Virchows Arch. A Path. Anat. and Histol. 389, 287–305 (1980)

*Virchows Archiv A*


# Ultrastructure of Blastic Crisis in Osteomyelofibrosis

## A Report of 2 Cases with Some Unusual Features*

J. Thiele, K.F. Vykoupil, and A. Georgii
Institute of Pathology, Hannover Medical School, Hannover, Federal Republic of Germany

**Summary.** The clinical and morphological findings are presented in two patients suffering from myelofibrosis and osteomyelosclerosis which terminated in an acute blastic crisis. Clinical follow-up data and light microscopy of the bone marrow however, revealed a chronic megakaryocytic-granulocytic myelosis (CMGM) with progression into myelofibrosis during the course of disease. In one patient the blastic transformation involved predominantly basophils, and in the other, neutrophils, with an accompanying abnormal proliferation of megakaryocytes in both cases. Electron microscopy of this cell population demonstrated remarkable atypicalities of the neutrophilic, basophilic and megakaryocytic cell lines. These abnormalities consisted of a nuclear-cytoplasmic asynchrony and a partial arrest of maturation, sometimes resulting in bizarre cell forms. Our investigations support the hypothesis of a mixed cellularity type of myelosis with a gradual and insiduous progression into osteomyelofibrosis/-sclerosis and a potential blastic crisis. In the evolution of blastic crisis all cell lines may be transformed, but with predominance of one population – basophils and neutrophils in our two cases – in addition to atypicalities of megakaryocytes.

*Names of drugs (cytostatics):* Myleran®-Busulfan-(Wellcome); Puri-nethol®-6-Mercaptopurin-(Wellcome); Adriblastin®-Adriamycin-HCL-(Farmitalia) (Doxorubicin-HCL); Vincristin®, Lilly-Vincristinsulfat-(Lilly); Methotrexat®, Lederle-Amethopterin-(Lederle).

**Key words:** Myeloproliferative disease – Osteomyelofibrosis – Bone marrow biopsy – Blast crisis – Ultrastructure – Basophilic leukaemia.

## Introduction

The aetiology and nosological position of myelofibrosis (MF) and osteomyelosclerosis (OMS) in the system of haematopoietic disorders has been debated

* Supported by the Deutsche Forschungsgemeinschaft (DFG, grant Ge 121/19)

*Offprint requests to:* Jürgen Thiele, M.D., Institute of Pathology, Medical School Hannover, Karl-Wiechert-Allee 9, D-3000 Hannover 61, Federal Republic of Germany

0340-1227/80/0389/0287/$03.80

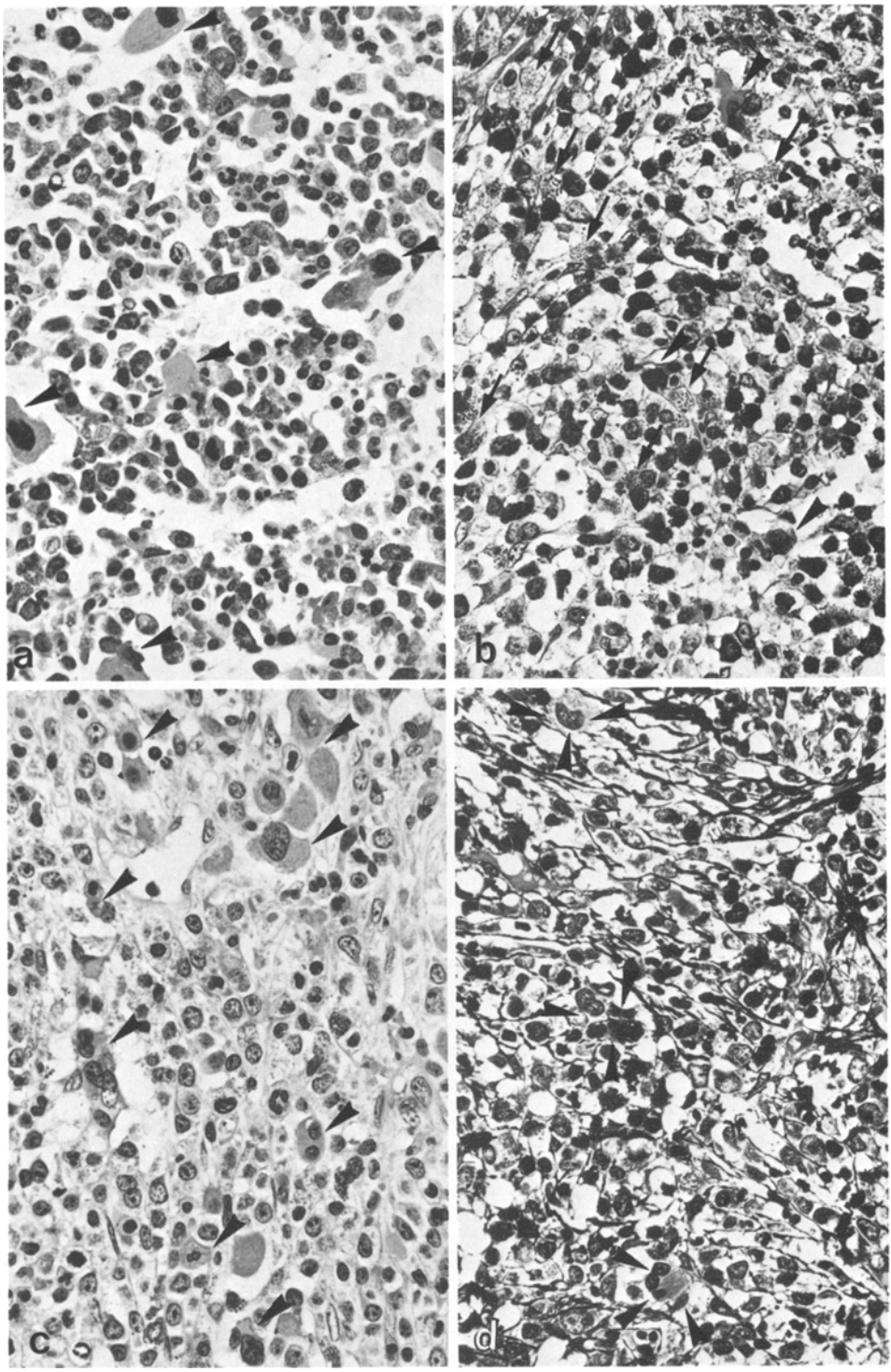
for many years (review by Block et al. 1975). Besides the primary (idiopathic) MF of unknown or reactive cause which may closely resemble experimentally induced lesions (review by Hunstein and Hauswaldt 1974), most haematologists regard MF/OMS as some kind of neoplasia involving the bone marrow. Controversy about the nature of this disorder has let to the emergence of two principle standpoints: *firstly* that MF/OMS follows an acute (malignant) or chronic course and is an entity of the myeloproliferative syndrome (Dameshek 1951) as stated by Bouroncle and Doan (1962), Silverstein et al. (1973), Lewis and Szur (1963), Lubin et al. (1976), Bearman et al. (1979). *Secondly* that MF/OMS actually presents a chronic a- or subleukaemic myelosis with accompanying fibrosis, a concept which was proposed by Heller et al. (1947), Taylor and Simpson (1950), Krauss (1966) and Hickling (1968) and further widely supported by the findings of Georgii and coworkers (review by Georgii 1979). In the majority of cases MF is thought to occur in the natural course of a subtype of chronic myelogenous leukaemia (CML), chronic megakaryocytic-granulocytic myelosis (CMGM), as was firstly emphasized by Georgii and Vykoupil (1972, 1976).

Blastic crisis may develop in all stages of progressive MF during the course of this disease and frequently results in a lethal outcome. A review of the pertinent literature suggests that several patients reported as idiopathic or malignant (acute) MF, are really examples of a blastic transformation of CMGM, rather than a distinctive clinicopathological entity (Bird and Proctor 1977; Fabich and Raich 1977; Cheng 1979; Den Ottolander et al. 1979).

The fine structure of acute blastic crisis in MF enables a more thorough investigation of the relationships between CMGM, MF and acute myelogenous leukaemia by studying the light and electron microscopy of the bone marrow in those patients with a well documented clinical course.

Consequently, our investigation has concentrated on the ultrastructure of blasts involved in this malignant transformation, the possible atypicality of megakaryopoiesis, and the development of fibres. In addition, the fine structure of the basophils is described in a case of acute basophilic crisis arising from CMGM and accompanied by MF.

