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Hairy Cell Leukemia

Bone Marrow Findings in 24 Patients*

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Summary. In 24 patients with hairy cell leukemia, histological and fine structural findings from biopsies of the bone marrow are reported and their validity is compared with other diagnostic procedures available. Diagnosis by *light microscopy* of anterior iliac crest biopsies obtained by the method of myelotomy is possible with a high degree of accuracy. The differentiation of hairy cell leukemia from other myelo- or lymphoproliferative disorders based on cytomorphology as well as patterns of growth is emphasized. Morphological differences between fibrosis in this entity and other lesions such as malignant lymphomas, Hodgkin's disease, osteomyelofibrosis and -sclerosis are emphasized.

Electron microscopy of the bone marrow shows single fibroblastic cells with numerous slender cytoplasmic processes randomly dispersed among the hairy cells. These fibroblasts are probably responsible for the synthesis of the reticulin and collagen fibres in their surroundings. Moreover fine structure of the hairy cells demonstrates pinocytic activity but no apparent phagocytosis in contrast to the phagocytic reticulum cells (histiocytes, macrophages). In the bone marrow the precursor cells and the many immature forms of hairy cells exhibit an overall lymphocytoid appearance during their maturation, suggesting a lymphocytic origin.

Key words: Bone marrow biopsy — Hairy cell leukemia — Differential diagnosis — Myelofibrosis — Electron microscopy.

Among the neoplastic disorders of the bone marrow, hairy cell leukemia (HCL) has been established as a definite clinical and pathologic entity. Until recently this haemoblastosis was described under various headings such as primitive histiolymphocytosis, lymphoid myelofibrosis, chronic reticulolymphocytic leukemia, reticulum cell leukemia and leukemic reticuloendotheliosis (review by Mende et al., 1975). Following the demonstration of "hairy cells" (HCs) by Schrek and Donnelly (1966) this disease has attracted the interests of many authors (review by Möbius et al., 1975). Several of these reports deal with clinical findings (review by Flandrin et al., 1973), electron microscopy of HCs (review by Daniel and Flandrin, 1974), with cytochemistry (review by Schaefer et al., 1975) and with other functional and cytological characteristics (review by Haak et al., 1974). Despite all these efforts it has not been unequivocally decided which category the proliferating cells of HCL belong to, whether they are of lymphocytic or histio-monocytic origin.

Clinical symptoms are vague. HCL is often characterized by an unexplained anemia or pancytopenia, splenomegaly, an unsuccessful bone marrow aspiration (dry tap) and pathologically by HCs in the peripheral blood with an infiltration

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of liver, spleen, bone marrow, and even lymph nodes. In many patients the clinical diagnosis is rather difficult to establish probably because of the insiduous onset and the invariably chronic course of this disease. For this reason reliance must be placed on the distinctive bone marrow biopsy findings. The extensive morphological study of Burke et al. (1974) does not take this diagnostic approach into consideration. Other authors emphasize the difficulties in the differentiation of HCL particularly when accompanied by fibrosis from other myelofibrotic disorders (Düllmann et al., 1974; Mende et al., 1975; Möbius et al., 1975). Bone marrow biopsies from patients with HCL are found to offer a reliable diagnostic tool as will be shown in our 24 patients. In addition, electron microscopy of large pieces of intact bone marrow obtained by the method of myelotomy presents some new features indicative of the origin of HCs, as well as for the development of fibres that simulate myelofibrosis in this disease.

Material and Methods

This study is based on the analysis of the bone marrow in 24 patients with HCL, the sex ratio is 14 (M): 10 (F), the mean age 55 years (range 31–74 years). The various clinical diagnoses preceding the biopsies of the bone marrow are given in Table 1. Some clinical symptoms suggestive of a haematological disorder are presented in Table 2. All bone biopsies were obtained by the method of myelotomy with a drilling device developed by Burkhardt (1966) which mills a bone cylinder up to 20 mm in length and 4 mm in width from the anterior iliac crest. Fixation and embedding was performed following the procedures described by Burkhardt (1966) and Vykoupil et al. (1976). To semithin plastic embedded sections the following staining methods were applied: Giemsa, silver impregnation for reticulin fibres after Gomori, trichrome stain after Goldner, periodic acid Schiff reagent (PAS), Prussian blue reaction for iron and naphthol-AS-D-chloroacetate-esterase (Leder, 1964).

For electron microscopy a second myelotomy was performed in 3 patients (cases: 15, 16, 17) and the bone cylinders dissected under a stereo microscope to seperate large pieces of intact bone marrow from the trabecula. Fixation was done in an aldehyde solution (Cotran and Karnovsky, 1968) and phosphate buffered osmium tetroxide (Millonig, 1961), with dehydration in ethanol and embedding in Epon (Luft, 1961). Thin sections were stained with uranyl acetate (Watson, 1958) followed by lead citrate (Venable and Coggeshall, 1965). In addition in 4 patients (cases: 15, 16, 17, 20) the spleen had been resected and was available for study in paraffin and acrylate embedded tissue blocks. In only one patient (case 22) was a post mortem examination performed. Further clinical data and laboratory findings confirmed the diagnosis HCL in all cases.

Results

Light microscopy of the bone marrow shows a diffuse or in very early cases a more patchy infiltration, by uniform mononuclear cells almost entirely replacing normal haemopoiesis (Figs. 1a and b). Small islands of granulopoiesis may be found between the neoplastic cells located mainly around the bony trabecula and the vessels. Megakaryocytes are extremely reduced, mast cells and plasma cells are increased in number and interspersed among the HCs, but they are never as numerous as in Waldenström's disease. Clusters of lymphocytes among the HCs are distinguishable by their compact arrangement and smooth outlines (Fig. 1c). Although bone marrow infiltration by HCs is very dense and homogenous in distribution, the characteristic loose and spongy arrangement of these cells is preserved (Figs. 1a–d, 2a). The reticulum like arrangement of HCs is

Table 1. Sex and age distribution of the 24 patients with "hairy cell leukemia" and their clinical diagnoses prior to bone marrow biopsy

