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Chronic Megakaryocytic-Granulocytic Myelosis – An Electron Microscopic Study

I. Megakaryocytes and Thrombocytes*

J. Thiele, A.-Ch. Ballard, A. Georgii and K.F. Vykoupil

Institute of Pathology, Medical School Hannover,
D-3000 Hannover, Federal Republic of Germany

Summary. The fine structure of the bone marrow in chronic megakaryocytic-granulocytic myelosis (CMGM) was studied in 5 nontreated patients to investigate possible malignant proliferation of megakaryocytes and the role of megakaryopoiesis in fibrillogenesis, terminating in osteomyelofibrosis. In comparison with normal megakaryopoiesis there is an enormous increase of the megakaryocytic cell line and many immature and atypical forms are seen. Most conspicuous are microforms, nuclear-cytoplasmic disorganization and nuclear inclusions. Asynchrony of maturation causes abnormal thrombocytogenesis with premature detachment of platelets resulting in immature and peculiar giant forms of thrombocytes. Besides megakaryocytes appearing superficially normal the maturation anarchy of many cells is so severe that by analogy with observations in other leukaemic cells these abnormalities are thought to be representative of a malignant growth. Moreover, there is a striking accumulation of microfibrils and single collagen fibres around megakaryoblasts. Since these cells contain all those organelles commonly associated with fibre production the initial step for fibrillogenesis may therefore arise from the megakaryoblasts prior to platelet release, or any fibroblast proliferation.

Key words: Myeloproliferative disorder – Thrombocytes – Megakaryocytic-granulocytic myelosis – Bone marrow biopsy – Megakaryocyte lineage – Electron microscopy.

Introduction

There are several varieties of myeloproliferative disorder and some are characterized by proliferation of more than one cell line (Dameshek, 1951). The most conspicuous forms involve megakaryocytic and granulocytic cell

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Abbreviations: Chronic megakaryocytic-granulocytic myelosis: CMGM, Chronic granulocytic leukaemia: CGL, Myelofibrosis: MF, Osteomyelosclerosis: OMS, Demarcation system: DMS, Rough surfaced endoplasmic reticulum: RER

proliferation. On a clinical basis these chronic progressive disorders are classified as either chronic granulocytic leukaemia (CGL) with marked megakaryocyte proliferation, idiopathic (malignant) thrombocythaemia, chronic megakaryocytic leukaemia or chronic megakaryocytic-granulocytic myelosis (CMGM) depending on quantitative and qualitative features of megakaryopoiesis. The existence of an authentic CMGM – implying malignant growth of both the granulocytic and megakaryocytic cell lines – as a separate entity, is currently under discussion (Georgii and Vykoupil, 1972, 1976). It is frequently confused with a temporary phase of CGL during which there is associated megakaryocytic proliferation.

With the increased application and improvement of bone biopsy (myelotomy) and tissue processing techniques, CMGM has been shown to occur more frequently than was anticipated from observations based exclusively on sternal puncture, particularly in patients with a so called dry tap (Schäfer et al., 1975; Georgii and Vykoupil, 1976; Hill and Schäfer, 1976). Further, follow-up studies indicate that there is a progressive relationship between megakaryocytic proliferation, myelofibrosis (MF) and osteomyelosclerosis (OMS) (Block et al., 1975; Fisher and Fölsch, 1975). From a preliminary survey of a small number of patients from which bone biopsies were taken at intervals, myelosclerosis with myeloid metaplasia (agnogenic myeloid metaplasia) is not thought to be a completely separate entity, but possibly in the majority of cases, to represent a terminal or "burnt out" stage of CMGM (Georgii and Vykoupil, 1976). These preliminary results in patients with CMGM suggest subdivision of this myeloproliferative disorder into the following stages: I. atypical hyperplasia, II. early MF, III. full MF, IV. OMS (Georgii and Vykoupil, 1976).

To provide support for the above proposal, we studied *firstly* the question whether the megakaryocytic proliferation was truly a malignant one and not merely a concomitant hyperplasia provoked by the neoplastic granulocytic growth, and *secondly,* whether the microenvironment of the megakaryocytes included evidence of early fibre formation that could lead to MF.

These points are studied by examining large, intact areas of bone marrow under the electron microscope to form quantitative and qualitative assessments of cell proliferation and morphology.

Case Reports

Five patients were studied, 4 females, 1 male, aged between 50 and 60 years. They were separated into 2 groups by their clinical and morphological features.

The first group, consisting of 3 patients, presented with symptoms of a latent haematological disorder; subleukaemic white blood cell counts (20–30,000/cu mm), marked thrombocythaemia (600–1,000,000/cu mm) erythrocyte counts of 4–4,500,000/cu mm and a haemoglobin level of 11–13 g/100 ml. Differential blood counts apparently established CGL with many myeloblasts and promyelocytes. Sternal puncture however demonstrated a large quantity of immature megakaryocytes. The megakaryocytic and granulocytic series were shown by methacrylate embedded iliac crest biopsies to have undergone atypical hyperplasia whereas erythropoiesis was relatively normal. There was no MF. These findings are consistent with a stage I of CMGM (Georgii and Vykoupil, 1976).

The second group (2 patients) entered hospital suffering from abdominal pain, caused by enlargement of the spleen and liver, and having slight anaemia, (red cell count about 3,000,000/cu mm, haemoglobin 8–10 g/100 ml). Clinical findings seemed to be consistent with CGL: white blood

cell count 70–50,000/cu mm (25% neutrophils, 10% metamyelocytes, 8% myelocytes, 3% promyelocytes, 39% myeloblasts, 3% eosinophils, 10% lymphocytes, 2% blast cells in one of the patients) and a thrombocyte count of about 200,000/cu mm. As in the case of the first group patients, iliac crest biopsies demonstrated atypical proliferation of the granulocytic and megakaryocytic series but in contrast, group 2 patients showed discrete MF initiating around sinus walls (sinus wall sclerosis) and large clusters of immature megakaryocytes. This group belonged therefore to stage II CMGM with early MF (Georgii and Vykoupil, 1976). Prior to taking iliac crest biopsies, the patients received no special treatment and in particular, no cytostatics.

Material and Methods

For electron microscopy the iliac crest biopsies were performed following the method of Burkhardt (1966). The bone cylinders were dissected under a stereo-microscope to separate large pieces of intact marrow from the bony trabecula.

Marrow specimens were immediately immersed and minced in the fixative (2.5% glutaraldehyde and 2% formaldehyde in 0.1 M sodium cacodylate buffer at pH 7.3).

Following aldehyde fixation for 90 min the samples were postfixed in 1% osmium tetroxide dissolved in phosphate buffer for 1 h, dehydrated in ethanol and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate after mounting on large unobstructed formvar-carbon films to obtain extensive surveys. Electron microscope: Siemens Elmiskop 1a.

As controls, bone biopsies were similarly processed the first from a 30-year old man having no haematological disorder, the second from a 35-year old female patient with a CGL and accompanying megakaryocyte proliferation.