**Fig. 1a–d.** Light microscopy. **a** *Patient A*, bone marrow biopsy in 1973 (A 604/73) showing a chronic megakaryocytic-granulocytic myelosis (CMGM) with many atypical megakaryocytes (*arrow heads*) between abnormal proliferating granulopoiesis. **b** Bone marrow in 1975 (A 1167/75) with an acute basophilic leukaemia and myelofibrosis. Basophils are indicated by arrows, micromegakaryocytes by arrow heads. **c** *Patient B*, bone marrow biopsy in 1973 (A 1033/73) with chronic megakaryocytic-granulocytic myelosis (CMGM) and obvious myelofibrosis. Between the abnormal megakaryocytes (*arrow heads*) many myeloblasts are detectable. **d** Bone marrow in 1975 (A 1376/75) with extensive myelofibrosis showing transgression into osteomyelosclerosis. Few micromegakaryocytes are still visible (*arrow heads*) between large blastic cells. Some collagen fibres are arranged in whorl-like or star-shaped formations. (Acrylate embedded semithin sections, a and c Giemsa stain, b and d silver impregnation after Gomori.) **a–d** ×280


a
b
c
d

## Patients and Methods

*Patients*

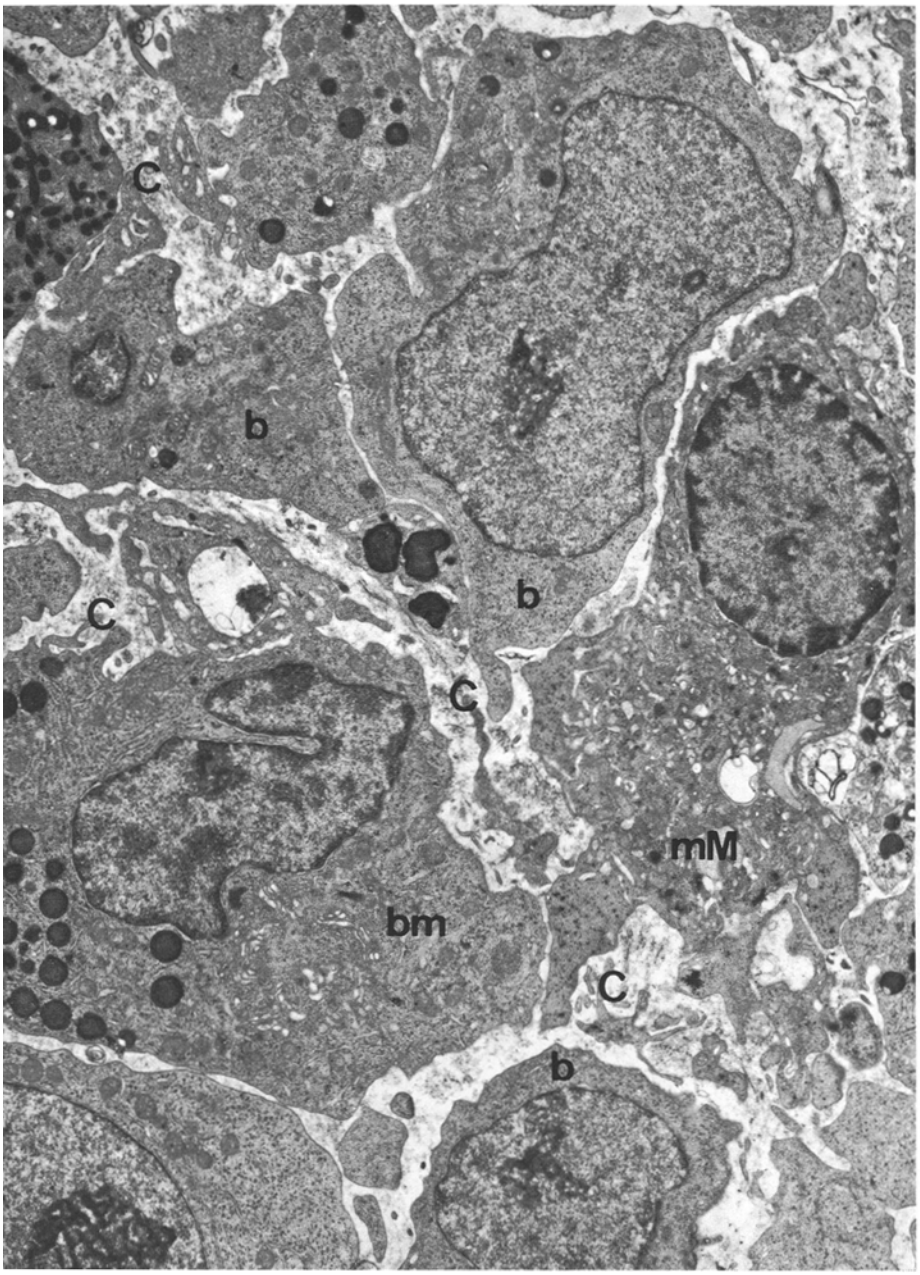
The clinical and morphological findings from core biopsies of the bone marrow were studied in two patients during a period of 2–3 years and are briefly summarized in Table 1.

**Table 1.** Survey of the principle clinical and morphological findings referring to the development of acute blastic transformation in the 2 patients with a chronic megakaryocytic-granulocytic myelosis (CMGM) evolving myelofibrosis/-sclerosis (MF/OMS). + : semiquantitative calculation of proliferating blood cell population to illustrate the distribution of different cell-lines (gradings from + → + + +); for gradings of myelofibrosis see Georgii and Vykoupil (1976)

| Two cases: | Patient A | | Patient B | |
|---|---|---|---|---|
| Clinical diagnosis: | CML | | CML | |
| Approximate duration of disease in years: | 4 | | 12 | |
| Histopathology: | CMGM | | CMGM | |
| Grading of Myelofibrosis: | 2 → 3 minimal MF → severe MF | | 3 → 4 MF → OMS | |
| Haematopoiesis | | | | |
| blasts (myeloblasts) | + + + | 30% | + + + | 40% |
| Granulopoiesis | | | | |
| basophils | + + + | 30% | 0 | – |
| neutrophils and precursors | (+) | 10% | + | 20% |
| Megakaryopoiesis | | | | |
| micro-forms and blasts | + + | 20% | + + | 20% |
| non blastic cells | + | 10% | + | 20% |

*Patient A* (Sch.F.), a 49-year-old man entered the hospital in June 1973 with the complaints of increasing fatigue and weight loss over about 2 years. Clinical and laboratory investigations including sternal puncture revealed chronic myelogenous leukaemia (CML). This diagnosis of uncomplicated CML was corrected and extended to CMGM with minimal MF (Fig. 1a) after the bone marrow biopsy (A 604/73). Grading of the MF corresponded to stage 2 of Georgii and Vykoupil (1976). Therapy was initiated by cytostatics (Myleran®) and continued during the next two years until June 1975 when the patient was readmitted to hospital. Laboratory findings were suggestive of an acute blastic crisis and therapy included several blood transfusions, cytostatics (Vincristin®, Purinethol®) and radiation of the spleen (300 r). Despite all therapeutic efforts the general condition of this patient deteriorated during the next 3 months. There was an increasing anaemia, several attacks of fever and spontaneously occurring flushing episodes. The leukocyte count increased during cytostatic therapy (after a short interval of depression) up to 27,000 cu/mm with a conspicuous basophil count of 33% and 19% of myeloblasts; the leucocyte alkaline phosphatase was very low (index 6). The trephine biopsy of the bone marrow (A 1,167/75) confirmed the acute blastic crisis of a secondary basophilic myelosis accompanied by MF (Fig. 1b) suspected clinically. Methotrexat® was given, but no remission was achieved and the patient died approximately two weeks later with septicaemia and generalised bleeding. Autopsy (SN 533/75) revealed changes corresponding to an acute blastic transformation of a chronic leukaemia with complete overgrowth of the bone marrow by blasts with associated MF. The cause of death was bilateral bronchopneumonia complicated by septicaemia.

*Patient B* (U.N.), a 34-year-old woman, known to be suffering from CML since about 1964, was successfully treated during a period of 9 years with Myleran®. In August 1973 she was readmitted




**Figs. 2, 3a–d, 4a–d, and 5a–c.** Electron microscopy of the bone marrow of *patient A* in 1975 (compare with Fig. 1b)

**Fig. 2.** Survey with demonstration of the three types of proliferating cells: Several large blasts (myeloblasts, *b*), a basophilic metamyelocyte with small granules and extensive Golgi apparatus (*bm*) and a micromegakaryocyte (*mM*). Between these cells small bundles of fibrils (*C*) consistent with myelofibrosis. ×7,000

to the hospital with severe anaemia, weakness and abdominal pain due to enlargement of the spleen and liver. Sternal puncture resulted in a dry tap and a core biopsy of the bone marrow (A 1,033/73), done for the first time in this patient, revealed CMGM with extensive MF and early blastic transformation (Fig. 1c). Therapy included antibiotics, Adriblastin® and several blood transfusions and was continued after a remission with Puri-nethol® and erythrocyte transfusions. Clinical and peripheral blood findings during this period of cytostatic therapy were satisfactory. In autumn 1975 there was clinical evidence of an early relapse with a white blood cell count of 9,000 cu/mm; leukocyte alkaline phosphatase was low (index 6). A second trephine biopsy of the bone marrow or "myelotomy" (A 1,376/75) showed a CMGM with marked MF and progression into OMS corresponding to stage 4 of Georgii and Vykoupil (1976) and associated with a blastic crisis (Fig. 1d). The patient died in July 1976; unfortunately no autopsy was performed.