Case	Initials	Sex	Age (years)	Clinical diagnosis	Case	Initials	Sex	Age (years)	Clinical diagnosis
1	S.G.	♀	59	CLL	13	M.A.	♂	65	OMF
2	B.S.	♀	66	PP	14	W.H.	♂	49	OMF
3	F.H.	♂	51	CLL	15	W.E.	♂	53	A
4	P.M.	♀	63	OMF	16	F.H.-D.	♂	48	A
5	Z.H.	♂	31	OMF	17	K.E.	♀	56	HCL
6	R.H.	♀	63	A	18	G.A.	♂	49	HCL
7	D.C.	♀	67	A	19	M.E.	♂	69	CLL
8	G.A.	♂	61	CLL	20	F.W.	♂	49	PP
9	F.M.	♀	47	OMF	21	H.M.	♀	51	CLL
10	A.H.-J.	♂	33	HCL	22	T.E.	♀	64	CLL
11	B.H.	♀	48	OMF	23	O.F.	♂	74	OMF
12	R.A.	♂	46	AL	24	F.L.	♂	65	A

CLL: chronic lymphocytic leukemia (6); OMF: osteomyelofibrosis (7); A: anemia (5); HCL: hairy cell leukemia (3); PP: pancytopenia (2); AL: acute leukemia (1)

Table 2. Frequency of some clinical symptoms presented by the 24 patients with hairy cell leukemia on admission which were suggestive of a haematological disorder

Clinical symptoms	Frequency	Clinical symptoms	Frequency
anemia	all	leukopenia	most (17/24)
splenomegaly	all	hepatomegaly	some (7/24)
dry tap (punctio sicca)	all	lymph node enlargement	some (7/24)
thrombocytopenia	all	leucocytosis	few (2/24)

probably caused by the many villous cytoplasmic processes of the HCs leading to a close attachment to adjacent cells, which is particularly conspicuous in very thin sections (Fig. 1d). This is a very distinctive pattern of growth that enables differentiation of HCL from other kinds of neoplastic cell infiltration of the bone marrow, such as chronic lymphocytic leukemia (CLL), myelo-monocytic, or myeloblastic leukemias, and malignant lymphomas (Figs. 2–3a–d): CLL is composed in its diffuse form of a homogenous small rounded population which never displays such a network like pattern of growth (Fig. 2b). The diffuse type of cellular proliferation in chronic myelo-monocytic leukemia (Fig. 2c) does not exhibit an uniform reticulum of neoplastic cells as observed in HCL. The malignant lymphomas, e.g. the lympho-plasmacytic (Fig. 2d) or the well differentiated lymphocytic types (Fig. 3a) are easily distinguished from HCL by their cytological characteristics, which in case of HCs are the many hair-like cytoplasmic protrusions seen in light microscopy. HCL does not form nodular arrangements, but shows a diffuse involvement of the bone marrow space. Consequently it should not be confused with any type of nodular lymphoma e.g. early CLL and other lymphocytic or follicle centre cell (FCC)-types of lymphomas (Figs. 3c and d). Reactive lymphatic hyperplasias (Fig. 3b) such as occur in cases of rheumatoid arthritis may be also distinguished from HCL by their germinal centres and

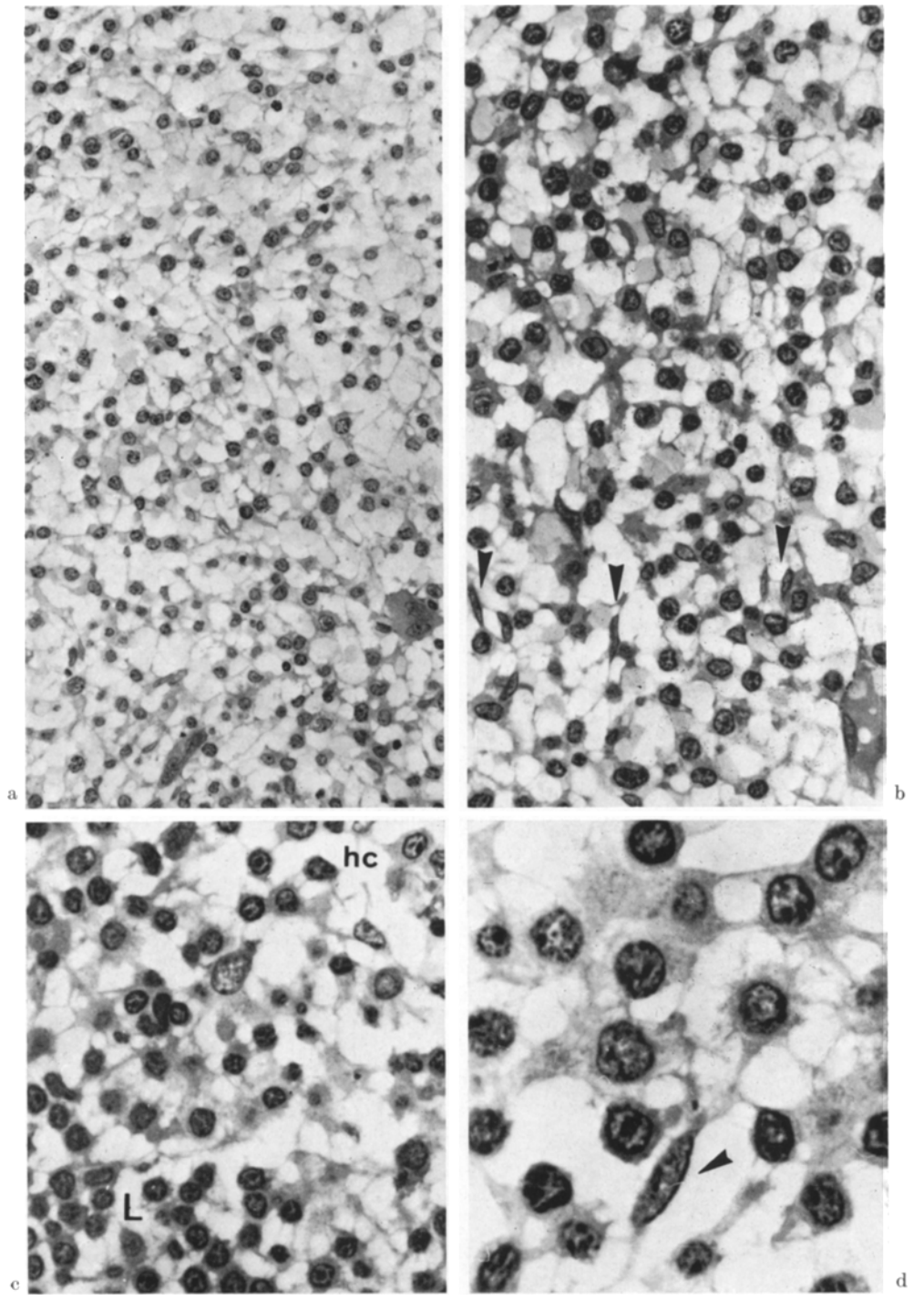


Fig. 1

nodular pattern. In conclusion, all these entities may be excluded by considering the distinctive patterns of growth as well as the striking differences in nuclear shape and size.

