# The Organization of Microtubules and Microtubule Coils in Giant Platelet Disorders

JAMES G. WHITE, MD, and JOHN J. SAUK, DDS

*From the Departments of Pediatrics, Laboratory Medicine, and Pathology, and Oral Genetics, School of Dentistry, University of Minnesota Health Sciences Center, Minneapolis, Minnesota*

**Normal human platelets are characteristically discoid in shape. The lentiform appearance is supported by a circumferential band of microtubules lying just under the cell membrane along its greatest circumference. Some of the cells from patients with giant platelet disorders are also disk-shaped, but the majority of their huge platelets are spherical. In the present study platelets from patients with the Gray platelet syndrome (GPS), May-Hegglin anomaly (MHA), and Epstein's syndrome (ES) were examined in thin sections and negatively stained whole mounts, and by indirect immunofluorescence with a monoclonal antibody to tubulin for determination of the organization of their microtubule systems. Many GPS platelets and some ES and MHA platelets were discoid and contained circumferential bundles of microtubules. The number of coils in the band was increased 10-20-fold. Giant spherical platelets also contained increased numbers of individual microtubules and coils, but they were not organized into circumferential bundles. Immunofluorescence revealed an organization of assembled tubulin in the huge cells, suggestive of balls of yarn. Failure of the microtubules to organize into a circumferential band may explain why the majority of the huge cells have a spherical form. (Am J Pathol 1984, 116:514-522)**

PLATELETS in circulating blood and in well-prepared samples of platelet-rich plasma have a characteristic discoid form.[1] A circumferential microtubule coiled on itself several times supports the lentiform appearance of the cells.[2-4] Large platelets from normal individuals and patients with idiopathic thrombocytopenic purpura are also discoid, and their form is supported by a circumferential bundle of tubules.

In many giant platelet disorders, however, this is not the case. Some of the platelets in such conditions as the May-Hegglin anomaly (MHA)[5,6] and Epstein's syndrome (ES)[7] are disk-shaped, but the majority of the huge cells are spherical in form. On the other hand, platelets from patients with the Gray platelet syndrome (GPS)[8] are often discoid, despite their increased size, although a substantial proportion are sphere-shaped.[9]

In the present study we have used electron-microscopic examination of thin sections and negatively stained whole mounts, together with indirect immunofluorescence, to evaluate the distribution of microtubules in normal platelets and giant cells from patients with MHA, ES, and the GPS. Normal platelets generally contained a single microtubule wound into a variable number of coils in one plane.[10,11] Some of the giant platelets, particularly from patients with GPS, also contained circumferential bundles in a single plane with a marked increase in the number of coils. Huge MHA and ES platelets contained increased numbers of single tubules and multiple coils in many planes, yielding an appearance resembling balls of yarn. The disorganization of the microtubule system may be a principal factor responsible for the spherical form of giant platelets in MHA, ES, and the GPS.

## Materials and Methods

### Patients

Most of the patients with giant platelet disorders available for study in our laboratory have been char-

Supported by USPHS Grants HL-11880, HL-16833, CA-21737, GM-22167, AM-17697 and a grant from the March of Dimes.

Accepted for publication April 27, 1984.

Address reprint requests to James G. White, MD, Department of Pediatrics, Box 490, Mayo Memorial Building, Minneapolis, MN 55455.