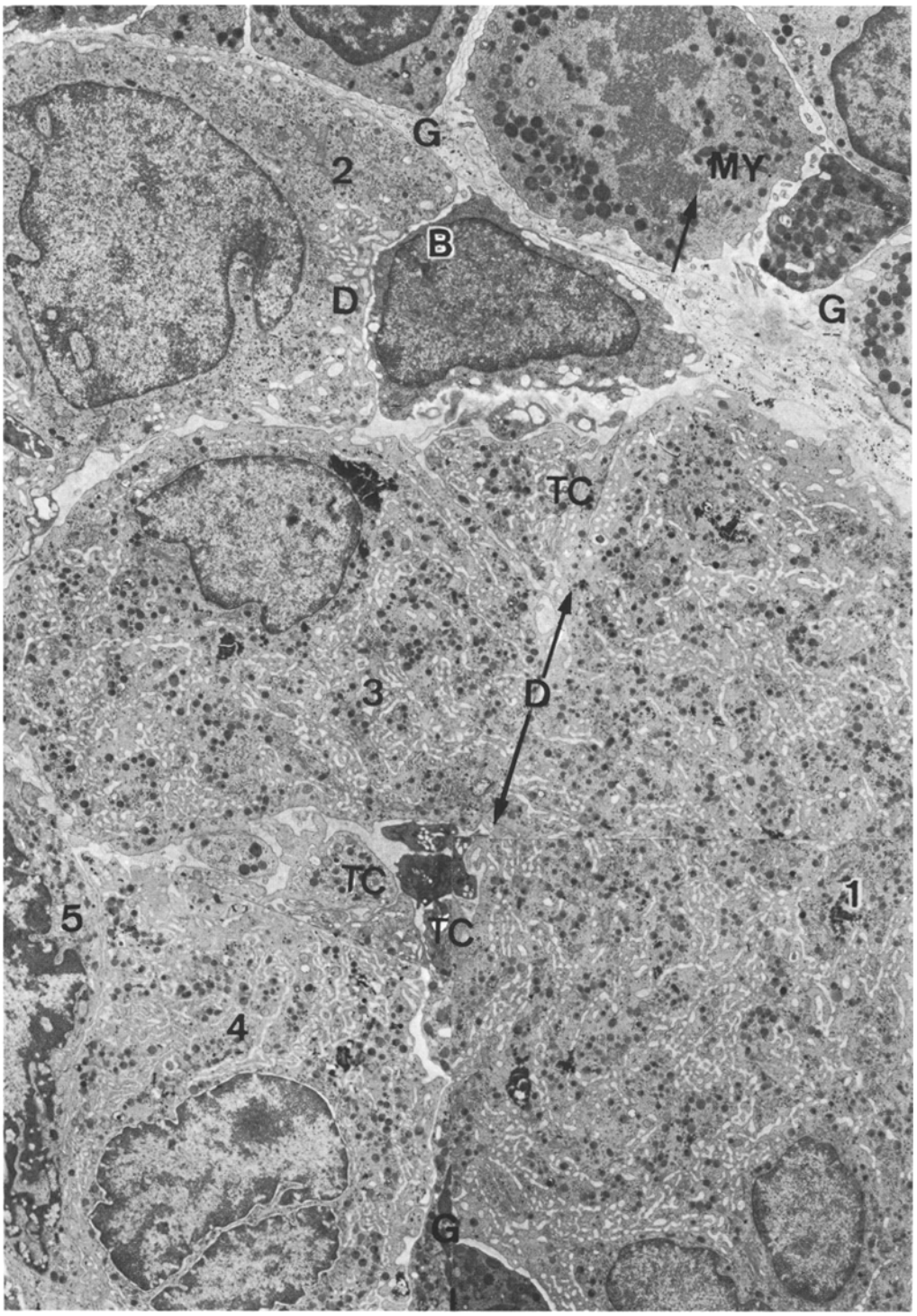
Results

General Survey

Electron microscopical surveys from the *first group of patients* show atypical proliferation of the megakaryocytic and granulocytic series with preponderance of precursor forms as well as numerous normally maturing cells. Both cell lineages, particularly the megakaryocytic series are noticeable because of many degenerative and abnormal forms. Erythropoiesis is slightly hyperplastic; lymphocytes, monocytes and plasma cells are only present in low numbers. Reticulum cells, in fairly large amounts, exhibit marked phagocytic activity around erythropoietic islands. Megakaryopoiesis is often localized around sinus walls or in large clusters with some interspersion of other cells, usually granulocytes (Fig. 1).

Numerous thrombocytes are scattered intercellularly and conspicuously accumulated around the sinuses. Even when fibrosis is not noticeable by light microscopy, a striking accumulation of microfilaments and collagen fibres can be detected predominantly around megakaryoblasts (Figs. 6, 7c).

The second group of patients shows similar atypical hyperplasia of haemic cells. In addition there is a proliferation of fibroblasts closely associated with collagen fibrils and microfilaments, adjacent to sinus walls (sinus wall sclerosis), the first confirmatory sign of MF (Fig. 2a). The abnormal microfilament and fibre accumulation surround the sinus walls. In many places, megakaryoblasts are seen protruding into the endothelial layer with resulting collapse of the sinus walls. Sometimes there is even intraluminal infiltration so that megakaryopoiesis leads to subtotal or total obliteration of the vascular bed (Fig. 2a).



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A. Megakaryocytes

1. Maturation and Degeneration. The most immature precursor of the megakaryocytic lineage is identified as a cell with a large, relatively rounded nucleus, one or more nucleoli, and a relatively small amount of cytoplasm. The cytoplasm is rich in polysomes, contains a few cisternae of the rough surfaced endoplasmic reticulum (RER), a Golgi apparatus including coated vesicles, and some mitochondria. The nuclear border is wavy whereas the cell outline is generally smooth although pseudopod-like cytoplasmic projections are observed. A few dense bodies are observable in some areas (Fig. 2a). This cells is only distinguished from myeloblastic and proerythroblastic precursor cells with difficulty. It is a little larger than the other types and is found among the clusters of maturing megakaryocytes. An even more primitive cell resembling the haemopoietic stem cell (haemocytoblast) as described by Dicke et al. (1973) is occasionally seen (Fig. 7a). The next step of megakaryopoiesis includes development of the demarcation membrane system (DMS). This is initiated at several localized areas of the cell periphery with growth of a spongy system of flattened cisternae (Fig. 1). At the same time the nucleus begins to indent, the Golgi apparatus develops extensively with budding off of granules along the length of the electron dense innermost saccule. Dilated cisternae of RER occupy large areas of the cytoplasm. The cellular membrane becomes highly scalloped due to production of many pseudopod like protrusions of the cytoplasm. Some of the latter contain densely packed microfilaments.

At this stage, there is an accumulation of microfibrils and single collagen fibres in the intercellular space surrounding the megakaryoblasts (Fig. 6).

The subsequent steps in megakaryocytic developement (promegakaryocytes, granular and thrombocytogenic megakaryocytes) are better documented than the very early and late forms, and we will therefore, only briefly describe these 3 stages. There is progressive nuclear lobulation, peripheral condensation of the chromatin, and regression of the nucleoli. The cell volume is much increased together with extensive development of the DMS, formation of specific granules by the Golgi apparatus and distinctive zonation of the cytoplasm. Finally the thrombocytes are delineated. For further reference see Jones (1960), Gautier et al. (1963); Zamboni (1965); Jean et al. (1971); Cawley and Hayhoe (1973).

Als platelet release approaches completion, the megakaryocyte consists of a hyperlobated nucleus, with much peripheral chromatin condensation and a cytoplasm, which contains dense accumulations of granules besides some DMS which probably produces the last platelets (Fig. 3a). Finally, the cytoplasm becomes devoid of all organelles terminating as so called naked nuclei which represent overaged or degenerative forms (Fig. 3b).