*Methods*

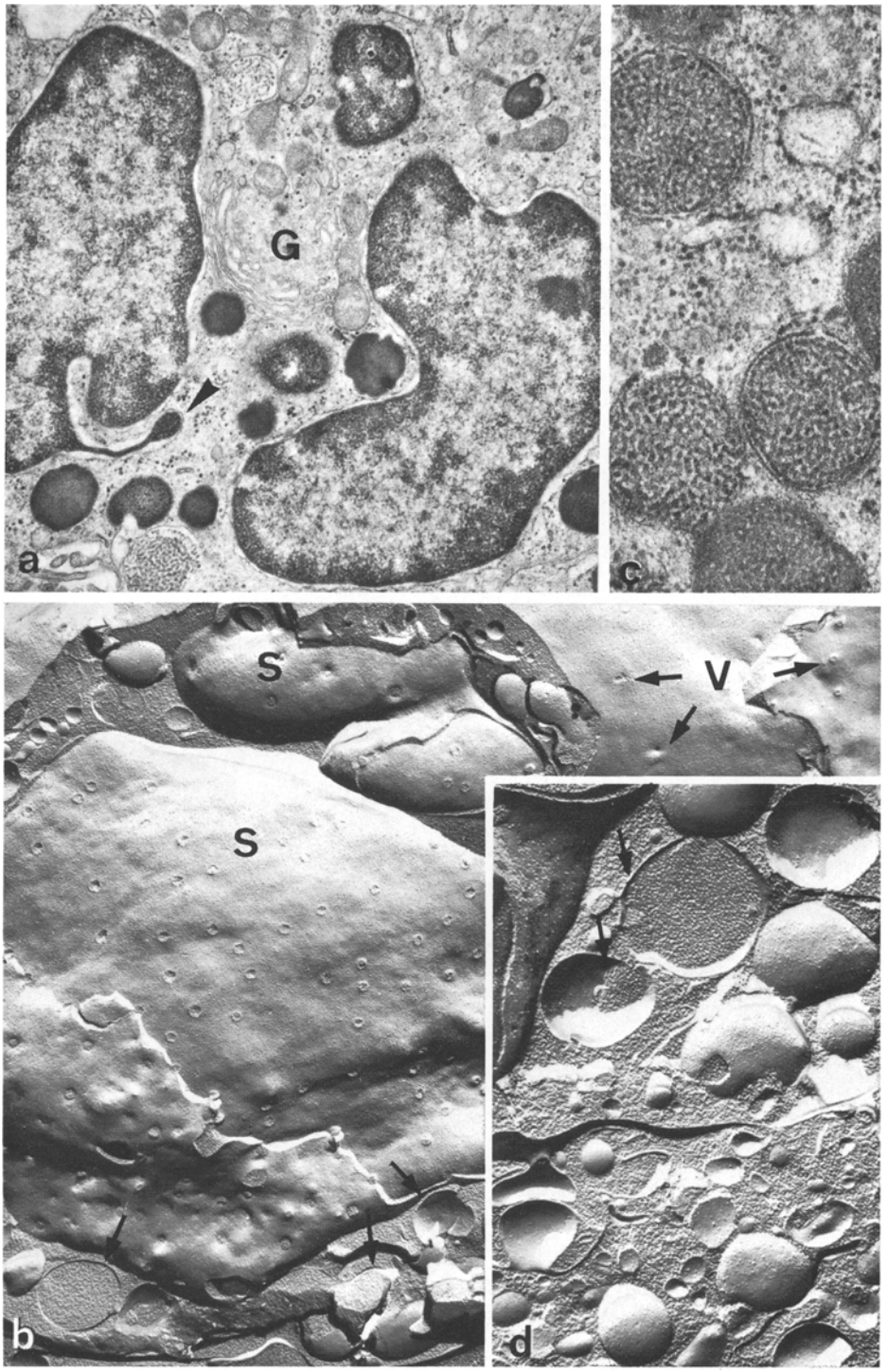
Core biopsies of the iliac crest were obtained and processed using techniques as described by Burkhardt (1966) and Vykoupil et al. (1976) for light microscopy. Fixation and embedding of the bone marrow blocks for electron microscopy, thin sections and freeze-fracture followed methods recorded elsewhere (review by Thiele et al. 1977a, b).

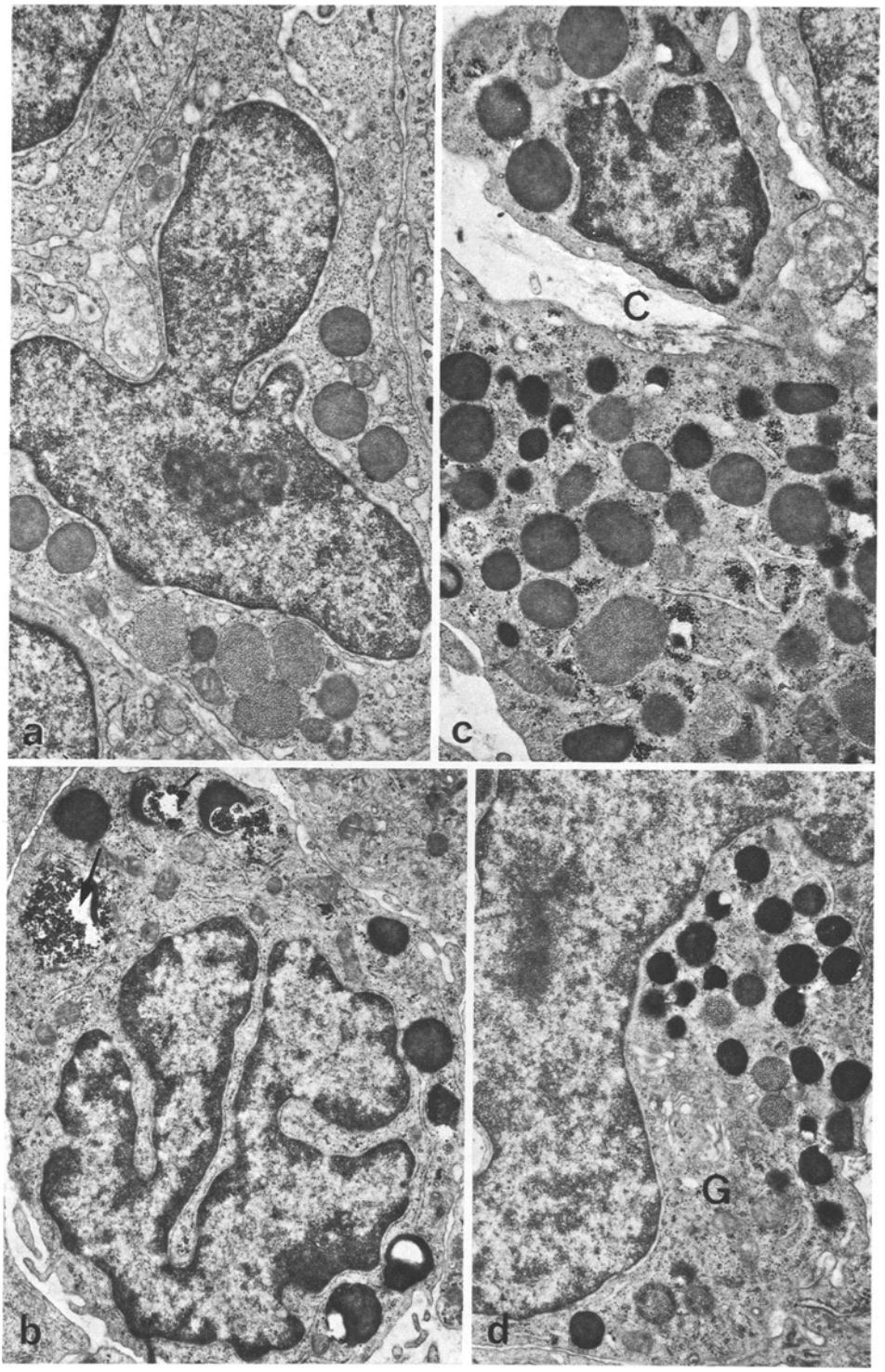
## Results

### *Patient A*

Electron microscopy of the bone marrow from this patient obtained about 2 weeks prior to death displayed a basophilic leukaemia associated with MF and blastic transformation in agreement to light microscopy (compare Fig. 1b with Fig. 2). Between the web-like arrangement of microfilaments and small bundles of collagen fibres, three types of proliferating cells were most conspicuous: basophils and their precursors, blasts and megakaryocytic cells (Fig. 2). Although increased in amount, *basophilic granulopoiesis* consisted of many apparently normal maturing cells (Fig. 3a). Microfilaments and single collagen fibres were almost equally distributed around basophils and atypical proliferating megakaryocytes (Figs. 2, 4c, 5b). Freeze-fracture of mature basophilic granulocytes generally exhibited findings similar to those made in thin sections (Fig. 3b). In particular, the granularity of the specific granules was demonstrated by a regularly particulated cleavage plane (Fig. 3c compare with Fig. 3d).