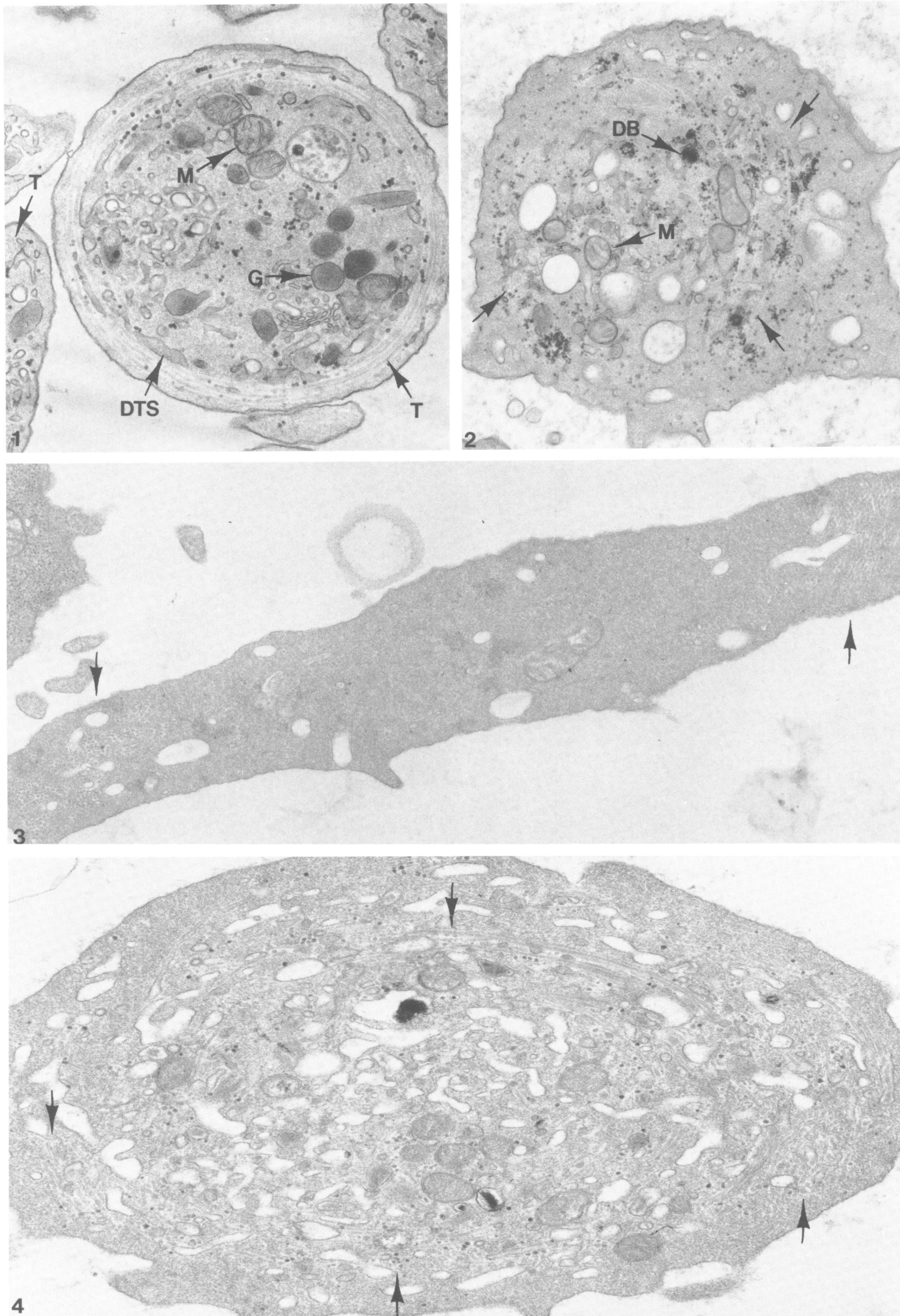
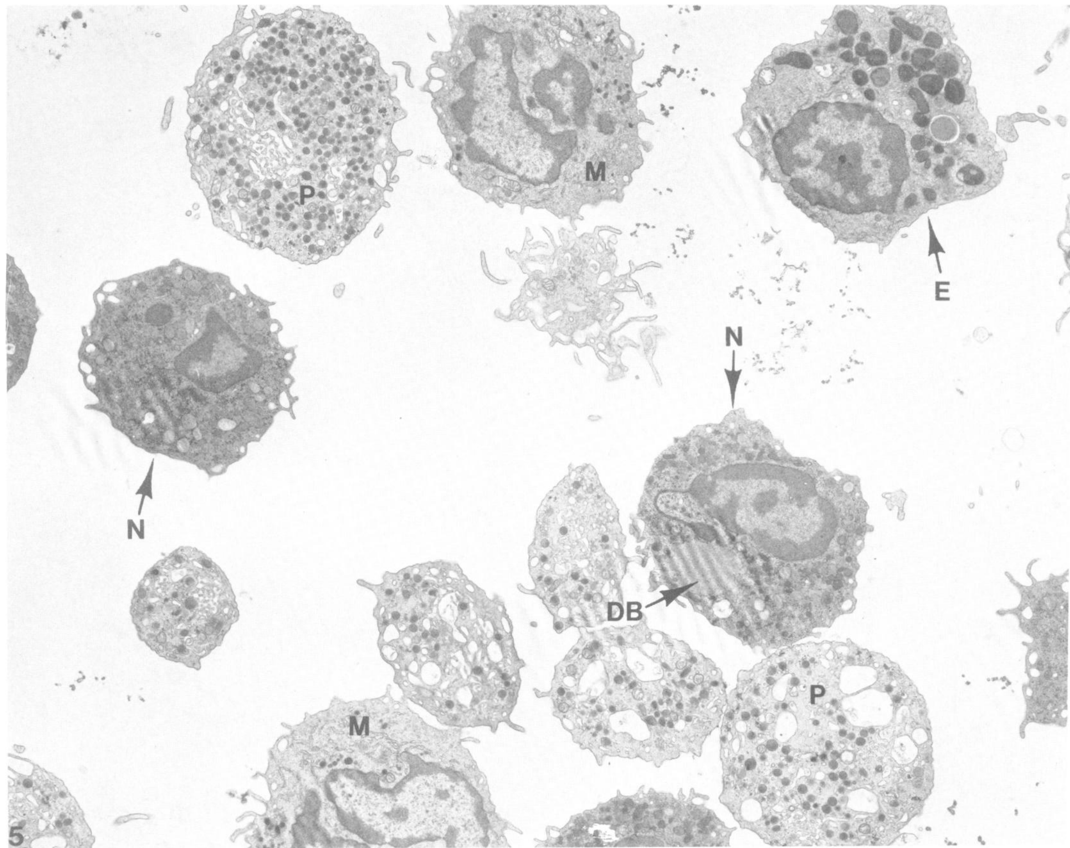
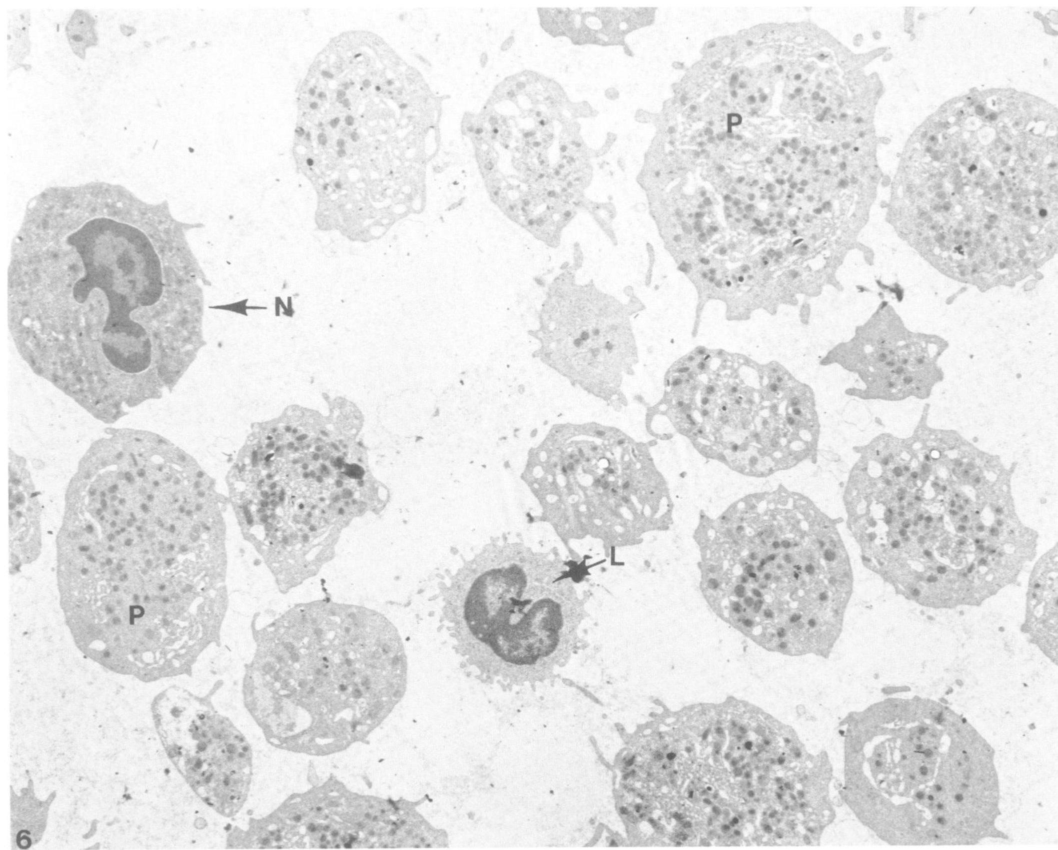