Fig. 1. Survey (reconstruction) with cluster of atypical maturing megakaryocytes. Apparently normal thrombocytogenic megakaryocyte (*1*), very early megakaryoblast (*2*) with early development of demarcation membrane system (*D*), micromegakaryocyte with heavy glycogen accumulation (*3*), platelet (*TC*) producing small megakaryocyte (*4*), and part of a degenerative form with hyperlobated nucleus (*5*). Surrounding cells of granulopoiesis (*G*) with immature blastic cell (*B*) and a myelocyte in mitosis (*MY*). ×4000

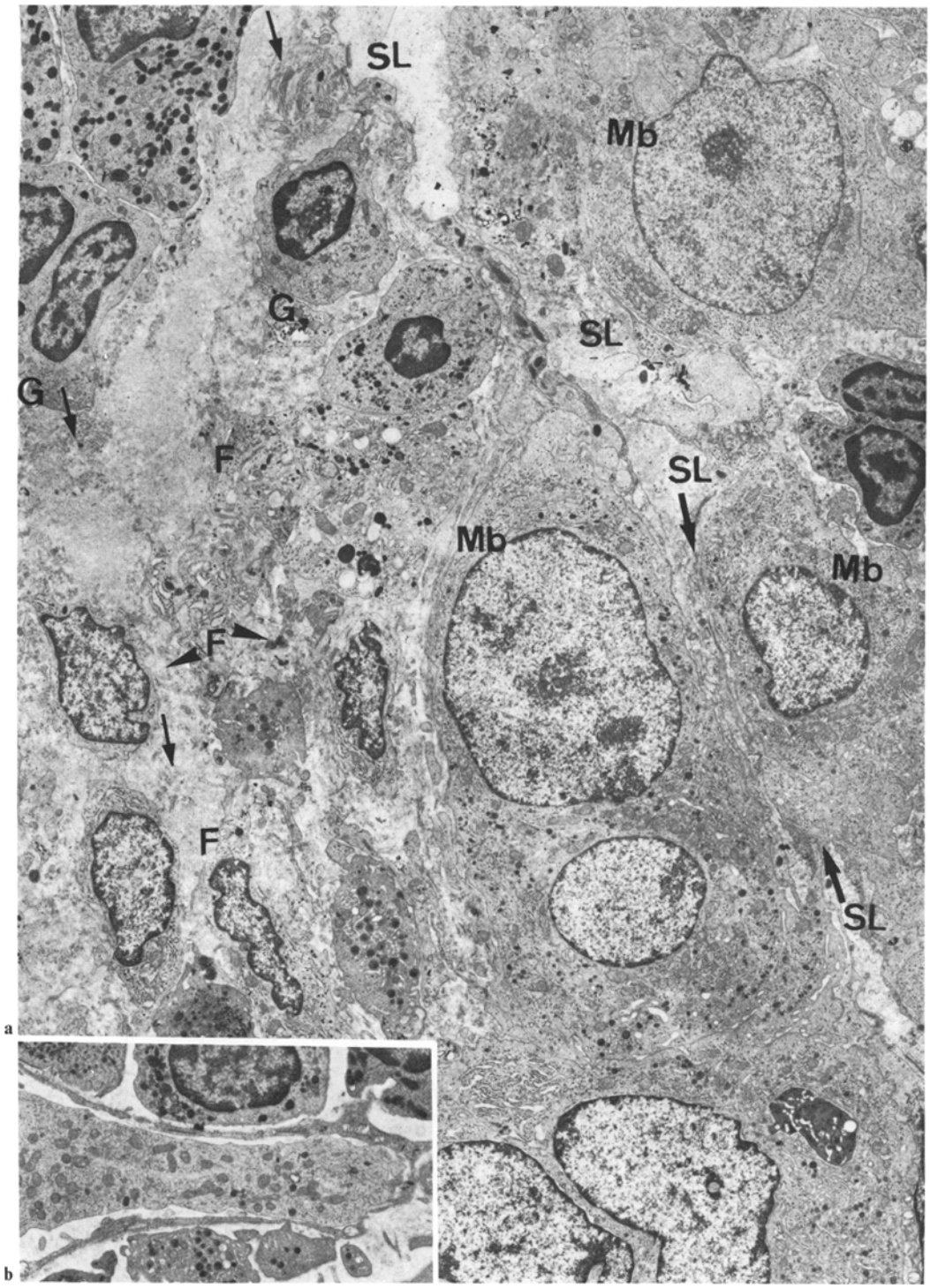


Fig. 2. **a** Survey (reconstruction) of sinus wall sclerosis in early myelofibrosis. Sinus lumen (*SL*) partially obliterated by extensive megakaryopoiesis with several early and late megakaryoblasts (*Mb*). Perisinusoidal fibroblastic cells (*F*) surrounded by microfilaments and bundles of collagen fibres (arrows), clusters of granulopoiesis (*G*). **b** Normal sinus wall for comparison with thin layer of sinus endothelium and part of a reticulum cell in the lumen. a ×4000, b ×4500

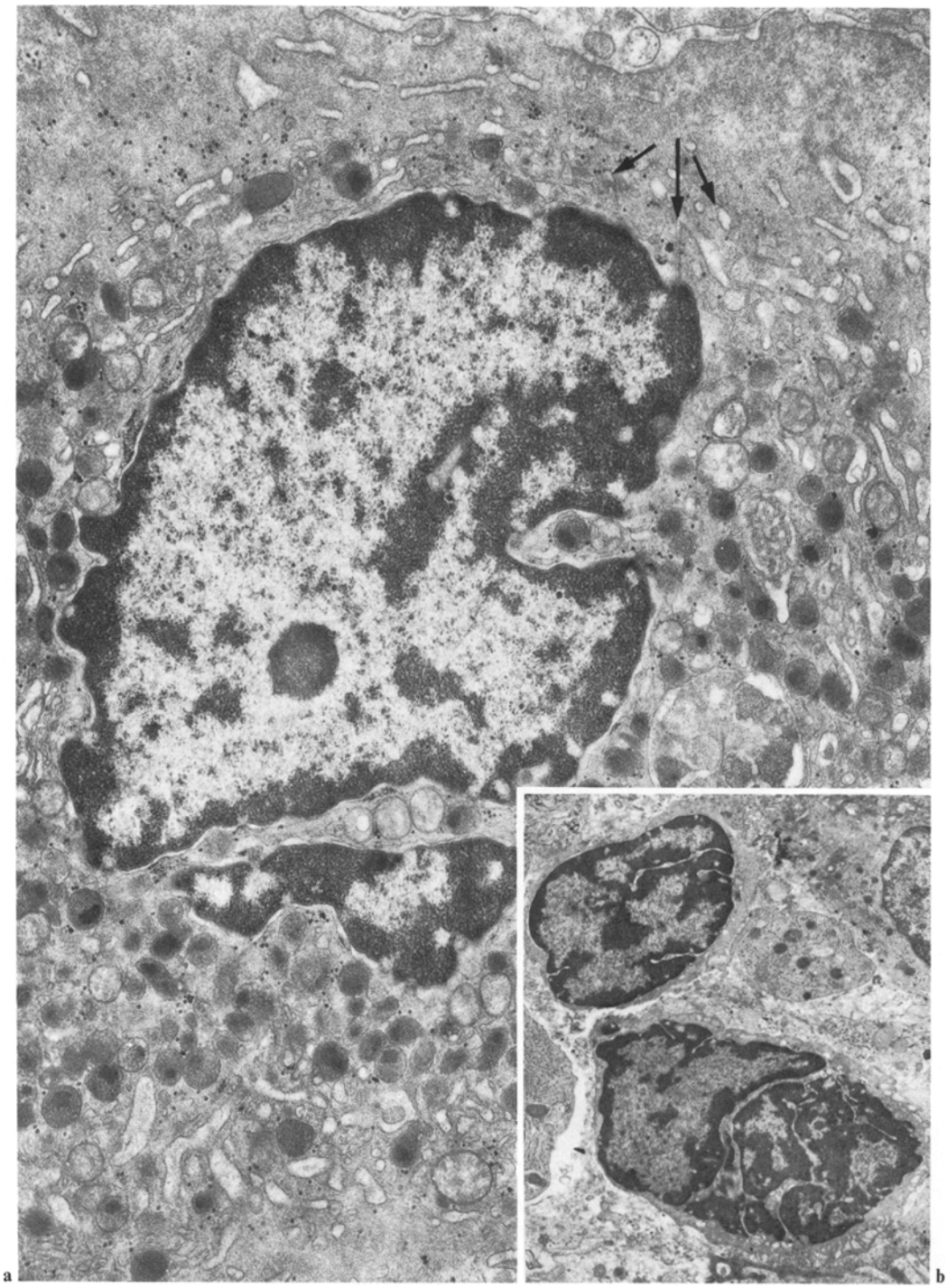


Fig. 3a and b. Megakaryocyte degeneration. **a** Regressive alteration with hyperlobated nucleus, condensed chromatin and nucleolus. Small portion of cytoplasm filled by specific granules (many of the bull's eye type) and cisternae and tubules of the DMS (arrows). **b** Two naked nuclei with hyperlobation and chromatin condensation surrounded by small margin of cytoplasm devoid of organelles corresponding to final step of megakaryocyte degeneration. a $\times 19{,}000$, b $\times 5000$