Fibrosis of the bone marrow is an other characteristic morphological feature of HCL, easily differentiated from other causes of myelofibrosis (Figs. 3–5a–d). There is a felt-like network of thin reticulin fibres which generally does not (or only in very late stage of this disease) give rise to coarse bundles of collagen (Fig. 4a). In comparison with the other types of sclerosing myeloproliferative disorders there is a very distinctive pattern of fibrosis with variable amounts of fibres and collagen that sometimes leads to a gross scarring of the bone marrow space (Figs. 5a–d). In contrast to HCL the nodular infiltrates by CLL (Fig. 4b), lymphoblastic and lymphocytic lymphomas (Fig. 4c), Waldenström's disease and chronic megakaryocytic-granulocytic myelosis (Fig. 4d) show a more patchy and

Figs. 1a—d. Light microscopy of hairy cell leukemia infiltrates of the bone marrow. Survey with a spongy, reticulum-like arrangement of mononuclear cells (a) interspersed with slender fibroblastic cells (b, arrow heads). "Hairy cells" (*hc*) are clearly distinguishable from lymphocytes (*L*) (c). High magnification shows slender cytoplasmic processes of "hairy cells" intermingled with a fibroblast (d, arrow head). (Giemsa stain). Light microscopy (Figs. 1–5a–d) was done on semithin (1–3 μ) sections, embedded in acrylate. a × 280, b and c × 450, d × 1,500

Figs. 2a—d and 3a—d. Hairy cell leukemia compared with bone marrow infiltrates of various leukemias and lymphomas (Giemsa stain)

Fig. 2 (a) Diffuse and spongy arrangement of hairy cell leukemia. (b) Chronic lymphocytic leukemia with diffuse infiltration by lymphocytic cells with a smooth outline. (c) Chronic myelo-monocytic leukemia with two different cell populations consisting of monocytes (arrow heads) and myelocytic elements. (d) Diffuse infiltration by a lympho-plasmacytic lymphoma with plasma cells (arrow) and a spongy arrangement of lymphocytes

Fig. 3 (a) Malignant lymphoma of a well differentiated lymphocytic type with diffuse infiltration of the bone marrow in contrast to HCL displaying a more compact pattern of growth. (b) Reactive nodular lymphatic hyperplasia of the bone marrow showing small lymph follicles with germinal centres surrounded by hyperplastic megakaryo- and granulopoiesis in a case of rheumatoid arthritis. (c) Nodular lymphocytic lymphosarcoma consisting of clusters of lymphocytes. (d) High magnification of the nodular lymphosarcoma with densely arranged lymphocytic cells; there are no cleaved follicle centre cells or centrocytes, respectively. 2a, 3a and 3d × 140, 2b × 170, 2d × 160, 3b × 90, 3c × 55, 2c × 350

Figs. 4a—d and 5a—d. Myelofibrosis of hairy cell leukemia compared with various myelosclerotic disorders (silver impregnation after Gomori)

Fig. 4 (a) Regular, felt like arrangement of thin reticulin fibres in hairy cell leukemia. (b) Chronic lymphocytic leukemia with irregular distribution of few coarse reticulin fibres. (c) Lymphosarcoma with very coarse reticulin and single collagen fibres in irregular distribution. (d) Chronic megakaryocytic-granulocytic myelosis (early myelofibrosis) with scattered reticulin and collagen fibres accompanied by a blastic crisis

Fig. 5 (a) Scarring of the bone marrow by infiltrates of Hodgkin's disease ("myelofibrose hodgkinienne"). (b) Osteomyelofibrosis in the course of chronic megakaryocytic-granulocytic myelosis (a later stage than in Fig. 4d) with atypical megakaryocytes between coarse bundles of reticulin and collagen fibres. (c) Osteomyelosclerosis (final stage of a chronic megakaryocytic-granulocytic myelosis) with gross scarring of the bone marrow and endophytic bone formation. (d) A higher magnification shows the intermingling of bundles of collagen fibres and myeloblastic cells in an acute blastic crisis. 4a–4d, 5a, b and 5d × 280, 5c × 140