**Figure 1** – Circumferential bundles of microtubles in normal human platelets. Cells in this illustration are from a sample of C-PRP fixed in glutaraldehyde and osmic acid containing potassium ferricyanide. The central platelet containing randomly dispersed granules (*G*), mitochondria (*M*) and other organelles has been sectioned in the equatorial plane. A circumferential band of microtubules (*T*) consisting of 4–5 coils supports the discoid form of the cell. An adjacent platelet cut in cross-section reveals 6–8 hollow-cored microtubule profiles (↑). (× 22,000) **Figure 2** – Gray platelet syndrome. The normal-sized platelet from a child with GPS contains normal numbers of mitochondria (*M*) and a dense body (DB), but is nearly devoid of granules. Groups of microtubules (↑) are spread unevenly in the cytoplasm and are not organized into a circumferential bundle. (× 17,000) **Figure 3** – Discoid GPS platelet. The giant cell cut in cross-section is supported by a massive circumferential bundle of microtubules (↑). Over 100 circular profiles are evident at each pole of the cell. (× 15,500) **Figure 4** – Giant GPS platelet. The cell is relatively discoid in shape and contains large numbers of microtubules (↑). The tubules are organized into bundles near the surface and deeper in the cytoplasm. Some of the tubules are cut in cross-section and project perpendicular to, as well as in, the plane of section. (× 19,000)


P
M
E
N
N
DB
P
M
5


6
P
N
P
L

acterized previously.[9,12] For the present investigation we used 1 of our 2 patients with the GPS, 2 patients with MHA, and 3 individuals from 2 unrelated families with ES. GPS platelets are big, but not as large as those observed in patients with MHA or ES. They are characterized by a virtual absence of $\alpha$ granules and their constituent proteins.[13] MHA consists of giant platelets and Dohle bodies in circulating granulocytes. Ultrastructural features of MHA platelets are essentially normal, but most of the huge cells have a spherical configuration.[14-16] ES is characterized by congenital deafness, hereditary nephritis, and giant platelets.[7] The huge platelets in this disorder appear virtually identical to those in individuals with MHA.

**General**

Blood was obtained from healthy adult donors and patients with various giant platelet disorders after informed consent. The samples were mixed immediately with citrate citric acid, pH 6.5 (93 mM sodium citrate, 70 mM citric acid, and 140 mM dextrose) in a ratio of 9 parts blood to 1 part anticoagulant. Platelet-rich plasma (C-PRP) was separated from whole blood by centrifugation at 100*g* for 20 minutes at 23 C.

**Ultrastructural Studies**

*Thin Sections*

Samples of C-PRP were combined with equal volumes of 0.1% glutaraldehyde in White's saline, pH 7.3 (a 10% solution of a 1:1 mixture of 1) 2.4 M NaCl, 0.1 M KCl, 46 mM $MgSO_4$, 64 mM $Ca[NO_3] \cdot 24$ $H_2O$, and 2) 0.13 M Na $HCO_3$, 8.4 mM $NaHPO_4 \cdot 7H_2O$, 3.8 mM anhydrous $KH_2PO_4$, and 0.1 g/l phenol red).[17] After 15 minutes at 37 C the samples were sedimented to pellets, and the supernatant was discarded and replaced with 3% glutaraldehyde in the same buffer. Fixation was continued at 4 C for 60 minutes. The cells were then washed in buffer and combined with 1% osmic acid in veronal acetate (0.02 NHCl and a 20% solution of a stock buffer containing 0.14 M sodium barbital, 0.145 M sodium acetate $\cdot$ $3H_2O$ and a 6.8% solution of a stock salt buffer containing 1.7 M NaCl, 54 mM KCl, and 18 mM $CaCl_2$). After exposure to the second fixative for 1 hour at 4 C, the cells were dehydrated in a graded series of alcohol and embedded in Epon 812. Contrast of thin sections cut from plastic blocks on an ultramicrotome was enhanced with uranyl acetate and lead citrate. Observations were made in a Philips 301 electron microscope.