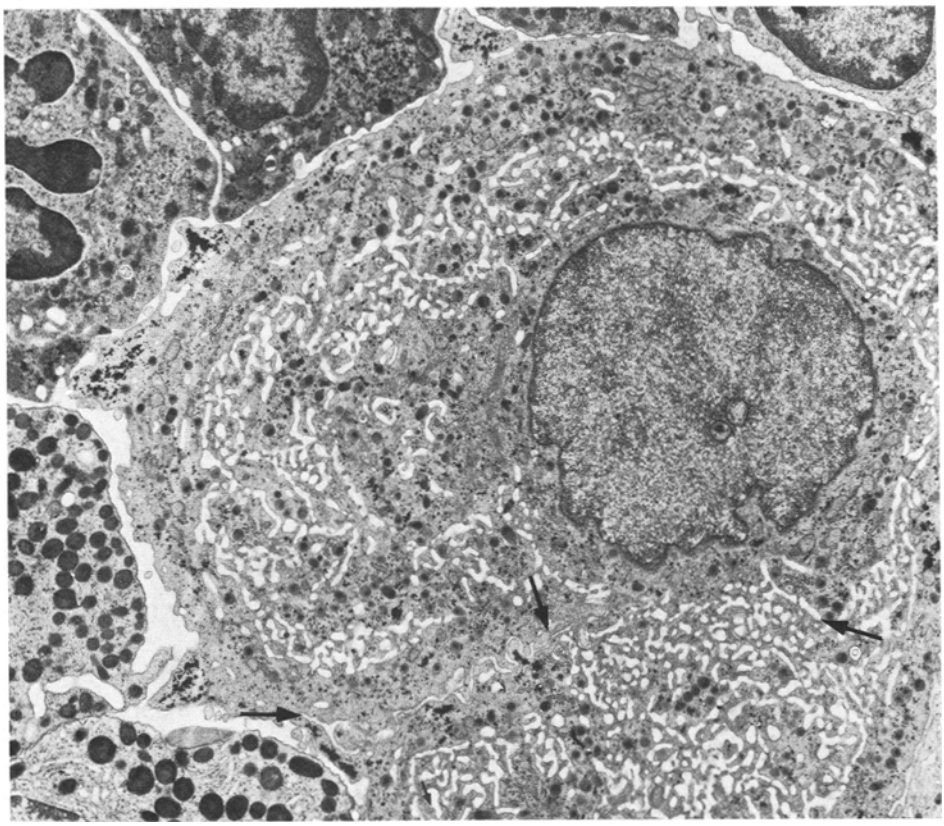


Fig. 4. Megakaryocyte atypia. Micro-megakaryocyte with immature nucleus (dispersed chromatin). DMS well developed with early premature segregation of platelets which contain only few specific granules (arrows). ×6500

2. Megakaryocytic Atypia. In contrast to the majority of megakaryocytes that follow an apparently normal pattern of development some exhibit striking atypia. These abnormalities are most frequently related to marked nuclear/cytoplasmic asynchrony as demonstrated by the common occurrence of micro-megakaryocytes of 8–15 μm diameter compared to normal dimensions of about 50 μm. These micro-megakaryocytes may lack or show a paucity of specific granules at a stage when the DMS is already fairly well developed (Figs. 1, 4) or platelet detachment has even taken place (Figs. 4, 7c). Megakaryocytes of normal size, but that possess a very large hyperlobated nucleus with minimal peripheral chromatin condensation, many nucleoli, and features of a fairly immature cytoplasm also occur. Alternatively, megakaryocytes show only a slightly indented nucleus resembling that of the promegakaryocytes but peripheral chromatin condensation and sometimes large clusters of glycogen typical of thrombocytogenic forms (Fig. 1). Other asynchronized patterns include extensive specific granule formation but only small primitive areas of DMS (Figs. 1, 7a).