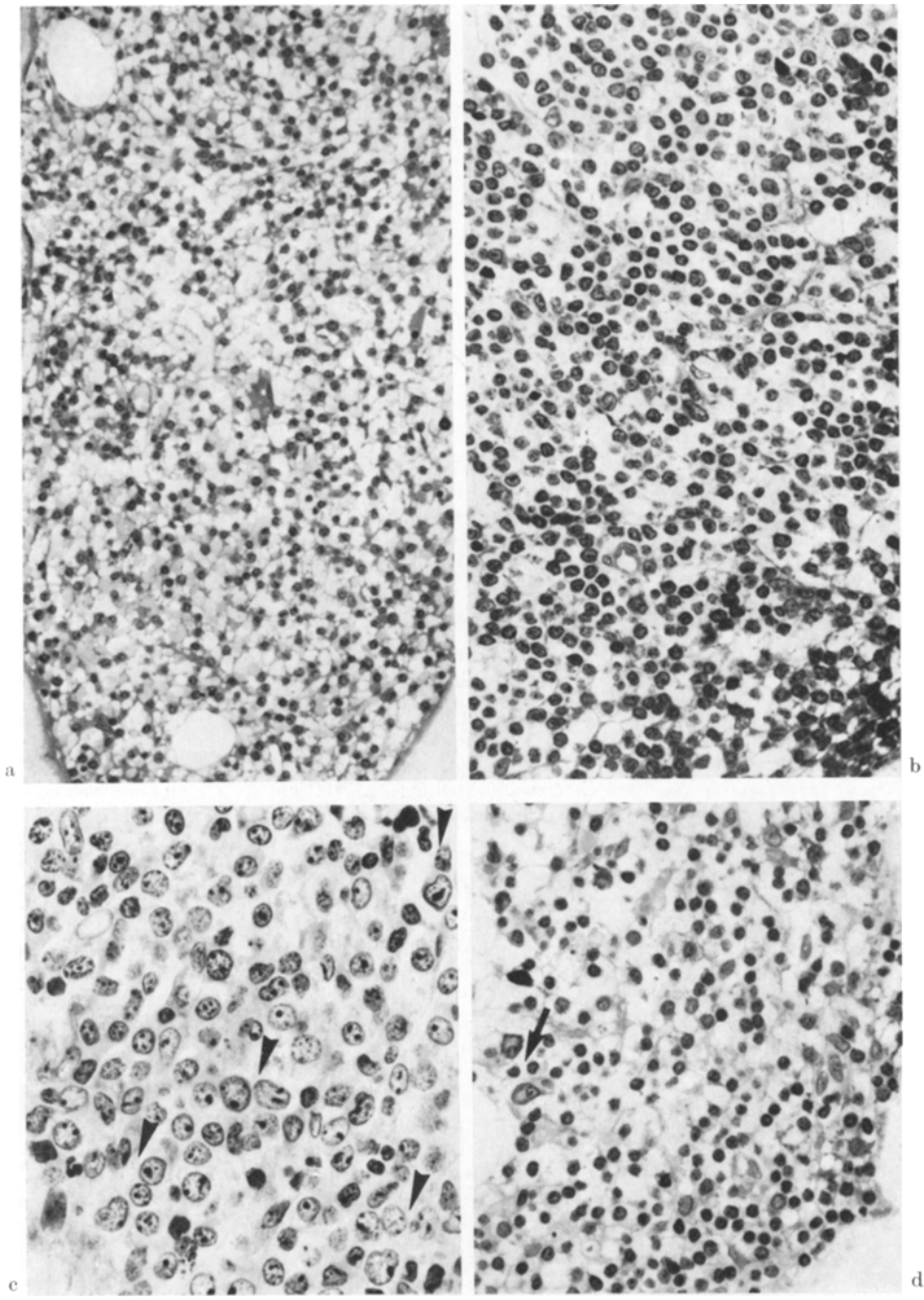
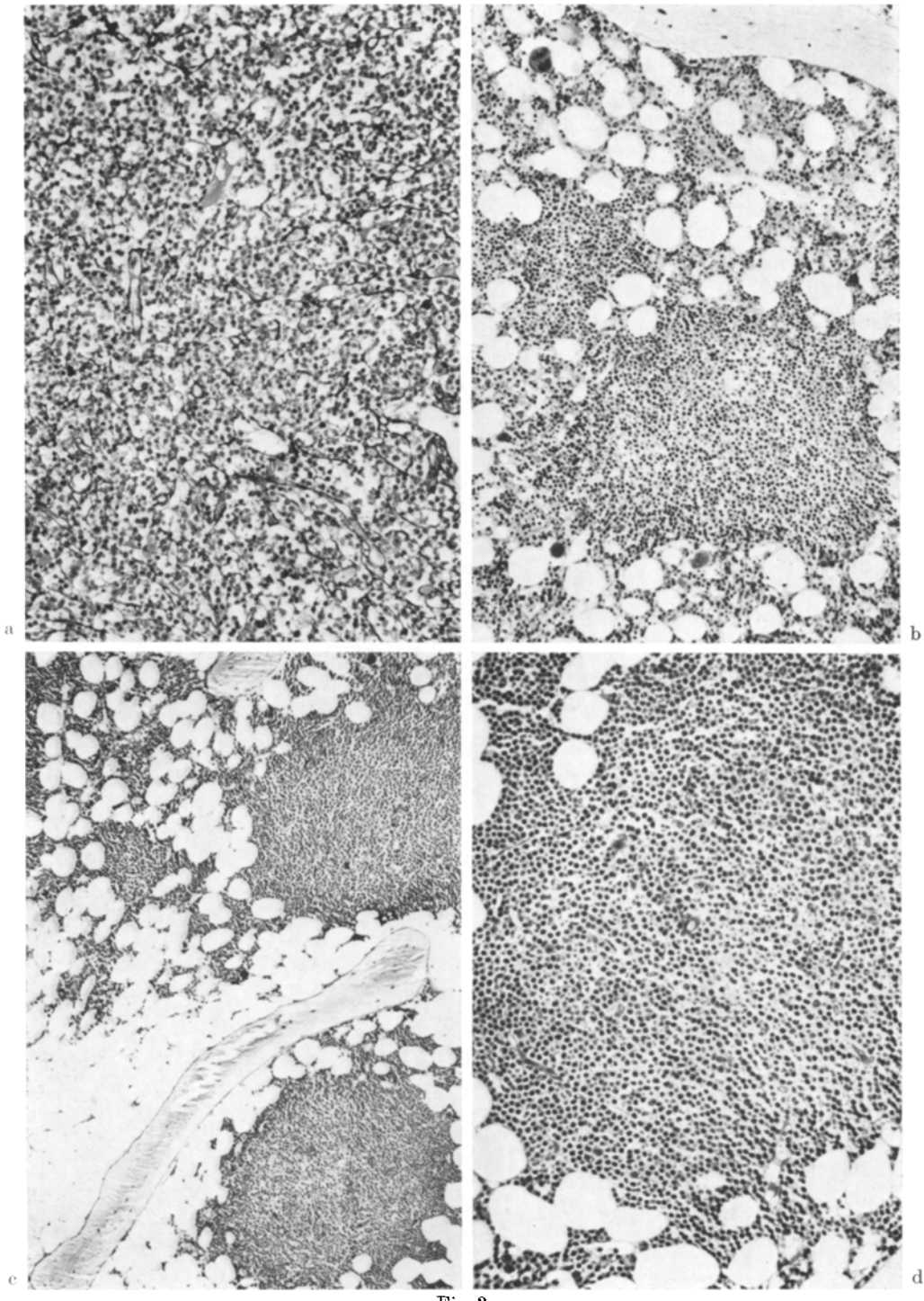