*Negative Stain Whole Mounts*

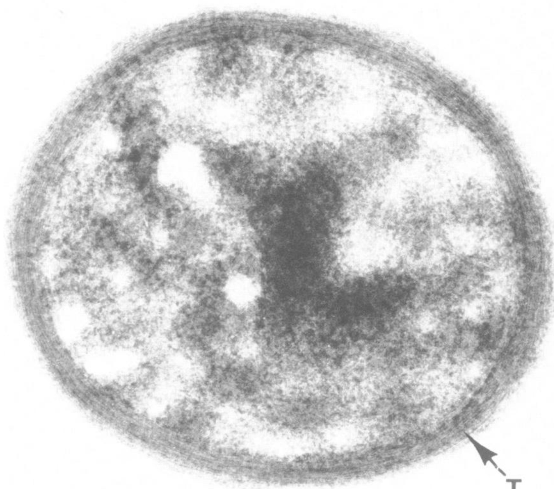
Samples of control and giant platelets were prepared for ultrastructural study after simultaneous fixation and detergent extraction followed by negative staining according to the method of Small,[18] as modified from Hoglund et al.[19] The triton X-100 glutaraldehyde mixture consisted of 0.5% triton X-100 and 0.25% glutaraldehyde. Detergent extraction and fixation were carried out at room temperature. Grids carrying spread platelets were washed briefly in Tris-buffered saline followed by a cytoskeleton buffer (NaCl, 127 mM; KCl, 5 mM; $Na_2HPO_4$, 1.1 mM; $KH_2PO_4$, 0.5 mM; $NaHCO_3$, 4 mM; glucose, 5.5 mM; $MgCl_2$, 2 mM; EGTA, 2 mM; PIPES, 5 mM, pH 6.0–6.1). Following washing in the cytoskeletal buffer, the cells were transferred to the triton X-100 glutaraldehyde mixture for 1 minute. After a brief wash in cytoskeleton buffer, the grids were stored on coverslips on the same buffer containing 2.5 glutaraldehyde for 2 hours before negative staining for electron microscopy. Staining in sodium silicotungstate was carried out at room temperature. Grids were rinsed two times in distilled water and transferred sequentially through 4 drops of bacitracin (40 mg/ml of water, Sigma Chemical Co., St. Louis, Mo) in a plastic Petri dish and drained briefly on the edge with filter paper. They were then passed through 4 drops of 3% sodium silicotungstate and then finally drained of excess stain and allowed to air dry.

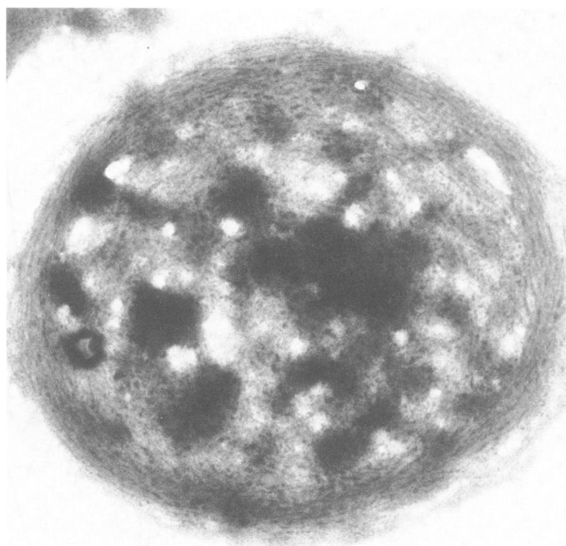
**Indirect Immunofluorescence[20]**

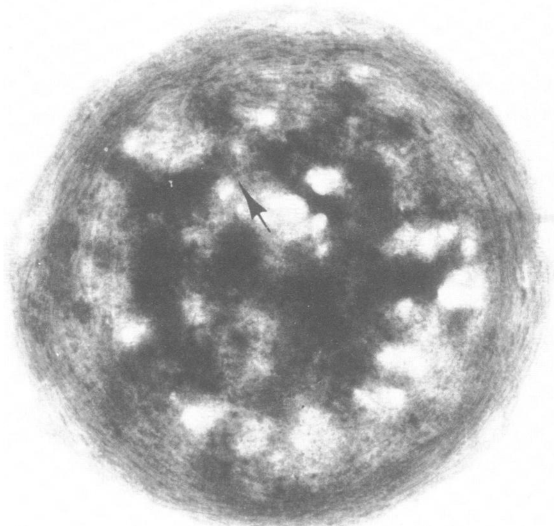
Samples of normal and giant C-PRP were mixed with an equal volume of the citrate anticoagulant and centrifuged to pellets. The platelets were resuspended in phosphate-buffered saline (PBS) (NaCl, 8 g/l; KClO, 2 g/l; $Na_2HPO_4$, 1.15 g/l adjusted to pH 7.2 with 1.0 M NaOH). Twenty microliters of washed platelets were placed on each of a number of polylysine-coated glass slides, which were inserted in a moist chamber to settle at room temperature. At intervals of 1, 5, 15 and 30 minutes, slides were fixed in methanol at −10 C for 10 minues and then immersed briefly (3 times for 3 minutes each) in acetone at −10

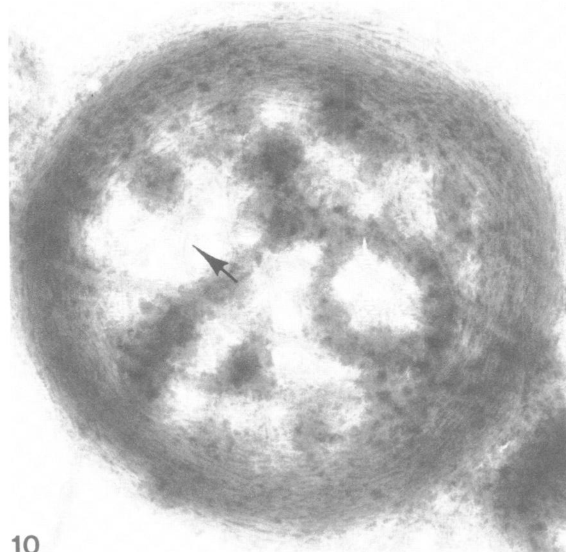
←

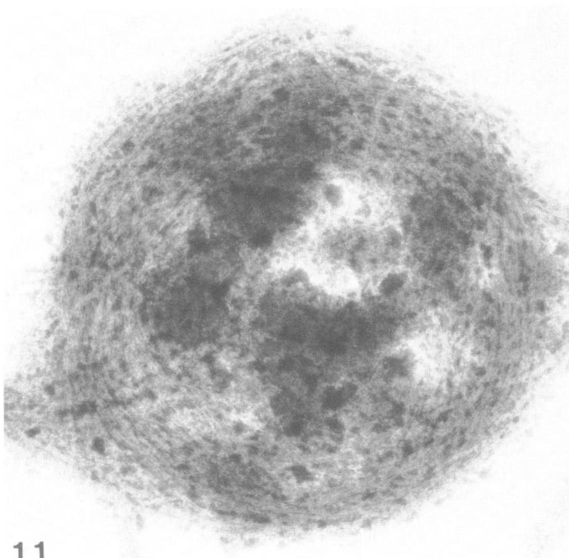
**Figure 5**—May-Hegglin anomaly. Two of the giant platelets (*P*) in this thin section appear similar in size to, if not larger than, the neutrophils (*N*), monocytes (*M*), and the eosinophil (*E*). A Dohle body (*DB*), characteristic of the MHA, is evident in one of the granulocytes. (×5000) **Figure 6**—Epstein's syndrome. Giant platelets in this disorder are similar to the huge cells from patients with MHA. Some of the platelets (*P*) are larger than lymphocytes (*L*) and neutrophils (*N*) in the same section. (×5000)