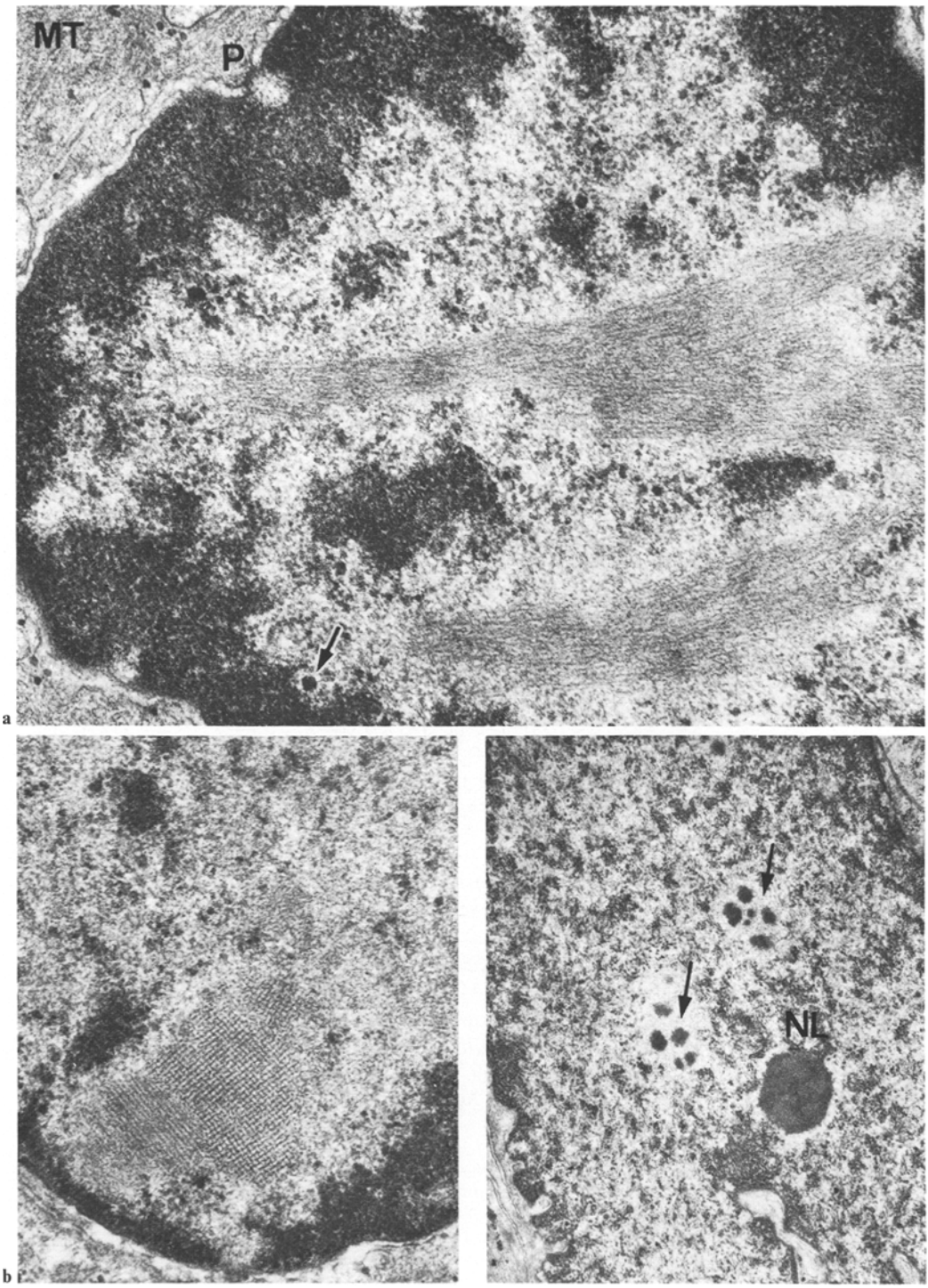


Fig. 5a–c. Nuclear inclusions. **a** Filamentous structures surrounded by clusters of chromatin. Large perichromatin granule (arrow), nuclear pore with dense annulus (*P*). In the cytoplasm many microtubules (*MT*). **b** Crosslinked and filamentous inclusion probably transverse section of a similar structure as in a. **c** Electron dense inclusions corresponding to large perichromatin granules (arrows) and possibly to condensed nucleolus (NL, compare with Fig. 3a). a ×42,000, b ×40,000, c ×16,000

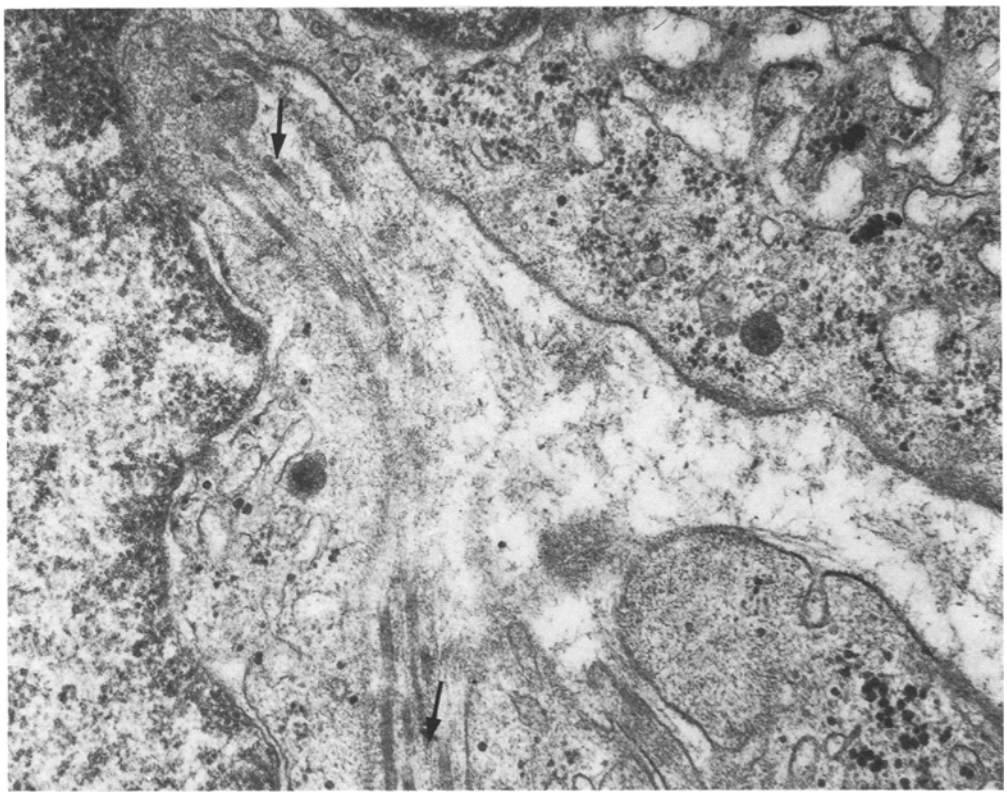
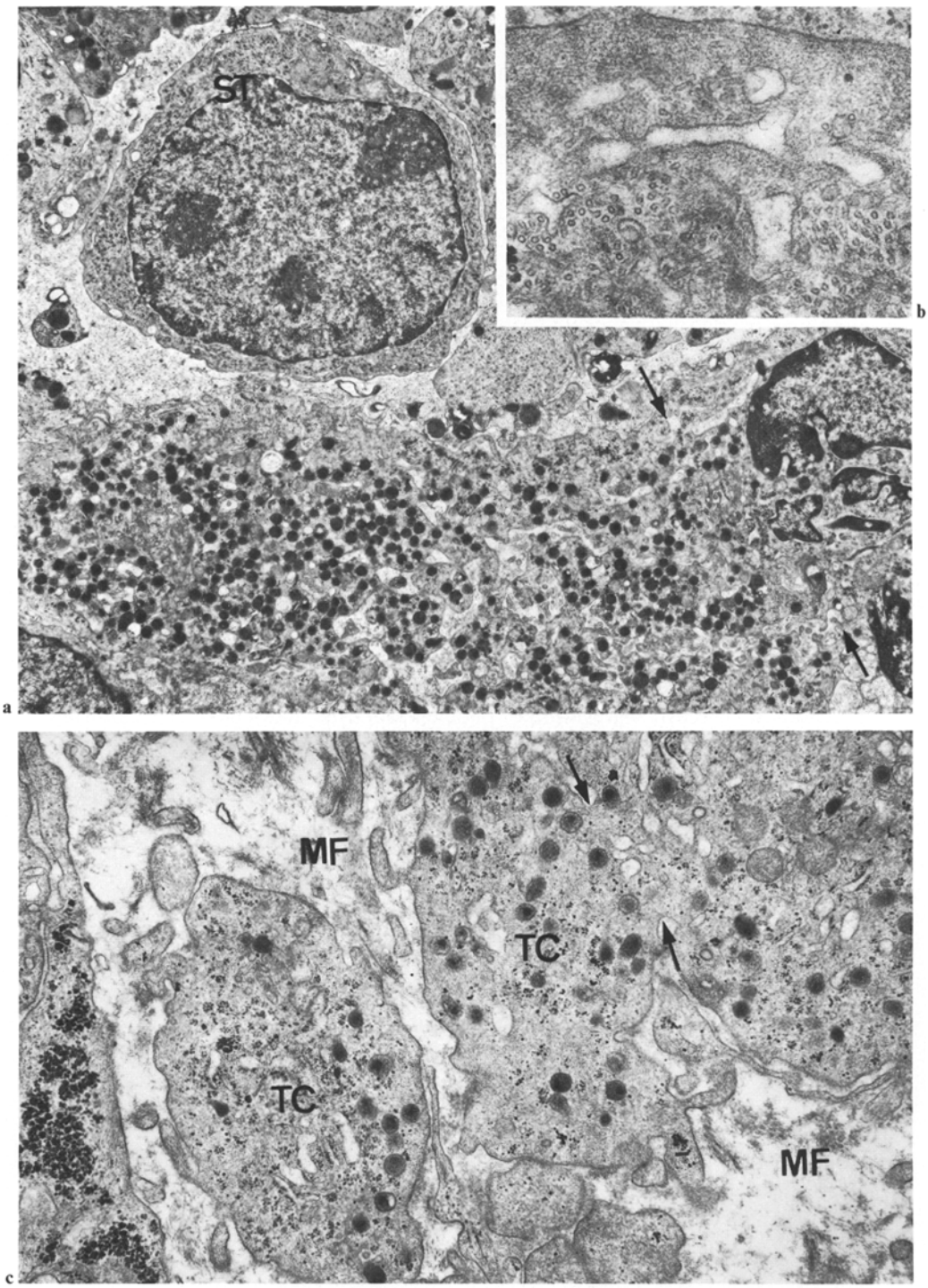


Fig. 6. Early myelofibrosis. Extracellular space of megakaryoblasts filled by a loose network of microfilaments intermingeled with single collagen fibres with periodical banding (arrows). ×40,000

The DMS exhibits further peculiarities besides those of asynchrony, the most striking being the presence of several very extensive dense compartments (Behnke, 1968).