Fig. 2



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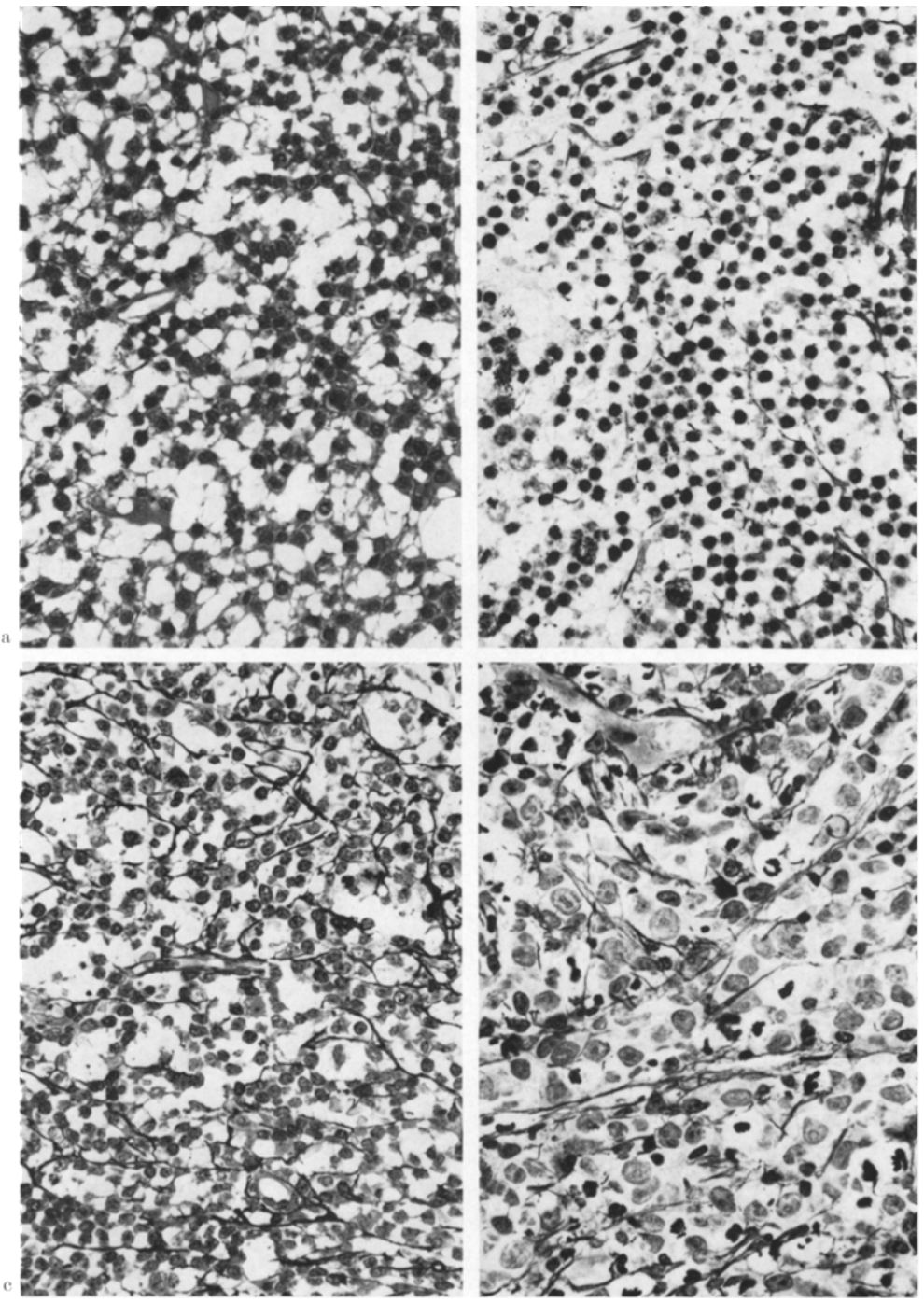
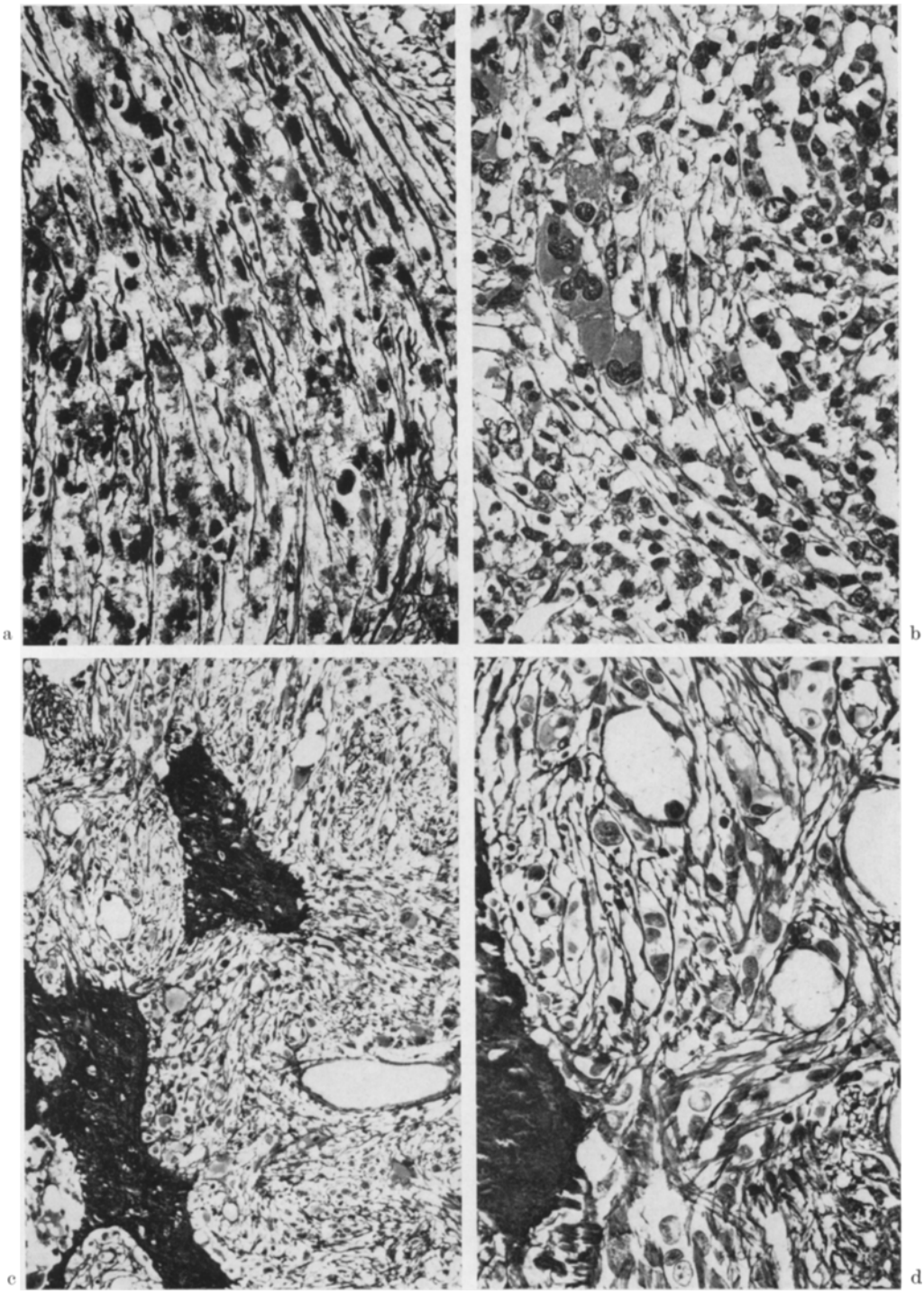