**Fig. 3a–d.** Normally developed basophilic granulocytes in thin sections and freeze-fracture replicas. **a** Mature basophil with segmented nucleus and drumsticklike nuclear appendage (*arrow head*). Large specific granules with granular content surrounding an extensive Golgi apparatus (*G*). **b** Freeze-fracture shows two exposed nuclear segments with many pores in en-face view (*S*). Large specific granules partially cleaved with granular surface (*arrow*). Plasma membrane with tiny openings of pinocytic vesicles (*V*). **c** Thin section with specific basophilic granules displaying a granular content. **d** A corresponding freeze-fracture replica with large tangentially and two crossly cleaved granules with coarse particulated surface (*arrow*) of a basophilic granulocyte (*upper half*). This is in contrast to the variety of granules of the secondary and specific (tertiary) type of a neutrophilic granulocyte (*lower half*). **a** and **b** ×16,000; **c** ×40,000; **d** ×30,000


G
a
c
S
V
S
b
d

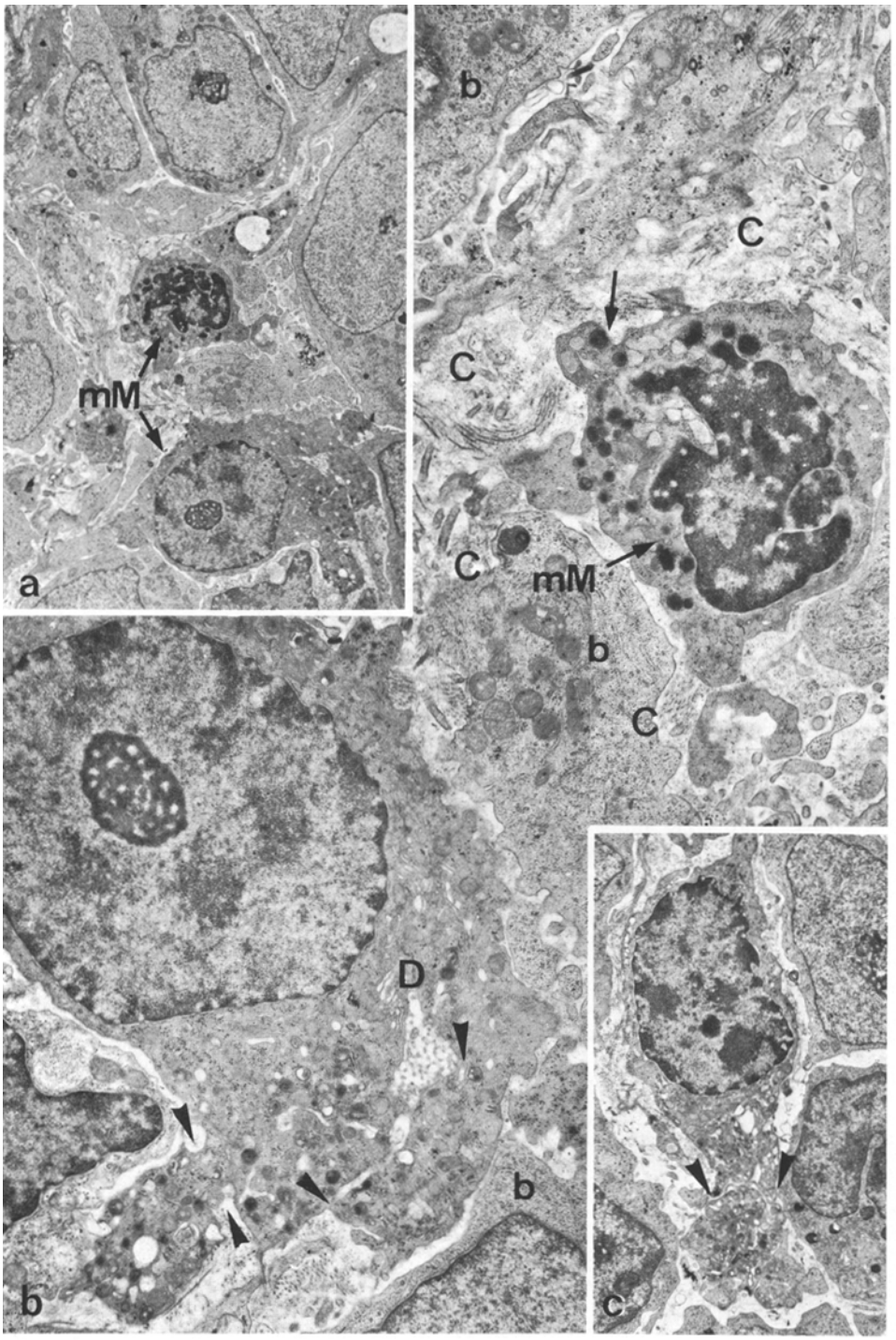

a
C
c
b
G
d

The most conspicuous atypicality however, was an extensive infiltration of the bone marrow by very primitive blast cells, characterised by a large nucleus and a small rim of cytoplasm containing only a few organelles (Figs. 2, 5a, b). Development towards the granulocytic cell line was indicated by the appearance of primary granules that characterised these cells as myeloblasts. The presence of primitive large progranules with a flocculent and granular content however, showed that many of these cells belonged to the basophilic series (Fig. 5a, c).

The normal process of basophilic granulopoiesis was very often altered by several abnormalities which occurred at different stages of maturation and consisted of disorganization of both granulogenesis and nuclear-cytoplasmic development. Most obvious were discrepancies of nuclear segmentation and maturation of cell organelles with atypicalities of specific granules (Fig. 4a–d). Besides the frequent finding of nuclear blebs especially in the more immature forms, there were cells with segmented mature nuclei and only a few primitive granules (progranules) in the cytoplasm (Fig. 4a). On the other hand nuclear segmentation itself was abnormal in some basophils. These displayed a cleaved or radial indentation with incomplete formation of chromatin bridges (Fig. 4b). Maturation anarchy was further demonstrated by remarkable aberrations of granulogenesis. A thorough examination not only showed a predominance of progranules in more mature basophils, but also a considerable variety in the amount and size of specific granules. The content of the granules in the basophils varied between being hypo- or hypergranular and exhibited micro- and macro-forms (Fig. 4c). In some basophil metamyelocytes very small dense homogenous granules resembling the primary (azurophil) or secondary granules of neutrophils (Fig. 4d) were frequently observed.

*Megakaryopoiesis* was atypical, since electron microscopy revealed many immature and numerous micro-forms, not recognizable by light microscopy (Figs. 2, 5a–c). These micromegakaryocytes showed abnormalities consistent with a maturation arrest. Remarkable was the lack of development of a normal demarcation membrane system. This was reduced in most cells to a structure identical to the so called dense compartment (Fig. 5b, review by Thiele et al. 1977b). Nuclear lobulation was deficient or even absent, despite marginal chromatin condensation (Figs. 2, 5a, c). There was a varying amount of specific granulation and evidence of atypical thrombocytogenesis (Fig. 5b, c). Abnormal platelet release sometimes produced giant thrombocytes containing numerous granules and vesi-