The most remarkable atypia associated with the nucleus is represented by nuclear inclusions. These are found mostly in immature and degenerating cells. There are several types, the most prominent consisting of filamentous structures as seen longitudinally (Fig. 5a) but having a crosslinked almost crystal lattice–like structure on transverse sections (Fig. 5b). Electron dense, rounded inclusions are also of frequent occurrence (Fig. 5c). Some cells contain very prominent

Fig. 7a–c. Atypia of thrombocytogenesis. **a** Very late megakaryocyte with hyperlobated nucleus and detachment of large (last?) platelet containing numerous specific granules (arrows). Above primitive blastic cell probably stem cell (*ST*) with large nucleus and several nucleoli. High nuclear-cytoplasmic ratio with small portion of cytoplasm showing many polysomes. **b** Marginal cytoplasm of a large platelet with abundance of irregularly arranged microbubules surrounding tubules of the surface connected system. **c** Very early granular megakaryocyte with premature segregation (arrows) of primitive thrombocytes (*TC*) containing many cisternae of the rough endoplasmic reticulum and only a few specific granules. The extracellular space filled by network of microfilaments (*MF*). a ×8000, b ×40,000, c ×16,000


ST
b
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perichromatin granules of an abnormally large size with distinctive halos as well as numerous ones of normal dimensions.

The Golgi apparatus is apparently normal surrounded by numerous dense granules (the future α-granules of thrombocytes), so calles "bull's eye" granules (Jean et al., 1963) which exhibit a smaller dense core inside the granule, mostly in excentric position (Fig. 8c).

Quantitatively however, there is a great increase in the proportion and number of "bull's eye" granules (Figs. 3a, 7a, 8a–c).

3. Early Myelofibrosis. Bundles of microfilaments and collagen fibrils are seen closely associated with megakaryoblasts even in the atypical hyperplastic bone marrow of early CMGM (Figs. 6, 7c). Their presence is not related to any obvious proliferation of normally accepted fibre producing (or fibroblastic) cells. Those appear in later stages of myeloid fibrillogenesis when sinus wall sclerosis is already noticeable (compare Fig. 2a with 2b). A web–like arrangement of microfilaments, without obvious periodicity but which are mingled with some larger fibrils (25–40 nm diameter) that sometimes show periodical banding, surrounds the cell membranes of most megakaryoblasts and promegakaryocytes (Fig. 6).

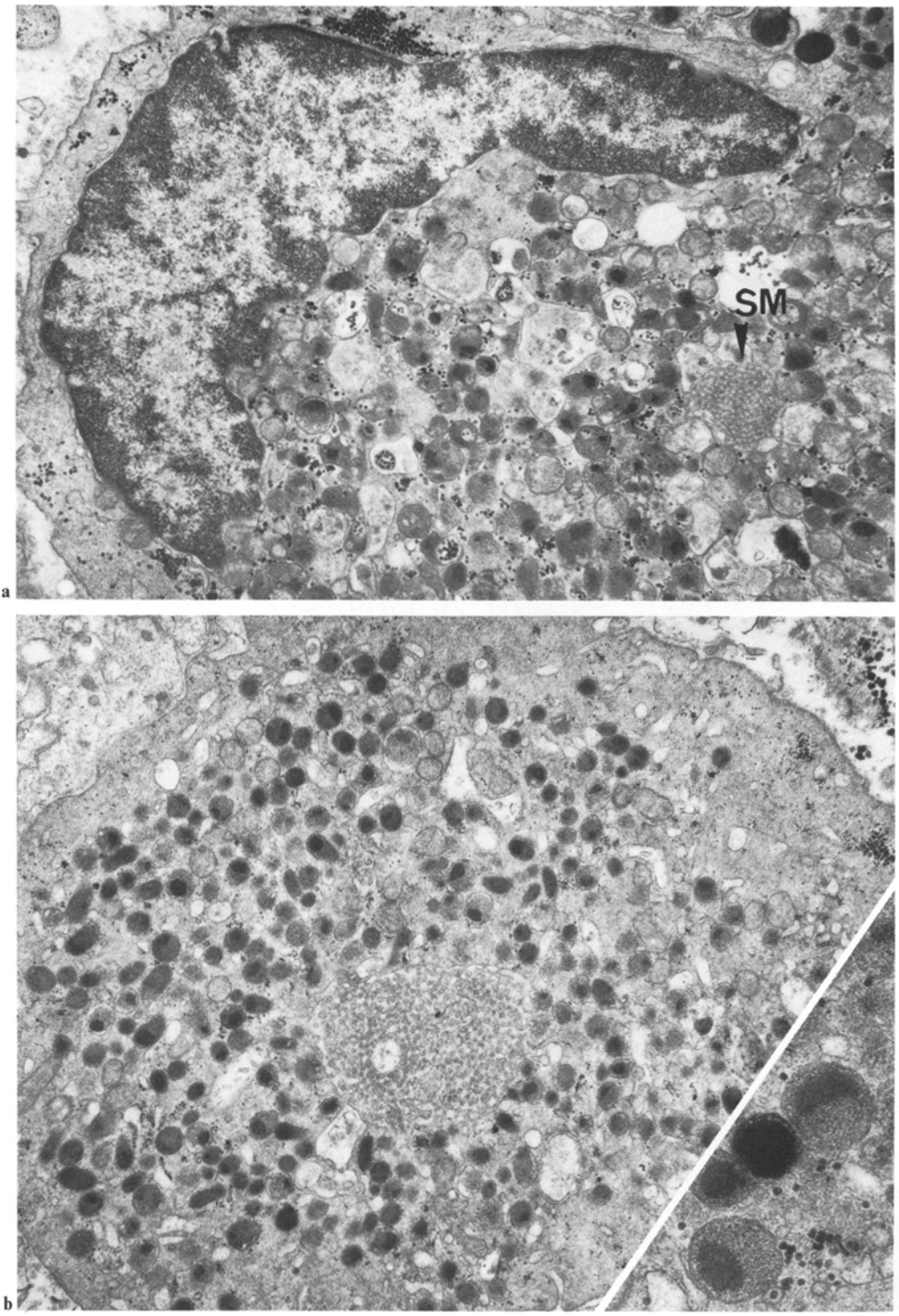
B. Thrombocytes

1. Platelet Release. Since the absolute number of megakaryocytes is greatly increased in CMGM, segregation of platelets from the many normally maturing megakaryocytes is very frequently observed.

The process follows that described by Gautier et al. (1963), Falcao and Gautier (1967), French (1967), Schulz and Schiller (1968), Behnke (1969) and the majority of released thrombocytes are normal in appearance if compared with those shown by Zucker-Franklin (1970), Hovig (1968) and Behnke (1967). As already briefly mentioned under megakaryocytic atypia however, abnormal separation of platelets results in grossly atypical forms. These consist of two types: firstly, there is an premature detachment of large cytoplasmic areas from promegakaryocytes producing immature thrombocytes (Fig. 7c) and secondly, delayed segregation from megakaryocytes almost at the degeneratory stage, leads to giant thrombocytes (Fig. 7a).