Fig. 4



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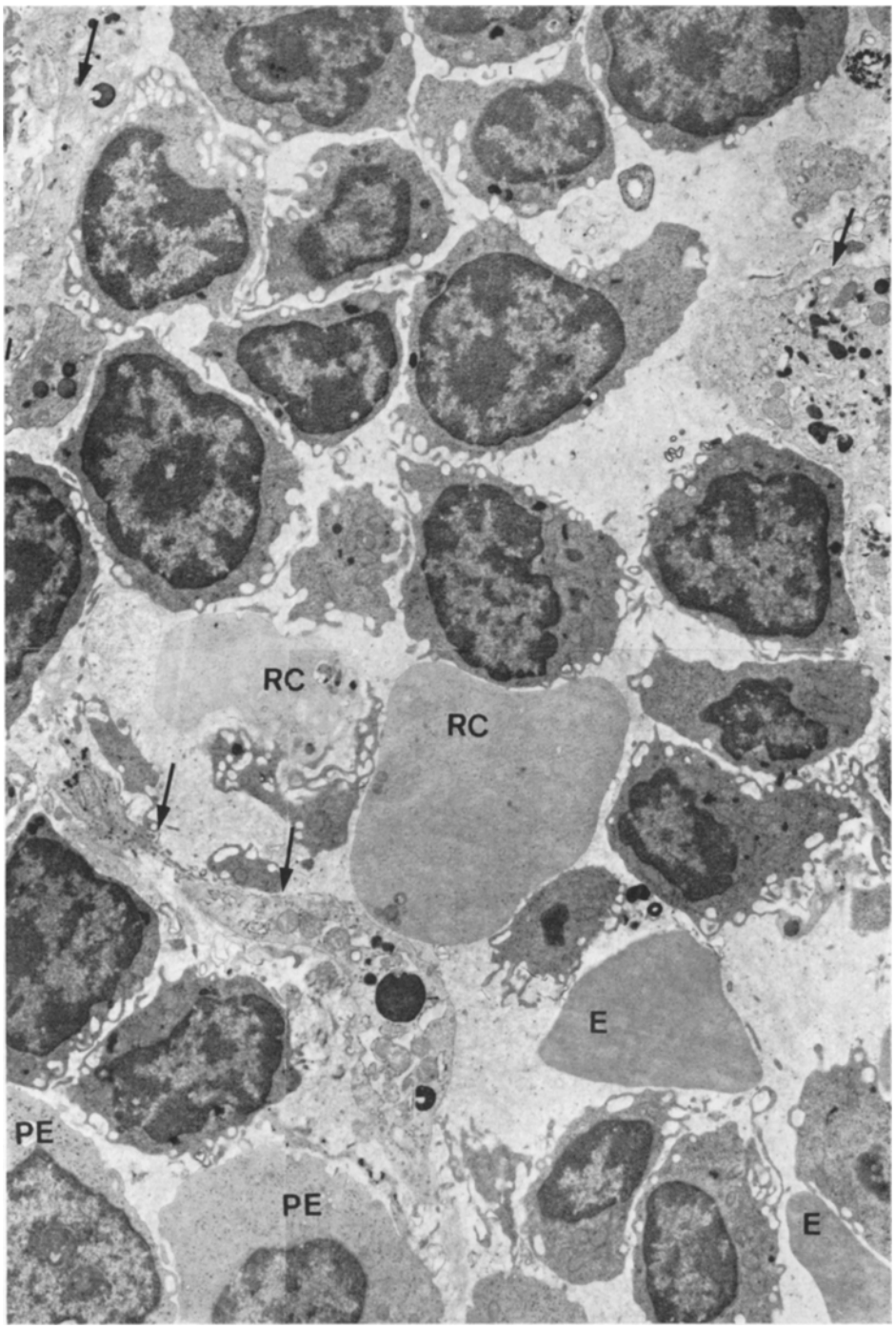

Fig. 6

irregular arrangement of scattered fibres. All these lesions may possibly be confused with the myelofibrosis of HCL. Fibrosis in Hodgkin's disease ("myelofibrose hodgkinienne", Duhamel et al., 1971; Fig. 5a) and in osteomyelofibrosis/-sclerosis accompanied by a blastic crisis (Figs. 5b–d) is much more scar like. In all these disorders the correct diagnosis can be reached by consideration of the proliferating cell-type and the patterns of myelofibrosis.

Electron microscopy confirms our light microscopic findings of a loose arrangement of villous mononuclear cells lying in small clusters in and around the sinuses (Fig. 6). In other areas immature forms and precursor cells are seen. These resemble lymphocytic cells with a large nucleus containing randomly dispersed chromatin, a prominent nucleolus and a relatively small rim of cytoplasm (Fig. 7a). Between these cells bundles of microfilaments and some collagen fibres are present in close relation to slender processes of the fibroblastic cells which are scattered among the HCs (Figs. 6 and 7a). A continuous line of maturation is observed starting with a large blastic cell with a high nuclear-cytoplasmic ratio and terminating in the typical HC with numerous cytoplasmic processes (Fig. 7a). The cytoplasm of these cells show many polysomes, few mitochondria, a small Golgi apparatus and evidence of micropinocytosis, usually at the base of the microvilli projecting from the cell surface (Figs. 7b and 8).