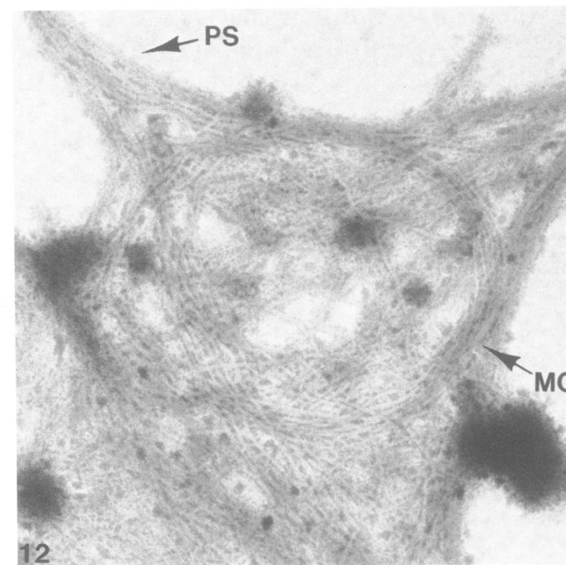

T
7


8


9


10


11


PS
MC
12

C. A stock monoclonal antibody to $\alpha$ tubulin (Amersham) was diluted to a concentration of 0.1 mg/ml in PBS with 1% bovine serum albumin (BSA). Slides were washed once with PBS and the platelet spots covered with 20–40 $\mu$l of the anti-tubulin antibody. After 30 minutes the slides were washed with PBS, and the spots were covered with a fluorescein-conjugated anti-immunoglobin antibody (Cappel). After incubation for another 30 minutes, the slides were washed well with PBS and mounted under coverslips in a solution of *p*-phenylenediamine–PBS–glycerin after the method of Johnson et al.[21] The slides were studied under phase contrast and phase fluorescence in a Zeiss photomicroscope equipped with an ultraviolet power source and appropriate excitation and barrier filters. Platelets were observed and photographed through epifluorescence optics and ×60 and ×100 planapo objectives.

## Results

### Thin Sections

Previous reports on the fine structure of platelets have described the circumferential bundle of microtubules and its relationship to discoid platelets.[2–4] Behnke and co-workers[10] demonstrated that the bundle was probably a single microtubule coiled on itself several times, and this work was confirmed by Nachmias.[11] In thin sections the microtubule consists of several coils lying just under the cell surface in platelets cut in the equatorial plane (Figure 1). In cross-section the bundle appears as a group of closely associated circular profiles at each pole of the cell. The number of profiles in normal sized platelets varies from 3 to 12 and may be as high as 18 in larger cells.

Platelets from our patient with GPS are larger than normal cells (Figure 2) and many approach in size those from patients with MHA or ES. Circumferential bundles of microtubules are apparent in some of the large discoid cells and consist of 20 to 100 coils (Figures 3 and 4). The largest GPS platelets, however, are spherical, rather than discoid, and lack the organized bundle of microtubules lying in a single plane.

MHA and ES platelets are, in general, very large, although some approach the size of normal cells (Figures 5 and 6). The near normal-sized MHA and ES platelets are discoid and contain circumferential bundles of microtubules. Most of the giant platelets, however, are spherical. Many microtubules are evident in these cells but do not appear to be organized in a single plane.

### Whole Mounts

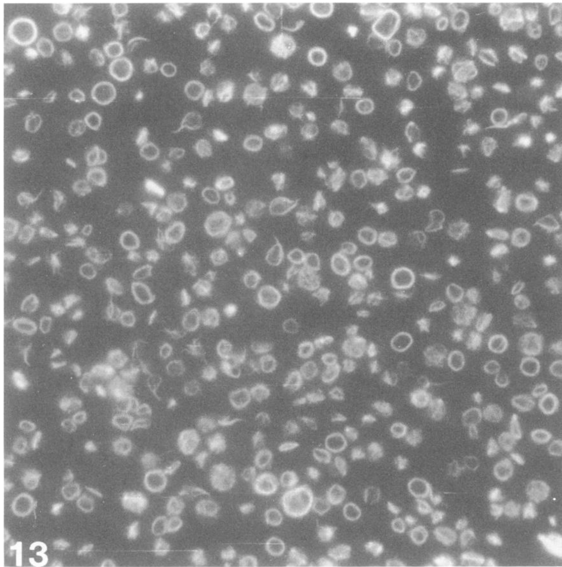
Many normal platelets were discoid in form 30 minutes after drops of C-PRP were placed on carbon-stabilized, formvar-coated grids (Figure 7). Negative staining of the cells after simultaneous glutaraldehyde fixation and exposure to triton X-100 revealed a coil of microtubules at the periphery of the cell. The exact number of coils in each microtubule bundle was difficult to determine, but appeared to range from 6 to 14. Most of the platelets exposed to surface activation for 30 minutes had undergone transformation to dendritic or spread forms. The microtubule coils in spead platelets were often constricted into tight rings.[22]

