ELECTRON MICROSCOPIC AND CYTOCHEMIC FINDINGS IN Di-GUGLIELMO'S SYNDROME AND IN OTHER FORMS OF LEUKEMIA

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The leukemic cell shows many peculiarities in contrast to normal ones. In particular, these are not specific for leukemia; by their quantity and combination they improve classification and understanding of this disease.

In order to avoid an exhaustive list of morphologic findings, a certain order of our results we shall try to achieve. In doing this we accept the risk of possibly over-simplifying or speculating.

Morphologic findings in leukemic cells can be divided into 3 groups according to increasing "specificity" (tab. 1).

I. "Degenerative" changes

These are found in cells, which have been disturbed in some way in their environment. Typical for these changes are vacuolization, formation of myelin-figures, rupture of the membranes of cells and of cell organelles. The following reasons for these findings in leukemic cells could be considered: disturbed interaction of cell organelles; impaired supply of high-energy-compounds, for instance by damage of mitochondria; superannuating of the cells.

II. Changes that are often observed in "malignant" cells

To this group primarily the peculiarities of the nucleus of a malignant cell may be mentioned: large and irregular nuclei; "nuclear pockets", conspicious nucleoli; evenly distributed and lose chromatin. It is only partially possible to explain these findings as an expression of a special activity of certain function: enlargement of the nuclear surface by "nuclear pockets", lose chromatin, large nucleoli for the purpose of an increased synthesis of DNA or of proteins. Other changes would rather indicate an impaired interaction of organelles or a compensatory hyperthrophy of the organelle-systems caused by disturbed partial functions: augmentation and enlargement of mitochondria, abnormally large Golgi-fields, extended bundles of fibrilles.

Table 1. Electron microscopic findings in leukemic cells.

I.	"degenerative" changes	1. vacuolization of cytoplasm and of cell organelles 2. myelin figures 3. breakage of membranes
II.	changes common to all kinds of "malignant" cells	1. polymorphism of cells 2. dissociation in maturation of nucleus and cytoplasm (immature nuclei; high nuclear-cytoplasmic ratio) 3. large, irregular nuclei; prominent nucleoli; "nuclear pockets" 4. large, irregular of augmented cell organelles, esp. mitochondria 5. intracytoplasmatic fibrills
III.	changes specific to leukemic cells	1. abnormal granules in myeloid cells 2. abnormal activities of enzymes 3. ferritin and ferruginous micelles in erythroblasts 4. transformation of mitochondria into cytosomes 5. abnormal deposits of PAS-positive material

III. Few changes are characteristic for certain forms of leukemia

As a rule, these are based upon the impairment of organelle-systems of correspondingly differentiated cells. So we find in structure and enzyme activity abnormal granules in all forms of myeloic leukemias; morphologic equivalents of a disturbed hemoglobin synthesis and an abnormal expulsion of nuclei in malignant erythroblasts; conspicious depositis of PAS-positive materials in cells containing glycogen.
Especially this last group of morphologic changes will be dealt with in the following.