2. Platelet Atypia. Thrombocytic atypia is generally associated with giant entities and hypertrophy of cytoplasmic structures. These cells sometimes attain a diame-

Fig. 8a–c. Atypical thrombocytes. **a** Nucleated thrombocytic cell or atypical degenerated megakaryocyte with part of a nucleus and cytoplasm filled by numerous specific granules predominantly of the bull's eye type. No DMS, but areas of smooth membrane system (*SM*) and glycogen accumulation. **b** Part of a giant thrombocyte with abundance of specific granules (bull's eye type) and hyperplasia of the smooth membrane system. Between the granules tubular portions of the surface connected system. **c** Specific granules of the platelet of the bull's eye type surrounding dense osmiophilic granule. a ×13,500, b ×16,000, c ×40,000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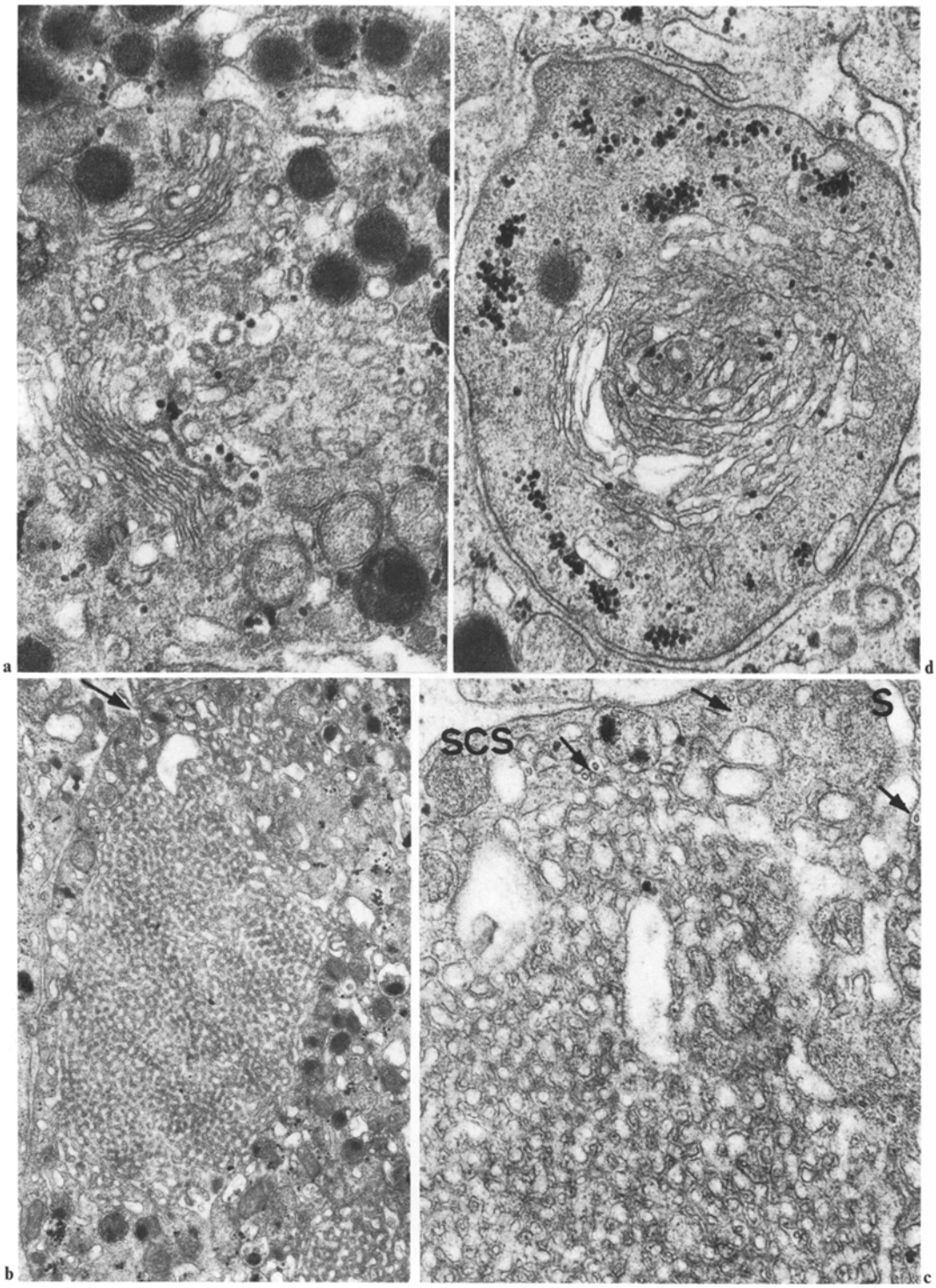
a
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ter of 20–30 μm, contain numerous organelles, dense bodies and membrane systems (Fig. 8a and b) and sometimes even retain a nucleus or nuclear relicts. In the latter cases, distinction between these nucleated thrombocytes and degenerating megakaryocytes is made only with difficulty (Fig. 8a compare with Figs. 3a and 7a). Peripheral bundles of microtubles show hypertrophy and disarray (Fig. 7b), and some platelets exhibit an extensive Golgi apparatus that apparently forms granules by budding off from the electron dense innermost sacculi (Fig. 9a).

Almost all giant platelets demonstrate an excessive proliferation of the surface connecting and dense tubular systems intermingled to produce the intramural interwoven membrane complex (review by White, 1972), probably equivalent to the dense compartment of the megakaryoblasts (Figs. 9b and c). The complex consists of a spongy system of perforated cisternae, occupying large areas of the platelet cytoplasm and surrounded by a dense population of granules. These granules, as in megakaryocytes are generally of the "bull's eye" variety (Figs. 8a–c) but sometimes, although relatively infrequently, resemble those known as "osmiophilic bodies" (Fig. 8c).

Other abnormalities not infrequently observed include an almost complete absence of cytoplasmic organelles replaced by hypertrophy of the dense tubular system (Fig. 9d), large glycogen accumulations or immature forms with large amounts of polysomes and RER (Fig. 7c).

Discussion

A. Megakaryocytes

Due to the pronounced proliferation of the megakaryocytic series in this myeloproliferative disorder; all stages of the life cycle of this cell line and variations in its microenvironment can be studied by electron microscopy. Atypia is confirmed by comparison with both the control cases and additionally by reference to pertinent literature (Huhn and Stich, 1969; Zucker-Franklin, 1970; Bessis, 1973; Cawley and Hayhoe, 1973). Light microscopic investigations of various myeloproliferative disorders reveal megakaryocytic abnormalities, particularly the production of microforms (Trautmann, 1961; Lobdell and Europa, 1962; Albrecht, 1969; Albrecht and Fülle, 1974). These alterations are regarded as due to severe disturbance in stem cell maturation resulting in diploid megakaryocytic cell lines and are comparable with a regression to lower levels of phylogenesis (Undritz and Nusselt-Bohaumilitzky, 1970). Work carried out by

Fig. 9. a Large Golgi apparatus of a giant thrombocyte with production of dense bodies and small vesicles budding off from the innermost saccule and surrounded by many specific granules. **b** Extensive complex of smooth membranes in a giant platelet with connection to the extracellular space (arrow). **c** High magnification of a membrane complex with tubules of the surface connected system (SCS) intermingeling with tubular structures (dense tubular system) and marginal microtubules (arrows). **d** Atypical thrombocyte with hypertrophy of smooth tubular system (dense tubular system?). Cytoplasm almost devoid of other organelles with few clusters of glycogen. a ×40,000, b ×16,000, c ×40,000, d ×30,000