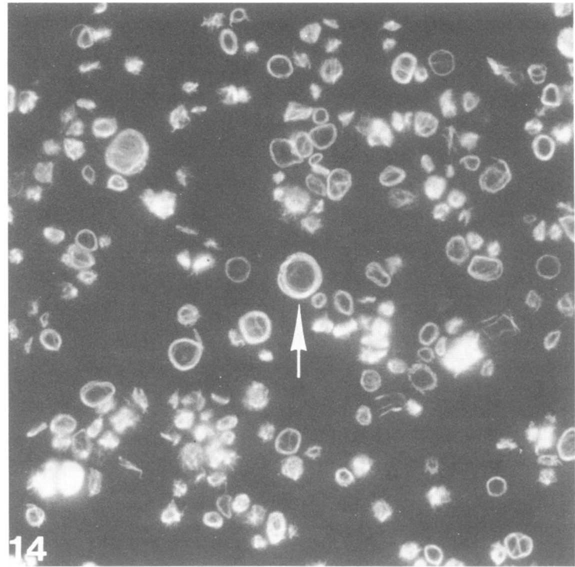
Except for their increased size, GPS platelets appeared similar to normal cells in whole mount preparations (Figure 8). However, the number of coils in circumferential bundles of microtubules was increased in larger discoid platelets. The increase in the number of microtubules in marginal bands and elsewhere in the cytoplasm was more striking in giant platelets from patients with MHA and ES (Figures 9–12). Huge discoid platelets in whole mounts for MHA and ES cells contained 40 to 50 coils in the circumferential bundle of microtubules. In activated platelets microtubules were prominent throughout the cytoplasm as well as in coils.

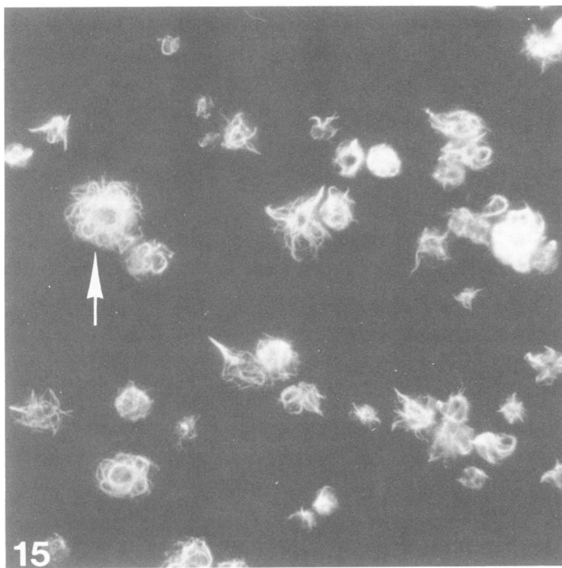
### Indirect Immunofluorescence

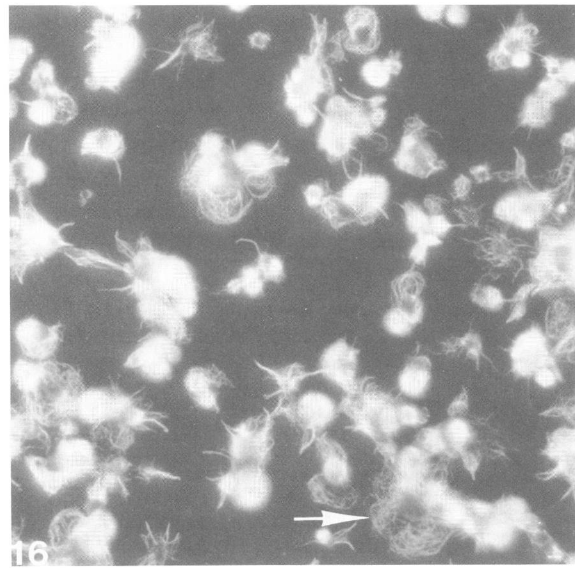
Examination of normal platelets stained for tubulin in the fluorescence microscope revealed bright rings of microtubules in discoid, dendritic, and spread cells (Figure 13). GPS platelets revealed similar fluorescent rings (Figures 14 and 15). However, they were often brighter, and many of the large platelets contained double rings of microtubule coils. A few of the smaller MHA and ES platelets contained bright rings of fluorescence in a single plane. The vast majority of huge cells, however, contained a marked increase in cytoplasmic microtubules and bright rings

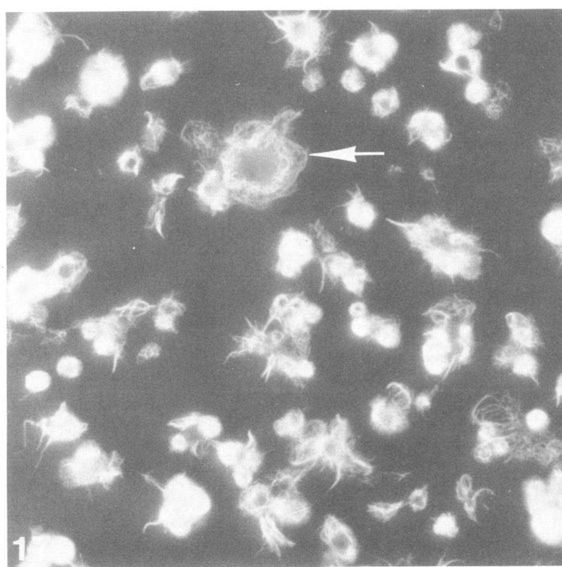
**Figure 7**—Normal human platelet prepared by the whole mount technique. The cell was fixed in glutaraldehyde and simultaneously treated with Triton X-100 before negative staining. A circumferential bundle of microtubules (↑) consisting of 6 coils is evident at the periphery of the detergent-resistant cytoskeleton. (×22,000) **Figure 8**—Detergent-resistant cytoskeleton of a platelet from the patient with GPS. Although not huge, the GPS platelet contains many more coils in its circumferential bundle of microtubules than the normal platelet in the previous illustration. (×20,000) **Figures 9 and 10**—Relatively normal-sized platelets from two patients with ES. The circumferential bundles of microtubules in these cells contain large numbers of individual coils. Individual microtubules (↑) are also evident in the cytoplasm. (**Figure 9**, ×16,500; **Figure 10**, ×16,500) **Figures 11 and 12**—Platelets from a patient with MHA. The cell in **Figure 11** is partially activated, and its cytoplasm is filled with microtubules. Another cell in **Figure 12** contains a concentric microtubule coil (*MC*) in the cell center and myriads of microtubules filling the cytoplasm and extending into pseudopods (*Ps*). (**Figure 11**, ×24,000; **Figure 12**, ×19,000)