**Fig. 4a–d.** Atypicalities of basophil maturation. **a** Basophilic metamyelocyte with early segmentation of nucleus, but primitive large granules with flocculent content (progranules). **b** Cleaved indentation (so called radial segmentation) of the nucleus in an apparently mature basophil. Large specific granules partially containing glycogen *(arrows)*. **c** Microform of a basophil with a few large granules *(upper portion)* and part of a large basophil with many granules of different size and density, i.e., maturation *(lower portion)*. Between these cells small bundles of fibrils *(C)*. **d** Basophilic metamyelocyte with very small mostly dense granules surrounding the Golgi apparatus *(G)*, some of which resemble primary or secondary granules of neutrophils. **a** ×12,000; **b** ×11,000; **c** × 13,000; **d** ×10,000


a
mM
b
C
C
C
mM
b
C
D
b
b
c

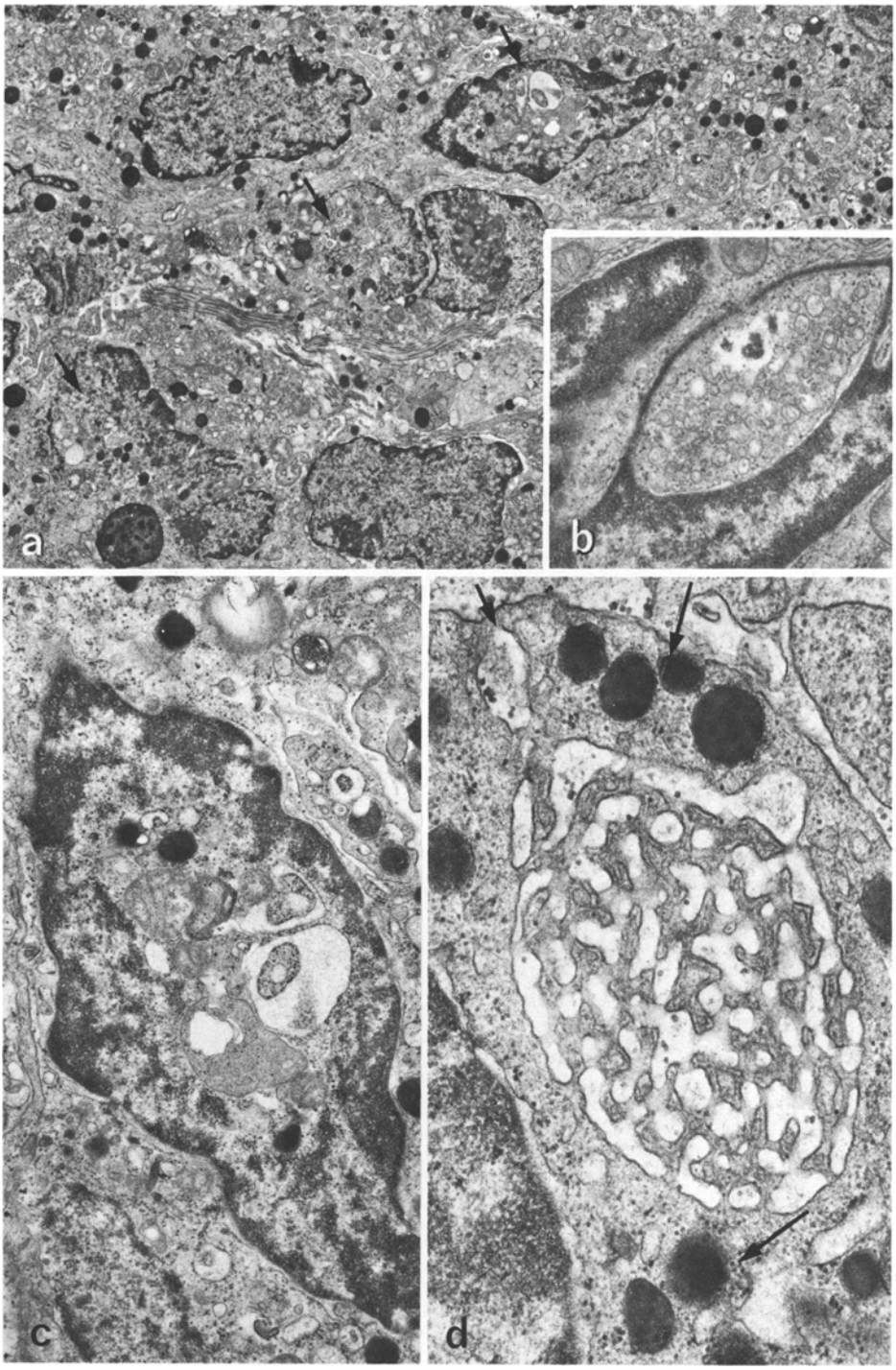
cles. In addition there were some cells with highly lobulated nuclei and condensed chromatin, possibly degenerate forms of micromegakaryocytes (Fig. 5b).

*Patient B*

In agreement with the findings by light microscopy (Fig. 1d), electron microscopy of the bone marrow in the second patient, performed approximately 8 months before death (autumn 1975) showed acute blastic transformation in OMS with multiple abnormal primitive cells surrounded by small bundles of collagen fibres (Fig. 6a). In most instances these myeloid precursor cells bore no resemblance to normal granulopoietic cells, but displayed striking abnormalities such as extensively developed nuclear blebs and loops (Fig. 6b). Sometimes there was a conspicuous nuclear-cytoplasmic disarrangement with an absent demarcation between cytoplasm and its organelles and nuclear chromatin. Thus the nucleus often contained dense bodies and mitochondria (Fig. 6a, c). Besides these numerous blasts there remained some areas of normal granulopoiesis with neutrophil promyelocytes and myelocytes. Another remarkable abnormality of granulocyte maturation was the presence of localised areas of smooth membranes in promyelocytes (Fig. 6d). These structures were connected with the extracellular space and were probably generated by infoldings of the plasma membrane. Sections at different levels revealed a spongy arrangement consisting of flattened interconnecting tubules.

*Megakaryocytes* existed as a considerable population of abnormal elements lying between large bundles of collagen fibrils (Fig. 7a, b) in addition to the various atypicalities of granulopoiesis and the many blast cells. These megakaryocytes were frequently very small and abnormal, so that they could be easily overlooked by light microscopy. The most primitive cell in megakaryopoiesis showed a large nucleus with flocculent chromatin dispersion, several large nucleoli and a relatively small amount of cytoplasm with numerous polysomes. These cells could be characterised as belonging to the megakaryocytic line by their few specific granules of the 'bull's eye' type. Further maturation resulted in the formation of many dense granules, but the demarcation system remained primitive or even absent in most cells (Fig. 7a). Auer rod-like bodies in abortive forms were observed infrequently in a few micromegakaryocytes (Fig. 7b). Mega-

**Fig. 5a–c.** Atypicalities of megakaryocyte maturation. **a** Survey with two micromegakaryocytes *(mM)* surrounded by bundles of fibres and blasts, sometimes containing primitive granules possibly of the basophilic type. **b** A high magnification showing an abnormal micromegakaryocyte *(mM)* with no demarcation system but some specific granules of the bull's eye type *(arrow)*. Below a larger megakaryocyte with a primitive demarcation system *(D)* and abnormal shedding of thrombocytes *(arrow heads)*. There are many bundles of collagen fibres in the surroundings *(C)* and fragments of blasts *(b)*. **c** Micromegakaryocyte with segregation of a thrombocyte (*arrow heads*), surrounded by blast cells, one with several primitive apparently basophilic granules. **a** and **c** × 5,000; **b** ×16,000




**Figs. 6a–d and 7a and b.** Electron microscopy of the bone marrow of *patient B* in 1975 (compare with Fig. 1d)

**Fig. 6. a** Survey with many bizarre blasts embraced by large bundles of collagen fibres. In several cells there is no separation between nuclear chromatin and cytoplasmic organelles *(arrow)*. **b** Abnormal nuclear bleb containing smooth tubular structures in the interior. **c** High magnification of a bizarre blast (from Fig. a) with a nucleus containing mitochondria, dense bodies and cisternae of the rough surfaced endoplasmic reticulum. **d** Promyelocyte with localised area of smooth membranes in connection with the extracellular space *(short arrow)*. This formation closely resembles the primitive demarcation system of megakaryoblasts. Primary granules are indicated by long arrows. **a** ×5,000; **b** ×8,000; **c** ×16,000; **d** ×40,000