Anomalies of Granules

The *primary granules* of myeloic cells are equipped with acid phosphatase and with peroxidase under the assistance of perinuclear space, rough surfaced endoplasmic reticulum and Golgi-cisterns [BAINTON e. a. 1968, 71]. This procedure can be made visible electron microscopically [methods: HUHN e. a. 1971]. Hereby poorly differentiated leukemic cells may be classified into a certain myeloic cell line before larger quantities of enzyme are synthesized, packed into granules and become visible by light microscopy. The enzymes, listed in table 2, were localized in the primary granules by means of biochemistry, cytochemistry or electron microscopy. In myeloic leukemias the following defects of enzyme activity could be demonstrated in neutrophils [SCHMALZL e. a. 1973].
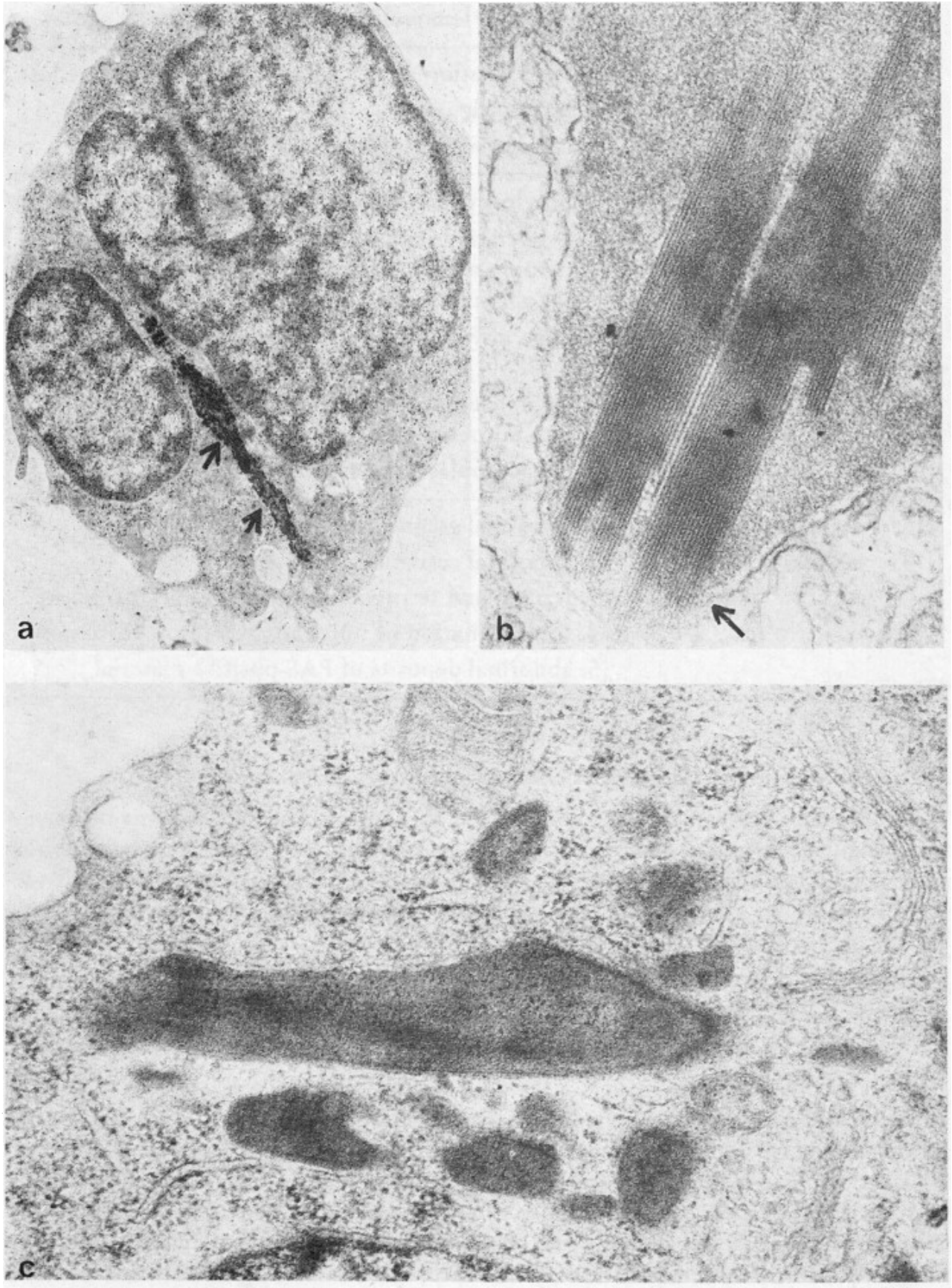
Fig. 1: Leukemic changes of primary granules a) myeloblast; activity of acid phosph. in Auer-rod b) cristalloid structures in prim. granules c) promyelocyte; Auer-rod and prim. granules.

