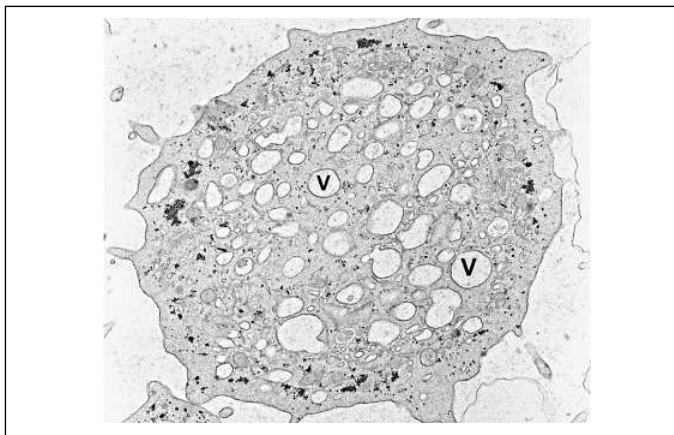
Figure 7: Thin section of a giant platelet from a patient with the gray platelet syndrome (GPS). Alpha-granules form in mega-karyocytes of GPS patients, but are unable to retain their contents. As a result, most of their platelets in circulating blood are devoid of alpha granules. Only their enclosing membranes remain as vacuoles (V). Magnification X 21,000.
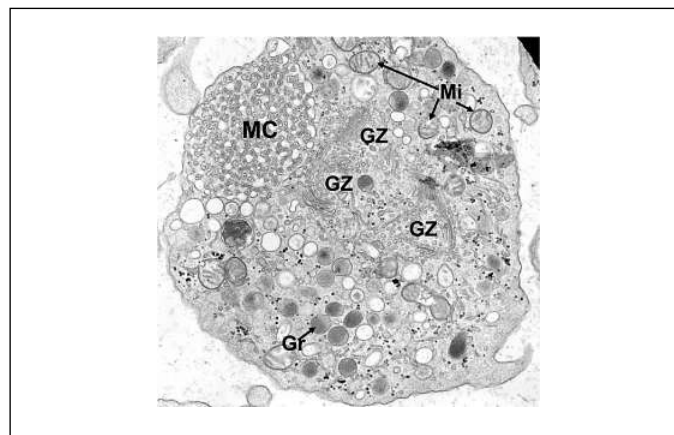

Figure 8: Thin section of a giant platelet from patient with the white platelet syndrome (WPS). The cells are hypogranular and may contain more mitochondria (Mi) often larger than the granules (Gr). A striking feature of WPS syndrome is the presence of large, fully functional appearing Golgi zones (GZ), at least three of which are present in this cell. The membrane complex (MC) made up of elements from the open canalicular system and dense tubular system of channels is a normal constituent. Magnification X 21,000.

for its definition. WPS platelets were clearly different from GPS platelets. Only two-thirds were hypogranular or agranular. Others contained a normal or near normal complement of alpha-granules. The most striking feature not visible in the light microscope was the presence of large, fully active Golgi complexes, as many as seven in a single platelet, in many of their circulating cells (Fig. 8). WPS platelets fail to complete making alpha-granules before leaving the megakaryocyte, and bring the equipment for finishing the job in circulating cells.