Queisser et al. (1972, 1974) and Smith et al. (1973) demonstrates the presence of diploid and abnormal megakaryocytes in patients with preleukaemia that progresses to acute leukaemia as well as in megakaryocytic leukaemia (Hossfeld et al., 1975; Queisser et al., 1976). The assumption is therefore made that micro-megakaryocytes can act as an indicator of neoplastic growth of the myeloid tissue. In addition Kass (1973) demonstrated enzymatic abnormalities in megakaryocytes of essential thrombocythaemia which presumably reflects the malignant nature of this cell lineage. Electron microscopy of the buffy coat cells of patients with chronic myeloproliferative disorders, some with associated MF comparable with our two groups, reveals circulating, atypical micro-megakaryocytes and giant thrombocytes in the peripheral blood (Maldonado et al., 1974; Maldonado, 1974; Breton-Gorius et al., 1972, 1973; Huhn and Ascher, 1975). The atypia described is almost identical to that seen in our study of the bone marrow. Comparable abnormalities of the megakaryocytes have been observed by Kinet-Denoel and Breton-Gorius (1973) and Roessner et al. (1975) and of the platelets by Zucker-Franklin (1975) in MF/OMS. Thus it is more than probable that the marrow represents the source of these atypical micro-megakaryocytes and large platelets, although, as the disease progresses, myeloid metaplasia can not be ruled out as the origin of the abnormal cells in the peripheral blood (Tavassoli and Weiss, 1973). To date, only one study, by Lagerlöf and Franzen (1972) has been carried out on the fine structure of bone marrow, but during CGL with associated megakaryocytic proliferation. This study does not show the atypia of megakaryopoiesis as demonstrated by Maldonado et al. (1974), Breton-Gorius et al. (1972, 1973) and Huhn and Ascher (1975) in the peripheral blood and by Roessner et al. (1975) in the bone marrow of a patient with aleukaemic megakaryocytic myelosis. These results support our suggestion that in certain haematological disorders, such as CGL concurrence of megakaryocytic proliferation is probably a transitory cyclic phase and not necessarily neoplastic (Vodopick et al., 1972). This probably applies to some of the cases of Lagerlöf and Franzen (1972) and to our second control case, since none of the atypia associated with megakaryopoiesis seen in our 5 patients with CMGM is observed in the CGL control. Our five patients with CMGM and those described by the authors above suffer, on the contrary, from a myeloproliferative disorder of the megakaryocytic and granulocytic cell lines. This disease is a mixed myelosis with or without various degrees of MF that partially undergo a chronic clinical course.

The nuclear inclusions we observed, particularly the large perichromatin granules and fibrillar structures, are of special interest in the context of neoplasms, as they have been detected in increased numbers in tumour cells (review by Bernhard, 1969). Additionally, 2 of our 5 patients (3 were not investigated) possess a typical Ph' chromosome which is occasionally found in megakaryocytic myelosis (Dougan et al., 1967; Hossfeld et al., 1975; Queisser et al., 1976).

B. Thrombocytes

The clinical course of CMGM is often characterized by severe thrombocythaemia. In some cases this causes lethal thrombosis whereas others suffer from

a haemorrhagic diathesis. Maldonado et al. (1974) describe an example of each. Enhanced megakaryocytic proliferation and maturation leading to increased amounts of apparently, normally functioning platelets, is responsible for the thrombocythaemia characteristic of early stages of CMGM (atypical hyperplastic stage, stage I of Georgii and Vykoupil, 1976). On the initiation of MF (stage II of Georgii and Vykoupil, 1976) thrombocytes become trapped between the bundles of collagen fibres. Consequently, those patients have normal or subnormal platelets counts in their peripheral blood.

A haemorrhagic diathesis is very closely related to primary malignant (idiopathic) haemorrhagic thrombocythaemia. This is a myelo-proliferative disease with abnormal growth of the megakaryocytic and granulocytic series (Ozer et al., 1960; Gunz, 1960; Mason et al., 1974) possibly identical to the atypical hyperplastic stage of CMGM. Bleeding tendency in the presence of normal or highly elevated platelet counts is probably due to impaired functioning of the thrombocytes (McClure et al., 1966; Didisheim and Bunting, 1966; Inceman and Tangün, 1972). This follows from the atypia observed which include giant platelets with abnormally high glycogen content (Jean et al., 1963; Hovig, 1968; Chan et al., 1971; Maldonado et al., 1974) or even complete lack of the granules (Bussi et al., 1966; Maldonado et al., 1974) which are thought to carry platelet factor 3 (review by Hovig, 1968). These abnormalities can also be observed in the bone marrow of CMGM and orginate from the atypical segregation of platelets.

C. Myelofibrosis

It is interesting to consider agnogenic myeloid metaplasia with associated myelosclerosis as being a late stage of CMGM in the context of studies on patients with MF. These point to a close relationship between fibrosis and neoplastic growth of megakaryocytes (Hill and Schäfer, 1976; Georgii and Vykoupil, 1976; Schäfer et al., 1976).

From fibrillogenesis studies (Schwarz, 1964; Ross, 1968) fibre producing cells are characterized by a large Golgi apparatus for the production of the carbohydrate moiety and an extensively developed RER for the protein part of the fibrils. The carbohydrate and protein are secreted into the extracellular space and polymerization to microfilaments and fibres takes place along the cell membranes. Megakaryoblasts are characterized by a highly active Golgi apparatus and extensive RER, Haust (1965) demonstrated microfibrils in the extracellular space in both collagen and elastic tissue and this also occurs around megakaryoblasts in the marrow. It is possible therefore that megakaryoblasts may be responsible for the initial step in collagen synthesis by providing a medium conductive to fibrillogenesis. Zucker-Franklin (1975) observed in MF/OMS defective membranes in plateletes and suggested that surface fragmentation, with release of coagulation factors from the platelet granules (especially factor 3), may enhance the coagulation system and lead to extracellular thrombosis and local ischemia. Fibrin deposits (so called fibrin stars) should, therefore be detected around the megakaryocytes as particularly emphasized by Lennert et al. (1975). We observed no conspicuous fibrin accumulation. In later stages

of MF, however, degenerating platelets are frequently seen and a possible leakage of biogenic amines (such as serotonin, histamin, catecholamin etc.) may play an aetiological role (Zucker-Franklin, 1975). Among these compounds serotonin deserves particular attention. It probably enhances the polymerization of collagen and elastin fibres from precursor compounds which may be secreted into the extracellular space by megakaryoblasts.

The close relation of megakaryopoiesis to the sinus walls is possibly one of the most important factors in MF development. With increasing proliferation of megakaryocytes the sinus walls are infiltrated by immature cells often followed by intraluminal megakaryopoiesis. This frequently causes partial occlusion of the vascular bed and disturbance of medullary circulation with consequent localized ischemia. There follows an activation of advential cells with fibroblast proliferation and fibre accumulation, the sinus wall sclerosis of light microscopy as an early stage of MF. MF then appears to proceed by a self perpetuating process similar to that seen in liver cirrhosis, ending in OMS (stage IV, Georgii and Vykoupil, 1976). The terminal stage of OMS is endophytic bone formation and is characterized clinically by enormous spleen and liver enlargement, severe pancytopenia and extramedullary haematopoiesis. MF and OMS are regarded as terminal stages in all cases of CMGM, provided the natural course of this disease is not interrupted by an acute blastic transformation (Fisher and Fölsch, 1975; Georgii and Vykoupil, 1976).

D. Stem Cell of Megakaryopoiesis

Due to the close association of megakaryopoiesis with sinus walls, megakaryocytic development from sinus endothelial cells is postulated (Burkhardt, 1968; Demmler et al., 1970). The choice of the sinus endothelial cell as a possible precursor was probably instigated by observation of areas where megakaryoblasts infiltrate and destroy the sinus wall leading to extensive intraluminal megakaryopoiesis. This hypothesis is based on light microscopy. We have never observed megakaryopoiesis electron microscopically arising from endothelial cells along intact sinus.

Our findings of blast cells resembling the so called stem cell (haemocytoblast), and of cells showing gradual maturation toward a megakaryocyte, support experimental findings in megakaryopoiesis (Yamashita and Helpap, 1974) and the concept of a common stem cell in haematopoiesis (review by Golde and Cline, 1974).

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