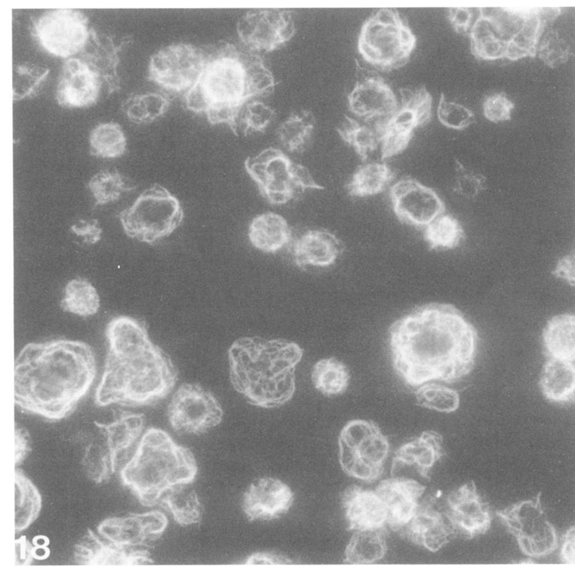

13


14


15


16




18

interwoven into the appearance of balls of yarn (Figures 16–18).

## Discussion

The present investigation has demonstrated that most of the giant platelets in patients with MHA and ES and many from individuals with the GPS are spherical, rather than discoid, and contain increased numbers of microtubules and microtubule coils arranged in a manner not observed in normal cells. Microtubules were first observed in platelets following introduction of the combined use of glutaraldehyde and osmic acid and fixation at room temperature, or 37 C.[2–4] The relationship between the circumferential bundle of microtubules and platelet discoid shape was recognized in the early reports, and experiments with chilling[23] and antimitotic agents[24] established that the marginal band served as a cytoskeletal support system to maintain the lentiform appearance. A study by Behnke and Zelander[10] employing the negative stain whole mount technique demonstrated that the circumferential bundle was in reality a single microtubule coiled on itself several times, and their observation was confirmed by Nachmias.[11] Behnke's work also demonstrated that platelets did not contain a circumferential bundle during development in the parent megakaryocyte, but formed it after release into the circulation.[25] The mechanism by which the precursor protein, tubulin, assembled into one microtubule wound into many closely associated coils in a single plane was not determined. It is also unknown how giant platelets develop in patients with ES, GPS, and MHA, because their megakaryocytes appear to be normal-sized.[7,8,15] Regardless of the mechanism, it is probable that the huge cytoplasmic fragments are released from the parent cell with incompletely assembled tubulin, just like normal platelets.[25] However, giant platelets do not develop the characteristic discoid form of normal cells. Instead the majority of the huge cells display a relatively spherical shape.

Findings of the present study indicate that the failure of most huge platelets to develop a discoid shape after release from the megakaryocyte is not due to a deficiency in assembled microtubules. Thin sections revealed large numbers of single microtubules in the cytoplasm of giant cells and numerous small bundles and coils near the surface or deep in the cytoplasm. Rare giant platelets were discoid and contained circumferential bundles consisting of massive numbers of microtubules.

Whole mount preparation supported the observations made on thin sections. Giant discoid and activated platelets contained large numbers of microtubules in their cytoplasm or organized into circumferential bundles. The platelets were 3–5 times normal size but often appeared to contain 10–20 times the number of microtubules found in control cells.

Evaluation of microtubules in the giant platelets by indirect immunofluorescence with a monoclonal antibody to $\alpha$ tubulin supported the observations made in thin sections and whole mounts of the giant cells. Microtubules were organized in single bright fluorescent rings in most of the control platelets.[20] Similar bright rings were evident in many of the smaller GPS platelets and some of the MHA and ES cells. The majority of giant platelets, however, contained many single tubules and multiple coils organized in a random fashion. The resulting appearance suggested a ball of yarn, rather than an organized bundle of microtubules, lying in a single plane. These findings suggest a relationship between the large mass of assembled, but unorganized microtubule polymer in giant platelets and persistence of their spherical form.

The reason microtubules present inside the huge spherical platelets seldom organized into a single circumferential band is unknown. It is possible that some factor necessary for organization of a single microtubule into closely associated coils is missing from the huge cells. Since some of the massive platelets do become discoid, however, this possibility is unlikely. More reasonable is the possibility that microtubules form from multiple foci, rather than a single center in giant platelets. This would account for the many single tubules, coils, and bundles observed in the large cells organized at random. Failure of the many microtubules to develop into a single bundle in one plane would foster the spherical shape observed in most giant platelets in this study.

The nature of the organizing center for the coiled circumferential microtubule in blood platelets is unknown. In many cells and in megakaryocytes centrioles serve as major centers for elaboration of microtubules.[25–27] Centrioles, however, are rare in platelets[28] and cannot serve an important function in

**Figure 13**—Indirect immunofluorescence of normal platelets stained with a monoclonal antibody to tubulin. Most of the cells contain bright rings indicative of circumferential bands of microtubules. (×300) **Figures 14 and 15**—Immunofluorescent localization of tubulin in platelets from the patient with GPS. Small GPS platelets are similar to normal cells with bright, single rings. Some of the large cells contain double rings (↑) lying in the same plane. The platelets in **Figure 15** are more activated than in **Figure 14** and contain multiple coils of microtubules (↑). (**Figure 14**, ×300; **Figure 15**, ×300) **Figures 16 and 17**—Localization of tubulin in ES platelets by indirect immunofluorescence. The intensity of fluorescence makes it difficult to see tubules and coils in many large cells, but multiple coils (↑) are evident in some. (×300) **Figure 18**—Platelets from a patient with MHA. Most of the giant platelets have large numbers of tubules and multiple coils. They resemble balls of yarn in the fluorescence microscope. (×300)

microtubule assembly after platelets leave the parent megakaryocyte. The only structure found in close association with the circumferential microtubule in normal platelets is a channel of the dense tubular system.[4] However, elements of this channel system are present throughout the platelet cytoplasm, and it is unlikely that their calcium-sequestering influence would favor assembly of the tubule at the platelet periphery, rather than the interior. Until the microtubule organizing center is recognized in normal platelets, it will not be possible to state with certainty that multiple centers are present in the giant cells.