Discussion

Clinical data of the 24 patients with HCL examined may suggest a slight male predominance (Bouroncle et al., 1958; Lee et al., 1969; Plenderleith and Vancouver, 1970; Flandrin et al., 1973; Katayama and Finkel, 1974; Naeim and Smith, 1974; Düllmann et al., 1974; Burke et al., 1974; Catovsky et al., 1974b). Most patients suffering from this haemoblastic disorder are in the 5th and 6th decade of life (mean age 55 years, range 31–74 years in our patients). The clinical symptoms presented on admission are vague, but splenomegaly and various degrees of pancytopenia are frequently seen (Flandrin et al., 1973; Burke et al., 1974). Unfortunately in these patients with the clinical signs and laboratory findings suggestive of a haematological disorder two difficulties prevent easy morphological diagnosis: lymph nodes are rarely enlarged and thus are not removed for histological evaluation. The frequently unsuccessful sternal aspiration of the bone marrow (so-called dry tap) makes cytological diagnosis impossible. In consequence two procedures remain for establishing a correct diagnosis. The first is the demonstration of the marker enzyme of the HCs, tartrate resistant acid phosphatase in "flagellated lymphocytes" of peripheral blood smears (Li et al., 1970; Yam et al., 1971; Yam et al., 1972). There are, however, several cases of proven HCL which do not show this marker enzyme (Schaefer et al., 1975). The second, a more reliable procedure, is the performance of a biopsy of

Fig. 6. Electron microscopic survey of the bone marrow in HCL. Mature HCs infiltrating an erythropoietic island: late pro-erythroblasts (*PE*), reticulocytes (*RC*), erythrocytes (*E*). Among the HCs slender processes of fibroblastic cells surrounded by a few microfilaments and collagen fibres (arrows). × 6,000