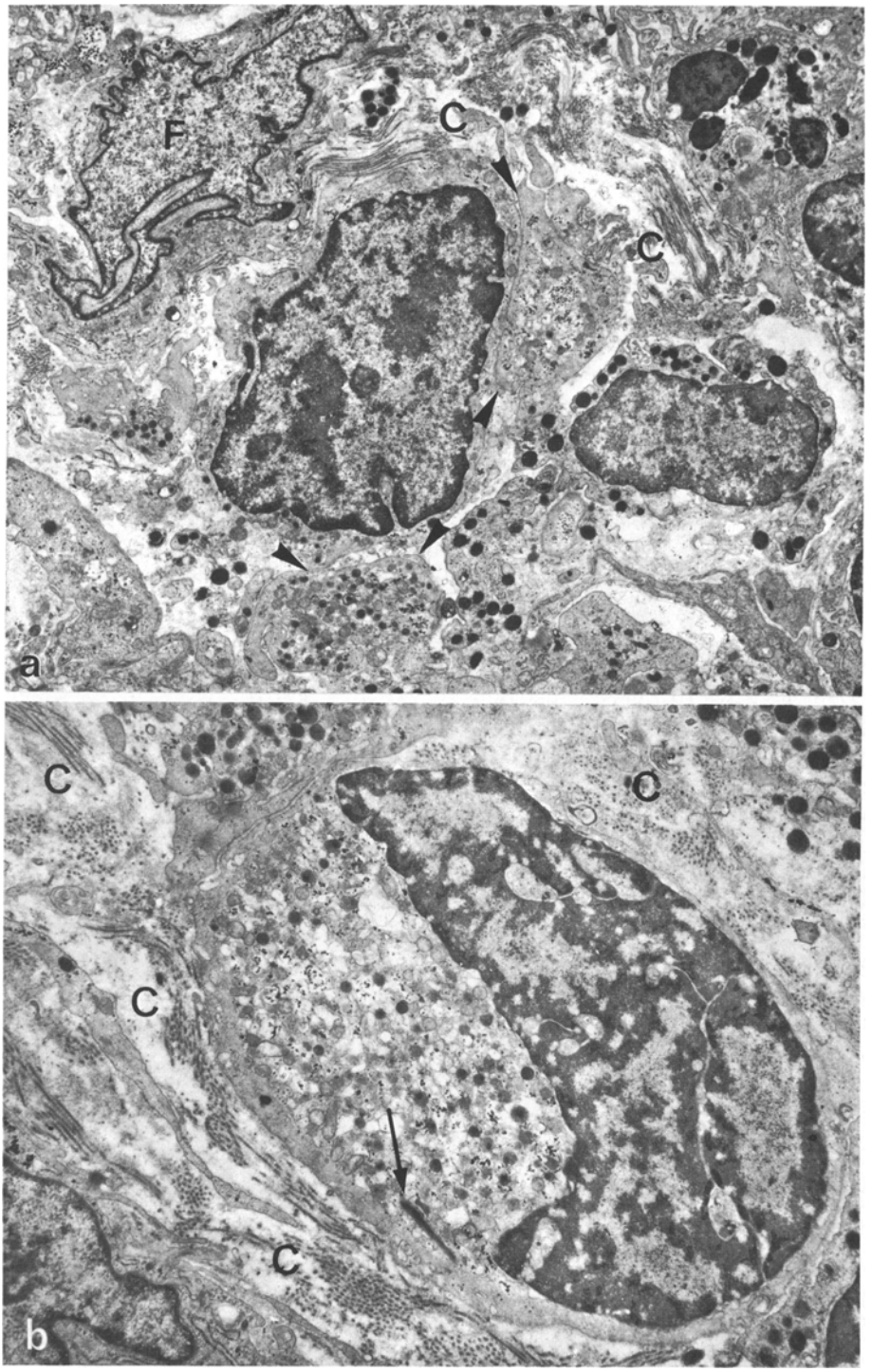



**Fig. 7a and b.** Atypical megakaryopoiesis. **a** Micromegakaryocyte (almost a naked nucleus) with small rim of cytoplasm devoid of organelles, but apparently shedding thrombocytes *(arrow heads)* and surrounded by coarse bundles of collagen fibrils *(C)* and a fibroblast *(F)*. **b** Hyperlobated megakaryocyte with some specific granules few cisternae of the demarcation system and an Auer rod-like body *(arrow)*. There are many collagen fibrils *(C)* in the extracellular space consistent with extensive myelofibrosis/osteomyelosclerosis. **a** ×5,000; **b** ×8,000

karyocyte proliferation was always closely connected with the appearance of fibroblastic cells and bundles of collagen fibrils, besides web-like arranged microfibrils (Fig. 7a, b).

## Discussion

Both patients studied suffered from a chronic myeloproliferative disease that should be called chronic megakaryocytic-granulocytic myelosis (CMGM), since there are two neoplastic cell lines involved in this subtype of CML (Georgii and Vykoupil 1976; Georgii 1979). Both were terminated by a blastic crisis and associated with varying degrees of MF and OMS. Light microscopy showed that blastic transformation predominantly involved the basophilic line in the one *(patient A)* and the neutrophilic series in the other patient *(patient B)*. In addition electron microscopy demonstrated proliferation of atypical megakaryocytic cells and various abnormalities in the other cell types.

Consideration of the clinical and morphological evidence available over a relatively long period suggests that the CMGM started in *patient A* approximately 4 years and in *patient B* about 10 years before evolution of the final stage of blastic crisis. This long survival is remarkable since it clearly exceeds the average survival data calculated for patients with CML receiving chemotherapy as in our cases (reviews by Jacquillat et al. 1975 and Sokal 1976). As stated by Georgii (1979) a prolonged prediagnostic period points to an insiduous onset and clinical findings are suggestive of a more prolonged natural course in CMGM, by comparison with chronic granulocytic leukaemia (CGL). In *patient A,* MF proceeded from a very slight degree to an overt fibrosis obliterating the bone marrow space (Table 1). Although cytostatics were used for quite a long time in the treatment of these patients, it has been presumed that the development and progression of MF are hardly influenced by this therapy (Schäfer et al. 1975).

Several authors (Allegra and Broderick 1971; Georgii and Vykoupil 1972, 1976; Estevez 1974; Maldonado et al. 1974; Breton-Gorius et al. 1978; Bain et al. 1977; Thiele et al. 1977a) have undoubtedly established the existence of a megakaryocytic-granulocytic myelosis or megakaryoblastic transformation of CML respectively, based on the morphological as well as the clinical findings. In a minority of patients this myeloproliferative disease may follow an acute course called acute megakaryocytic myelosis or acute (malignant) myelofibrosis (Guichard et al. 1956, Demmler et al. 1970; Den Ottolander et al. 1979). More frequently however, the course is chronic (Prechtel et al. 1977; Georgii 1979) and may then proceed to different stages of MF, or terminate in a blastic crisis. Many of these results are based on histomorphology of the bone marrow, and a review of the illustrations of the biopsies presented in the papers of Krauss (1966), Bird and Proctor (1977), Fabich and Raich (1977), Buyssens and Bourgeois (1977), Cheng (1979), Bearman et al. (1979) and Den Ottolander et al. (1979) leads to the assumption that at least some of those cases of so called idiopathic myelofibrosis or myelosclerosis closely resemble CMGM or a terminal blastic phase of this disorder respectively.

Despite the frequently used term 'aleukaemic megakaryocytic leukaemia' (Allegra and Broderick 1971), there is strong evidence that many of these myeloses actually present a leukaemic form, since micro-megakaryocytes in the peripheral blood smear may be erroneously mistaken for monocytic, blastic, mononuclear, or lymphocytic cells, when studied by light microscopy (Maldonado 1974; Huhn and Ascher 1975; Bain et al. 1977; Breton-Gorius et al. 1978).

The conspicuous development of MF during the course of CMGM is thought to be a non-specific response to an abnormal cellular proliferation as was stated by a panel of several scientists (review by Block et al. 1975), rather than an integral part of the neoplastic process (Sanerkin 1964). This statement is supported by chromosomal findings (Maniatis et al. 1969; van Slyck 1970; de la Chapelle et al. 1973), enzymatic studies (Fialkow et al. 1977; Jacobson et al. 1978), and clinical observations (Bird and Proctor 1977). Ultrastructural investigations extend this concept of a reactive fibrosis, since it suggests that a possibly important role is played by megakaryoblasts in the initial steps of fibrillogenesis. Atypical megakaryocytes are thought to destroy normal bone marrow circulation by intrasinusoidal infiltration with consequent scarring (Thiele et al. 1977a). Release of biogenic amines from fragmented abnormal thrombocytes may be an additional factor in initiating fibre formation, as emphasized by Zucker-Franklin (1975). All these assumptions take into account that fibrillogenesis and consequent MF are usually associated with megakaryocytic proliferation, as stated by several authors (Block et al. 1975) and finally results in a scirrhous-like (Lennert 1964) pattern of growth with endophytic bone formation (OMS).

The majority of *basophilic leukaemias* described previously (Rosenthal et al. 1977) may be classified as accelerated terminal phases of CML, or so-called secondary basophilic leukaemias (Parwaresch 1976b). This leukaemic proliferation of blood basophils has to be separated from tissue mast cell leukaemia or mast cell reticulosis (systemic malignant mastocytosis) as reviewed by Coser et al. (1980). The basophilic leukaemias which terminate CML may display a striking MF (Lennert 1956; Lennert et al. 1956; Lennert and Mohri 1971). Further, in CML an increased basophil count in the peripheral blood may be related to the later development of a blastic crisis and, as in our patient, to the transient flushing episodes which are thought to be due to an excessive histamine discharge (Jacquillat et al. 1975). Elevated values of histamine have been measured in CML in several patients, and basophil precursor cells shown to be present in increased numbers in the peripheral blood as observed by serial tissue cultures (Miyoshi et al. 1977; Denburg et al. 1980).