Leven and Nachmias[29] have provided evidence based on indirect immunofluorescence that microtubule coils may form in platelets before their separation from the megakaryocyte. On the basis of this observation, it is possible that organization of the microtubule may be a factor in establishing the size of putative platelets in the parent cell. Because giant platelets have increased numbers of microtubules, it is possible that failure of their organization into coils plays a role in the formation of platelets of giant size.

In conclusion, the present study has examined the state of microtubules in giant platelet disorders. A few near normal-sized GPS, MHA, and ES platelets and rare giant cells have a discoid form supported by a circumferential bundle of microtubules. Most of the huge cells in patients with these disorders, however, are spherical, rather than discoid. Increased numbers of single microtubules and microtubule coils are present in the giant platelets, but they are not organized into circumferential bundles. Failure of the microtubules to organize into a circumferential band may play a role in the development of giant platelets and explain why the huge cells have a spherical configuration in circulating blood.

## References


1. Eberth JC, Schimmulbusch C: Experimentelle untersuchungen uber thrombose. Virchows Arch [Pathol Anat] 1886, 103:38-87
2. Haydon GB, Taylor DA: Microtubules in hamster platelets. J Cell Biol 1965, 26:673-675
3. Bessis M, Breton-Gorius J: Les microtubules et les fibrilles dans les plaquettes etalées. Nouv Rev Fr Hematol 1965, 5:657-662
4. Behnke O: Microtubules in disc shaped blood cells. Int Rev Exp Pathol 1970, 9:1-92
5. May R: Leukocyteneinschulusse. Deutsch Arch Klin Med 1909, 96:1-6
6. Hegglin R: Über eine besondere Form gleichzeitiger konstitutioneller Veränderungen der Neutrophilen und Thrombozyten (Konstitutionelle polyphyle Reifungstörung). Arch Llaus-Stift VerebForsch 1945, 20:1-12
7. Epstein CJ, Schaud MA, Piel CF, Goodman JR, Bernfield MR, Kushner JH, Albin AR: Hereditary macrothrombocytopenia, nephritis and deafness. Am J Med 1972, 52:299-310
8. Raccuglia G: Gray platelet syndrome: A variety of qualitative platelet disorders. Am J Med 1971, 51:818-828
9. White JG: Ultrastructural studies of the gray platelet syndrome. Am J Pathol 1979, 95:445-462
10. Behnke O, Zelander T: Substructure in negatively stained microtubules of mammalian blood platelets. Exp Cell Res 1967, 43:236-243
11. Nachmias VT: Cytoskeleton of human platelets at rest and after spreading. J Cell Biol 1980, 86:795-802
12. White JG, Gerrard JM: Ultrastructural features of defective human platelets. Mol Cell Biochem 1978, 21:109-128
13. Gerrard JM, Phillips DR, Rao GHR, Plow EF, Walz DA, Ross R, Harker LA, White JG: Biochemical studies of two patients with the gray platelet syndrome: Selective deficiency of platelet alpha granules. J Clin Invest 1981, 67:1431-1440
14. Lusher JM, Schneider J, Mizukami I, Evans RE: The May-Hegglin anomaly: Platelet function, ultrastructure and chromosome studies. Blood 1968, 32:950-961
15. Godwin HA, Ginsberg AD: May-Hegglin anomaly: A defect in megakaryocyte fragmentation? Br J Haematol 1974, 26:117-961
16. Hamilton RW, Shaikh BS, Ottie NJ, Storch JN, Saleem A, White JG: Platelet function, ultrastructure and survival in the May-Hegglin anomaly. Am J Clin Pathol 1980, 74:663-668
17. White JG: Fine structural alterations induced in platelets by adenosine diphosphate. Blood 1968, 31:604-622
18. Small JV: Organization of actin in the leading edge of cultured cells: Influence of osmium tetroxide and dehydration on the ultrastructure of actin meshworks. J Cell Biol 1981, 91:695-705
19. Hoglund AS, Karlsson R, Arro B, Fredriksson E, Lindberg U: Visualization of the peripheral weave of microfilaments in glia cells. J Muscle Res Cell Motil 1980, 1:127-146
20. Debus E, Weber K, Osborn M: The cytoskeleton of blood platelets viewed by immunofluorescence microscopy. Eur J Cell Biol 1981, 24:45-52
21. Johnson DG, de C Nogueira Araujo GM: A simple method of reducing the fading of immunofluorescence during microscopy. J Immunol Methods 1981, 43:349-356
22. White JG, Burris SM: Morphometry of platelet internal contraction. Am J Pathol (In press)
23. White JG, Krivit W: An ultrastructural basis for the shape changes induced in platelets by chilling. Blood 1967, 30:625-635
24. White JG: Effects of colchicine and vinca alkaloids on human platelets: I. Influence on platelet microtubules and contractile function. Am J Pathol 1968, 53:281-291
25. Behnke O: An electron microscope study of the rat megakaryocyte: II. Some aspects of platelet release and microtubules. J Ultrastruct Res 1969, 26:111-129
26. Penington DB, Streatfield K, Roxburgh AE: Megakaryocytes and the heterogeneity of circulating platelets. Br J Haematol 1976, 34:639-653
27. Scurfield G, Radley MJ: Aspects of platelet formation and release. Am J Hematol 1981, 10:285-296
28. White JG: Platelet morphology, The Circulating Platelet. Edited by SA Johnson. New York, Academic Press, 1971, pp 45-121
29. Leven RM, Nachmias VT: Cultured megakaryocytes: Changes in the cytoskeleton after ADP induced spreading. J Cell Biol 1982, 92:313-323