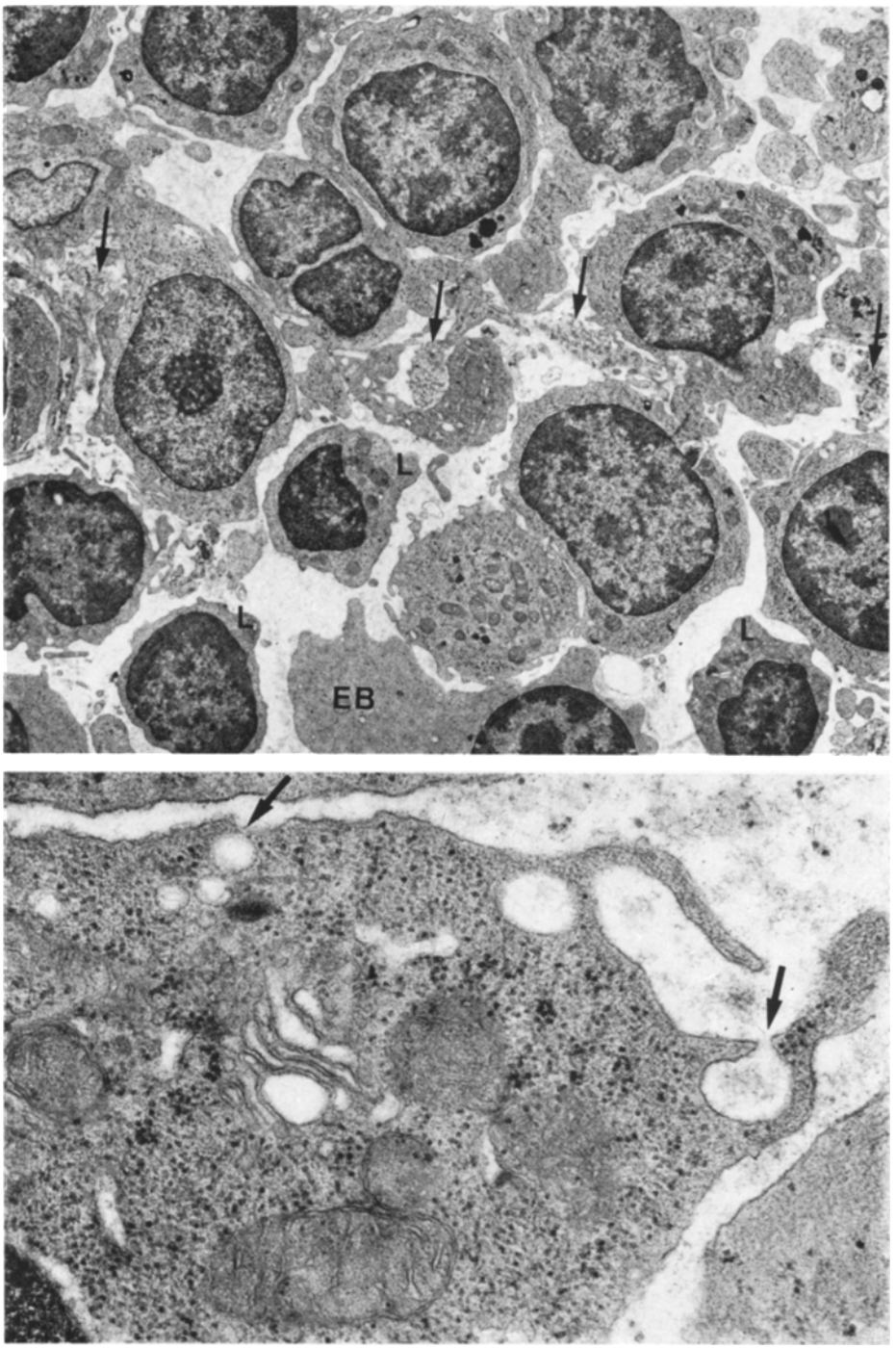

Fig. 7

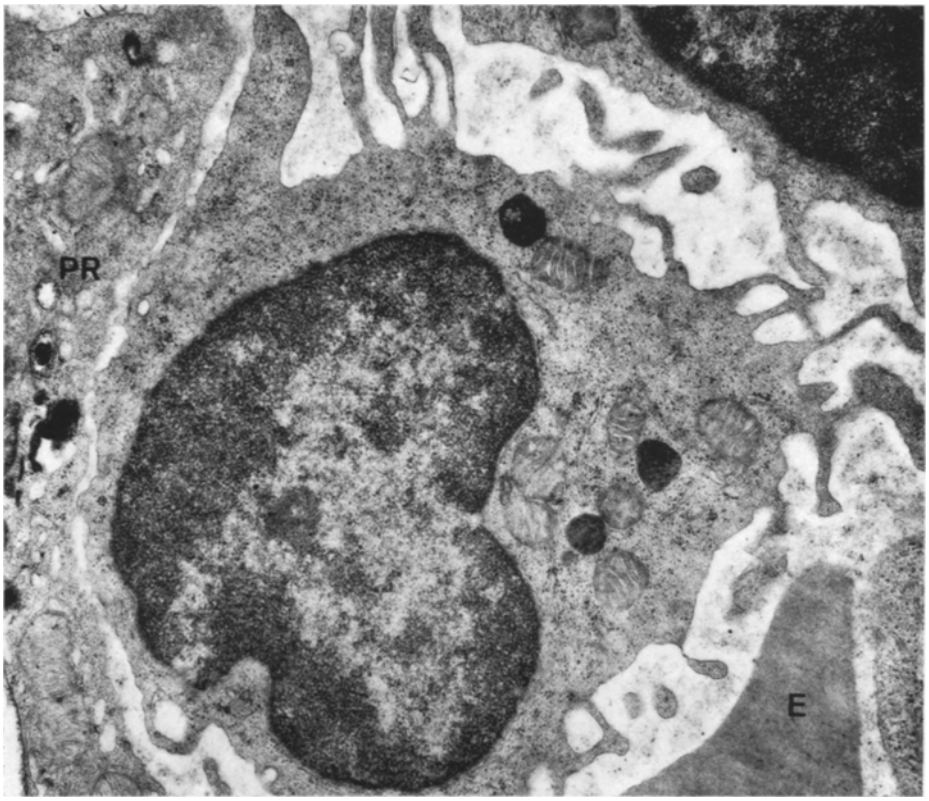

Fig. 8. "Hairy cell" with many cytoplasmic processes. The small portion of cytoplasm contains mitochondria, dense bodies (lysosomes?) and abundant polysomes. Nucleus with shallow indentations, flocculent chromatin and small nucleolus. Erythrocyte (*E*), phagocytic reticulum cell (*PR*). × 21,000

the bone marrow by the method of myelotomy which may confirm HCL or reveal another haematological disorder. According to our findings presented here, bone marrow biopsy has proven to be the most valuable method in achieving a correct diagnosis, even in those cases with an absent or questionable acid phosphatase reaction and no clinical evidence for HCL.

Light microscopy of acrylate embedded bone cylinders displays the diagnostic slender cytoplasmic processes of the HCs as seen in the peripheral blood smears in contrast to the smooth cellular outlines of lymphocytic and myelo-monocytic cells. In accordance with the unique pattern of the "lymphoid myelo-

Fig. 7. (a) Nest of immature HCs with a lymphocytoid appearance lying between bundles of reticulin and collagen fibres (arrows). Several small lymphocytes (*L*) and a late erythroblast (*EB*). (b) Surface of a HC showing slender cell processes and pinocytosis with vesicle formation (arrows). The cytoplasm with many free polysomes, a small Golgi apparatus and some mitochondria. a × 6,000, b × 46,000

fibrosis " described by Duhamel (1971), we would like to emphasize the specific appearance of myelofibrosis in this disease. It is responsible for the dry tap and is quite different and easily distinguishable from any other kind of sclerosing myeloproliferative disorders (compare Figs. 4–5a–d). There may be some doubts about this statement, since some authors have claimed an inability to differentiate the myelo- and lymphoproliferative disorders by morphometric analysis of the pattern or quantity of fibres (Lennert et al., 1975). Differences between material from necropsies and bone marrow biopsies have yielded new possibilities for investigation, especially when performed in early stages for diagnostic reasons or for clinical staging in patients with various lymphomas or myeloses.

In conclusion, iliac crest biopsies have proven to be the most valuable method in establishing the diagnosis of HCL, because the characteristic morphological features of this disease are present. The possibility of the simultaneous evaluation of remnants of hematopoiesis may favour this method in comparison with lymph node biopsies. Semithin sections of non-decalcified acrylate embedded marrow specimens give outstanding results for light microscopy, which is essential for diagnosis of this haemoblastosis. However, the method itself is not essential for recognizing HCs, since excellent paraffin wax embedding is also satisfactory.

Electron microscopy of mature HCs in the bone marrow confirms the results of several other authors, which were usually obtained by investigating buffy coat cells of peripheral blood (Trubowitz et al., 1971; Burns and Hoak, 1973; Daniel and Flandrin, 1974; Schnitzer and Kass, 1974; Catovsky et al., 1974 b; Katayama et al., 1972; Ghadially and Skinnider, 1972; Düllmann et al., 1974) or liver infiltrates (Fabre et al., 1974). In our 3 cases we were not able to detect the peculiar ribosome-lamellae complexes seen in various amounts in HCs (Katayama et al., 1972, 1973). The tri-dimensional structure of these tubular complexes has been analyzed by Daniel and Flandrin (1974) and their model resembles the cylindric inclusions of atypical plasma cells observed in certain cases of multiple myeloma (Krull et al., 1973). Analogous to plasmacytoma cells these ribosome-lamellae complexes may represent a disturbed synthesis of immunoglobulins which are not secreted into the extracellular space but deposited in the area of the rough surfaced endoplasmic reticulum. Fibre formation in HCL is probably induced by HCs and mediated by the fibroblasts randomly dispersed among those cells. A similar dispersion of fibroblastic cells with surrounding microfilaments and bundles of collagen fibres could not be detected in CCL or lymphosarcoma infiltrates nor in a case of chronic myelo-monocytic leukemia (Georgii and Thiele, 1976). Even in the very early stages of chronic megakaryocytic-granulocytic myelosis, fibres are not usually present in such amounts and particularly with such a regularity of distribution (Thiele et al., 1976). In HCs micropinocytosis may be observed frequently, but no gross phagocytosis comparable with the histiocytic-phagocytic reticulum cells is seen. Micropinocytosis may be at least partially responsible for microvilli formation of the plasma membrane. The absence of a phagocytosis even in the most immature forms of HCs agrees with the findings of many other authors made in mature HCs (review by Daniel and Flandrin, 1974). In contrast to these observations Katayama et al. (1972) and Ghadially and Skinnider (1972) found erythrophagocytosis in some HCs. In vitro phagocytic capacity of latex and various bacterial particles was demonstrated by Flandrin et al.

(1973); Daniel and Flandrin (1974) and v. Heyden et al. (1976) in contrast to the findings of a negligible phagocytosis by Beachey (1969); Rubin et al. (1969); Schrek and Donnelly (1966); Yam et al. (1968); Berg and Brandt (1970); Mitus et al. (1961) and most recently by Catovsky et al. (1974a ,b). The controversy concerning the phagocytic activity of HCs in vivo directly concerns the problem of the origin of HCs. From the morphological point of view the "hairiness" seems to resemble the surface structures of lymphocytes (Sullivan et al., 1974; Schnitzer and Hammack, 1974), rather than normal and leukemic monocytes as stated by (Golomb et al., (1975). If their functional activity is compared with the monocyte-macrophage series of cells they do not form rosettes with sheep erythrocytes, show poor adhesion and negligible phagocytosis in vivo. On the other hand there are surface bound immunoglobulins and there is no phytohaemagglutinin (PHA) stimulation (Haak et al., 1974; Catovsky et al., 1974a; Mende et al., 1975). These functional characteristics indicate that the HCs probably are of lymphocytic origin closely resembling the B lymphocyte and that therefore HCL should be classified as a lymphoproliferative disorder.

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