Clinical and light microscopy findings in the patients recorded by Lennert et al. (1956) and Rosenthal et al. (1977) may be compared with our *patient A*. Our patient however, was actually shown to suffer from an acute basophilic crisis of CMGM as confirmed by the sequential biopsies of the bone marrow and the clinical course. MF in these cases of secondary basophilic leukaemia is thought to originate from release of biogenic amines, hyaluronic acid and related compounds from the specific granules of the atypical proliferating basophilic granulocytes (Lennert et al. 1956). As discussed above, this may not be the only mechanism in the development of MF, since atypical proliferation of megakaryocytes and fragmentation of abnormal thrombocytes may be another

aetiological factor (Zucker-Franklin 1975). On the other hand, no conspicuous accumulations of fibrin (so called fibrin stars) could be detected in these and other cases of MF in resin embedded bone marrow specimens (Thiele et al. 1977a) which were emphasized by Lennert et al. (1975) as the source of fibrillogenesis.

Malignant growth of our cell population with accompanying MF/OMS is established for certain by the blastic transformation of the granulocytic lineage (review by Pedersen 1973). Additional signs of a malignant proliferation are the atypical cellular maturation with the formation of abnormal granules, such as the abortive Auer rods in megakaryocytes, and the nuclear blebs which may be an expression of aneuploidy (Ahearn et al. 1974). This very frequent occurrence represents a disturbance of DNA synthesis which may be at least partially due to the cytostatic therapy (Stalzer et al. 1965; Ahearn et al. 1967). Further evidence for the suggested malignant granulo- and megakaryopoiesis are the striking atypicalities of nuclear-cytoplasmic maturation leading to anarchy of cellular differentiation, particularly of granulogenesis, which may be observed in various kinds of leukaemic cells (review by Bessis and Breton-Gorius 1969). Special attention should be directed towards the unique sponge like complexes of smooth membranes in the atypical promyelocytes of *patient B*, resembling the dense compartments of megakaryoblasts (review by Thiele et al. 1977b) and structures described by Breton-Gorius et al. (1976) in promyelocytes in so called preleukaemia evolving into acute myeloid leukaemia.

In contrast with the elevated leukocyte alkaline phosphatase recorded in CML with associated MF/OMS (Gralnick et al. 1971) or acute and chronic MF with myeloid metaplasia (Mitus and Kiossoglou 1968), we found low values in both our patients which is rather exceptional among the many other cases of CMGM and MF/OMS in our own material. This may be due to the blastic transformation which produces many atypical granulocytes with deficient, abnormal or even absent specific granules (Bohinjec 1976), thus creating a cell population resembling more an acute leukaemia with its frequent low phosphatase index.

There are several clinical reports dealing with basophilic leukaemias as discussed above (Barlas 1954; Lennert et al. 1956; Quattrin et al. 1959; Schubert and Martin 1968a, b; Quattrin 1973; Rosenthal et al. 1977), but electron microscopy of these leukaemic basophils and their cellular maturation has very rarely been described. In general agreement with the results of Parwaresch (1976a, b), we encountered most of the abnormalities that have already been recorded in leukaemic cells of others than the basophilic line (see above). In comparison with normal basophilic granule development (review by Zucker-Franklin 1967) specific granules in neoplastic basophils display much variation in size, inner structure, and density, thus confirming the observations of Parwaresch (1976b). Disorganization of maturation was particularly striking in relation to nuclear segmentation, where so-called radial indentation was present, a frequent finding in other leukaemic cells (Norberg and Söderström 1967).

Our clinical and morphological observations support the concept of a myelofibrosis accompanying chronic megakaryocytic-granulocytic myelosis with a potential evolution of a blastic crisis involving several lines, but with a predomi-

nance of one population (basophilic and neutrophilic granulocytes in our two patients).

*Acknowledgements.* We are indebted to Dr. T. Wolff, Hannover *(patient A)* and Dr. K. Mainzer, Hamburg *(patient B)* for providing the clinical data and the bone marrow specimens of their patients. We thank Prof. E. Reale, Hannover, for his support of our ultrastructural investigations. The excellent technical assistance of Mrs. M. Rißmann and Ms. H. Glinzer is acknowledged.

## References

Ahearn MJ, Lewis CW, Campell LA, Luce JK (1967) Nuclear bleb formation in human bone marrow cells during cytosine arabinoside therapy. Nature (London) 215:196–197

Ahearn MJ, Trujillo JM, Cork A, Fowler A, Hart JS (1974) The association of nuclear blebs with aneuploidy in human acute leukemia. Cancer Res 34:2887–2896

Allegra SR, Broderick PA (1971) Acute aleukemic megakaryocytic leukemia: report of a case. Am J Clin Pathol 55:197–105

Bain B, Catovsky D, O'Brien M, Spiers ASD, Richards HGH (1977) Megakaryoblastic transformation of chronic granulocytic leukaemia. An electron microscopy and cytochemical study. J Clin Pathol 30:235–242

Barlas O (1954) A propos d'un cas de leukémie chronique à basophiles. Sang 25:147–156

Bearman RM, Pangalis GA, Rappaport H (1979) Acute ("malignant") myelosclerosis. Cancer 43:279–293

Bird T, Proctor SJ (1977) Malignant myelosclerosis. Myeloproliferative disorder or leukemia? Am J Clin Pathol 67:512–520

Bessis M, Breton-Gorius J (1969) Pathologie et asynchronisme de développement des organelles cellulaires au cours des leucémies aiguës granulocytaires. Etude au microscope électronique. Nouv Rev Franc d'Hematol 9:245–278

Block M, Burkhardt R, Celloul N, Demmler K, Duhamel G, Georgii A, Kirsten WH, Lennert K, Nézelof C, Te Velde J (1975) Pathology and morphology (Working paper). In: Bernhard S, Saar U (eds) Proceedings of the Dahlem Workshop on Myelofibrosis-Osteosclerosis-Syndrome. Advances in the Biosciences 16. Pergamon Press Vieweg, Berlin, p 219–254

Breton-Gorius J, Coquin Y, Vilde JL, Dreyfus B (1976) Cytochemical and ultrastructural studies of aberrant granules in the neutrophils of two patients with myeloperoxidase deficiency during a preleukemic state. Relationship to abnormal bactericidal activity. Blood Cells 2:187–209

Breton-Gorius J, Reyes F, Duhamel G, Najman A, Gorin NC (1978) Megakaryoblastic acute leukemia: identification by the ultrastructural demonstration of platelet peroxidase. Blood 51:45–69

Bohinjec J (1976) Zytochemie der Blasten beim Blastenschub der chronischen myeloischen Leukämie und der Osteomyelosklerose. In: Stacher A, Höcker P (eds) Erkrankungen der Myelopoese. Leukämie, myeloproliferatives Syndrom, Polyzythämie. Urban & Schwarzenberg, München-Berlin-Wien, p 384–386

Bouroncle BA, Doan CA (1962) Myelofibrosis. Clinical, hematologic and pathologic study of 110 patients. Am J Med Sci 243:698–715

Burkhardt R (1966) Präparative Voraussetzungen zur klinischen Histologie des menschlichen Knochenmarkes. Blut 14:30–46

Buyssens N, Bourgeois NH (1977) Chronic myelocytic leukemia versus idiopathic myelofibrosis. A diagnostic problem in bone marrow biopsies. Cancer 40:1548–1561

Cheng DS (1979) Idiopathic myelofibrosis without splenomegaly. Cancer 43:1761–1765

Coser P, Quaglino D, De Pasquale A, Colombetti V, Prinoth O (1980) Cytobiological and clinical aspects of tissue mast cell leukaemia. Br J Haematol 45:5–12

Dameshek W (1951) Some speculations on the myeloproliferative syndromes. Blood 2:372–375

De la Chapelle A, Vuopio P, Brogström GH (1973) The origin of bone marrow fibroblasts. Blood 41:783–787

Demmler K, Burkhardt R, Prechtel K (1970) Megakaryoblastische Myelose. Klin Wochenschr 48:1168–1173

Denburg JA, Wilson WEC, Goodacre R, Bienenstock J (1980) Chronic myeloid leukaemia: evidence for basophil differentiation and histamine synthesis from cultured peripheral blood cells. Br J Haematol 45:13–21

Den Ottolander GJ, Te Velde J, Brederoo P, Geraedts JPM, Slee PHT, Willemze R, Zwaan FE, Haak HL, Muller HP, Bieger R (1979) Megakaryoblastic leukaemia (acute myelofibrosis): a report of three cases. Br J Haematol 42:9–20

Estevez JM, Urueta EE, Moran TJ (1974) Acute megakaryocytic myelofibrosis. Case report of an unusual myeloproliferative syndrome. Am J Clin Pathol 62:52–59

Fabich DR, Raich PC (1977) Acute Myelofibrosis. A report of three cases. Am J Clin Pathol 67:334–338

Fialkow PJ, Jacobson RJ, Papayannopoulou T (1977) Chronic myelocytic leukemia: clonal origin in a stem cell common to the granulocyte, erythrocyte, platelet and monocyte/macrophage. Am J Med 63:125–130

Georgii A (1979) Histopathology of bone marrow in human chronic leukemias. In: Neth R, Gallo RC, Hofschneider P-H, Mannweiler K (eds) Modern trends in human leukemia III. Springer, Berlin-Heidelberg-New York, p 59–70

Georgii A, Vykoupil KF (1972) Pathologische Anatomie der megakaryocytären Myelose. In: Gross R, van de Loo J (eds) Leukämie. Springer, Berlin-Heidelberg-New York, p 25–28

Georgii A, Vykoupil KF (1976) Histologisch-bioptische Klassifizierung myeloproliferativer Erkrankungen. In: Stacher A, Höcker P (eds) Erkrankungen der Myelopoese. Leukämie, myeloproliferatives Syndrom, Polyzythämie. Urban & Schwarzenberg, München-Berlin-Wien, p 47–58

Gralnick HR, Harbor J, Vogel C (1971) Myelofibrosis in chronic granulocytic leukemia. Blood 37:152–162

Guichard A, Fayolle J, Alex R, Revol A (1956) Myélose aleucémique décalcificante à plaquettes et à mégacaryocytes; la leucémie à plaquettes. Sang 27:337–351

Heller EL, Lewisohn MG, Palin WE (1947) Aleukemic myelosis. Chronic non leukemic myelosis, agnogenic myeloid metaplasia, osteosclerosis, leuko-erythroblastic anemia and synonymous designations. Am J Pathol 23:327–365

Hickling RA (1968) The natural history of chronic non-leukaemic myelosis. Quart J Med 37:267–279

Huhn D, Ascher S (1975) Mikrokaryoblastenschub bei chronischer Myelose. Acta Haematol 53:183–190

Hunstein W, Hauswaldt Ch (1974) Die Osteomyelofibrose. Klin Wochenschr 52:305–317

Jacobson RJ, Salo A, Fialkow PJ (1978) Agnogenic myeloid metaplasia: a clonal proliferation of hematopoietic stem cells with secondary myelofibrosis. Blood 51:189–194

Jacquillat C, Chastang C, Tanzer J, Briere J, Weil M, Pereira-Neto M, Gemon-Auclerc MF, Schaison G, Domingo A, Boiron M, Bernard J (1975) Facteurs de prognostic de la leucémie myéloïde chronique. A propos de 798 observations. Nouv Rev Franc Hématol 15:229–240

Krauss S (1966) Chronic myelocytic leukemia with features simulating myelofibrosis with myeloid metaplasia. Cancer 19:1321–1332

Lennert K (1956) Eine mastocytoide Osteomyeloreticulose (Mastzellenreticulose). IV. Kongreß der Europ. Ges. Hämatologie, Freiburg, Sept. 1955. Springer, Berlin-Göttingen-Heidelberg, p 573–575

Lennert K, Köster E, Martin H (1956) Über die Mastzellenleukämie. Acta Haematol 16:255–272

Lennert K (1964) Die pathologische Anatomie der Osteomyelosklerose. X. Tagung der Dtsch Ges Hämat Tübingen, Oktober 1964

Lennert K, Mohri N (1971) Zur Pathologie der Leukämien und malignen Lymphome im Kindesalter. Verh Dtsch Ges Path 55:216–269

Lennert K, Nagai K, Schwarze E-W (1975) Patho-anatomical features of the bone marrow. In: Videbaek A (ed) Clinics in haematology. WB Saunders Com Ltd, London-Philadelphia-Toronto, p 331–351

Lewis SM, Szur L (1963) Malignant myelosclerosis. Br Med J 2:472–477

Lubin J, Rozen S, Rywlin AM (1976) Malignant myelosclerosis. Arch Intern Med 136:141–145

Maldonado JE, Pintado T, Pierre RV (1974) Dysplastic platelets and circulating megakaryocytes in chronic myeloproliferative disease. I. The platelets: ultrastructure and peroxidase reaction. Blood 43:797–809

Maldonado JE (1974) Dysplastic platelets and circulating megakaryocytes in chronic myeloproliferative disease. II. Ultrastructure of circulating megakaryocytes. Blood 43:811–820

Maniatis AK, Amsel S, Mitus WJ, Coleman N (1969) Chromosome pattern of bone marrow fibroblasts in patients with chronic granulocytic leukaemia. Nature (London) 222:1278–1279
Mitus WJ, Kiossoglou KA (1968) Leukocytic alkaline phosphatase in myeloproliferative syndrome. Ann NY Acad Sci 155:976–979
Miyoshi I, Uchida H, Tsubota T, Kubonishi I, Hiraki S, Kitajima K (1977) Basophilic differentiation of chronic myelogenous leukaemia cells in vitro. Scand J Haematol 19:321–326
Norberg B, Söderström N (1967) "Radial segmentation" of the nuclei in lymphocytes and other blood cells induced by some anticoagulants. Scand J Haematol 4:68–76
Parwaresch MR (1976a) Ultrastruktur und Zytochemie der leukämischen Blutbasophilen. In: Stacher A, Höcker P (eds) Erkrankungen der Myelopoese. Leukämie, myeloproliferatives Syndrom, Polyzythämie. Verlag Urban & Schwarzenberg, München-Berlin-Wien, p 405–406
Parwaresch MR (1976b) The human blood basophil. Morphology, origin, kinetics, function and pathology. Springer, Berlin-Heidelberg-New York
Pedersen B (1973) The blastic crisis of chronic myeloid leukaemia: acute transformation of a leukaemic condition? Br J Haematol 25:141–145
Prechtel K, Beil E, Kronseder A (1977) Megakaryozytäre Myelose. Klinik und Morphologie. Dtsch med Wochenschr 102:853–857
Quattrin N, Dini E, Palumbo E (1959) Basophile Leukämien. Blut 5:166–187
Quattrin N (1973) Leucémies aigues à basophiles. Nouv Rev Franc Hématol 13:745–754
Rosenthal S, Schwartz JH, Canellos GP (1977) Basophilic chronic granulocytic leukaemia with hyperhistaminaemia. Br J Haematol 36:367–372
Sanerkin NG (1964) Stromal changes in leukaemic and related bone marrow proliferations. J Clin Pathol 17:541–547
Schäfer R, Schneider H-M, Hill K (1975) Histologisch-bioptische Knochenmarksbefunde bei behandelten und unbehandelten Myelosen. Verh Dtsch Ges Path 59:478
Schubert JFC, Martin H (1968a) Beobachtungen an sieben Kranken mit "Blutmastzell-Leukämie". Klin Wochenschr 46:929–936
Schubert JFC, Martin H (1968b) Beobachtungen bei Blutmastzell-Leukämie. Blut 18:35–39
Silverstein MN, Brown AL Jr, Linman JW (1973) Idiopathic myeloid metaplasia. Its evolution into acute leukemia. Arch Intern Med 132:709–712
Sokal JE (1976) Evaluation of survival data for chronic myelocytic leukemia. Am J Hematol 1:493–500
Stalzer RC, Kiely JM, Pease GL, Brown AL Jr (1965) Effect of 5-Fluorouracil on human hematopoiesis. Cancer 18:1071–1078
Taylor HE, Simpson WW (1950) Bone marrow fibrosis developing in aleukemic myelosis. Blood 5:348–357
Thiele J, Ballard A-Ch, Vykoupil KF, Georgii A (1977a) Chronic megakaryocytic-granulocytic myelosis: an electron microscopic study. I. Megakaryocytes and thrombocytes. Virchows Arch. A Path Anat and Histol 373:191–211
Thiele J, Ballard A-Ch, Georgii A (1977b) Freeze fracture of the normal and pathologic megakaryocytic lineage in chronic megakaryocytic-granulocytic myelosis. Virchows Arch. B Cell Path 23:33–51
Van Slyck EJ, Weiss L, Dully M (1970) Chromosomal evidence for the secondary role of fibroblastic proliferation in acute myelofibrosis. Blood 36:729–735
Vykoupil KF, Thiele J, Georgii A (1976) Histochemical and immunological techniques on acrylate embedded bone biopsies. Blut 32:215–218
Zucker-Franklin D (1967) Electron microscopic study of human basophils. Blood 29:878–890
Zucker-Franklin D (1975) Ultrastructural studies of hematopoietic elements in relation to the myelofibrosis-osteosclerosis-syndrome, megakaryocytes and platelets (MMM or MOS). In: Bernhard S, Saar U (eds) Proceedings of the Dahlem Workshop on Myelofibrosis-Osteosclerosis-Syndrome. Advances in the Biosciences 16. Pergamon Press Vieweg, Berlin, p 127–141

Accepted September 25, 1980