1. decrease or loss of the activity of peroxidase, of naphthol-ASD-chloracetate-esterase and/or of acid phosphatase;
2. isolated decrease or loss of peroxidase;
3. isolated decrease or loss of naphthol-ASD-chloracetate-esterase.

Primary granules of leukemic cells frequently show pecularities of their *fine structure*: they may contain cristalloid inclusions which penetrate their membrane transforming into Auer-bodies (fig. 1). Auerbodies as a rule exhibit activity in acid phosphatase, usually in peroxidase and naphthol-ASD-chloracetate-esterase [ACKERMANN 1950; FISCHER e. a. 1966; HUHN e. a. 1968]. Further on, primary granules are abnormally enlarged, may conglomerate or may appear as small, commalike structures (fig. 2).

The *secondary neutrophil granules* differ from the primary ones by their structure and enzyme activities (table 2). The neutrophils of a patient suffering from monocytic leukemia exhibited an excessive augmentation of alkaline phosphatase-activity extending to granulocyte precursors. In some cases of myeloblastic leukemia activity of alkaline phosphatase was demonstrated in myeloblasts [MALASKO-WA e. a. 1968; SCHUBERT e. a. 1968]. Concerning their *fine structure*, secundary granules contain conspicious amounts of laminar systems or cristalloid structures (fig. 2b).

Monocytic leukemia can be considered as a special form of malignancy of the myeloic cells [HUHN e. a. 1971; SCHMALZL e. a. 1968]. According to the degree of differentiation the leukemic monocyte obtains, we may differentiate between a *promonocytic* and a *monocytic* form. *Promonocytic leukemia* is characterized by undifferentiated blasts containing few granules and low enzyme-activity restricted to the perinuclear space and the rough surfaced endoplasmic reticulum. In *monocytic leukemia* the leukemic cell equals the normal monocyte according to contents in organelles, enzymes and to the ability of phagocytosis.

A third form of monocytic leukemia may be delimitated [SCHMALZL e. a. 1972]. The leukemic cells in this disease can be identified as monocytes, but in addition concerning their fine structure and enzyme-activities they show characteristics of granulocytic cells. In one case like this we found Auer-bodies. This special form of monocytic leukemia was termed *monomyelocytic leukemia*.

Summarizing the hitherto existing findings, we can make the following statements:

1. The formation of primary and secondary granules represents a specific activity of myeloic cells, reflecting the degree of maturation. The content in granules and in enzymes in leukemic cells determines the cytoplasmic differentiation of the leukemic cell population.
2. Different disturbances of granule-formation frequently occur in leukemic cells:
   a) reduction of granules in a cell;
   b) elevation or reduction of enzyme-activity in morphological (qual. and quan.) normal granules;
   c) morphologic (qual.) abnormal granules with normal enzyme activity.
3. In acute (myeloic) leukemias mature granulocytes may show defects of their granules.

Table 2. Activity of different enzymes in granules of neutrophils and monocytes (Schmalzl e. al. 1973).

	Granules in Neutrophils			Granules in Monocytes	
	Primary Granules		Secondary Granules		
bce	acid phosphatase	bce	alkaline phosphatase	bce	acid phosphatase
b	acid ribonuclease	bc	lysozyme (muramidase)	b	acid ribonuclease
b	acid desoxyribonuclease	bc	lactoferrine	b	cathepsin
b	cathepsin	bc	naphthylamidase	bc	ß-glucuronidase
bc	ß-glucuronidase			bc	lysozyme (muramidase)
bce	peroxidase			bce	peroxidase
bc	NaF-resistant NASE			bc	NaF-sensitive NASE
bce	arylsulfatase			c	NASDCE
(bce)	neutral proteases (NASDCE)				
b	bactericidal proteins				

b: localized to granules by biochemical methods
c: cytochemical or immunological demonstration
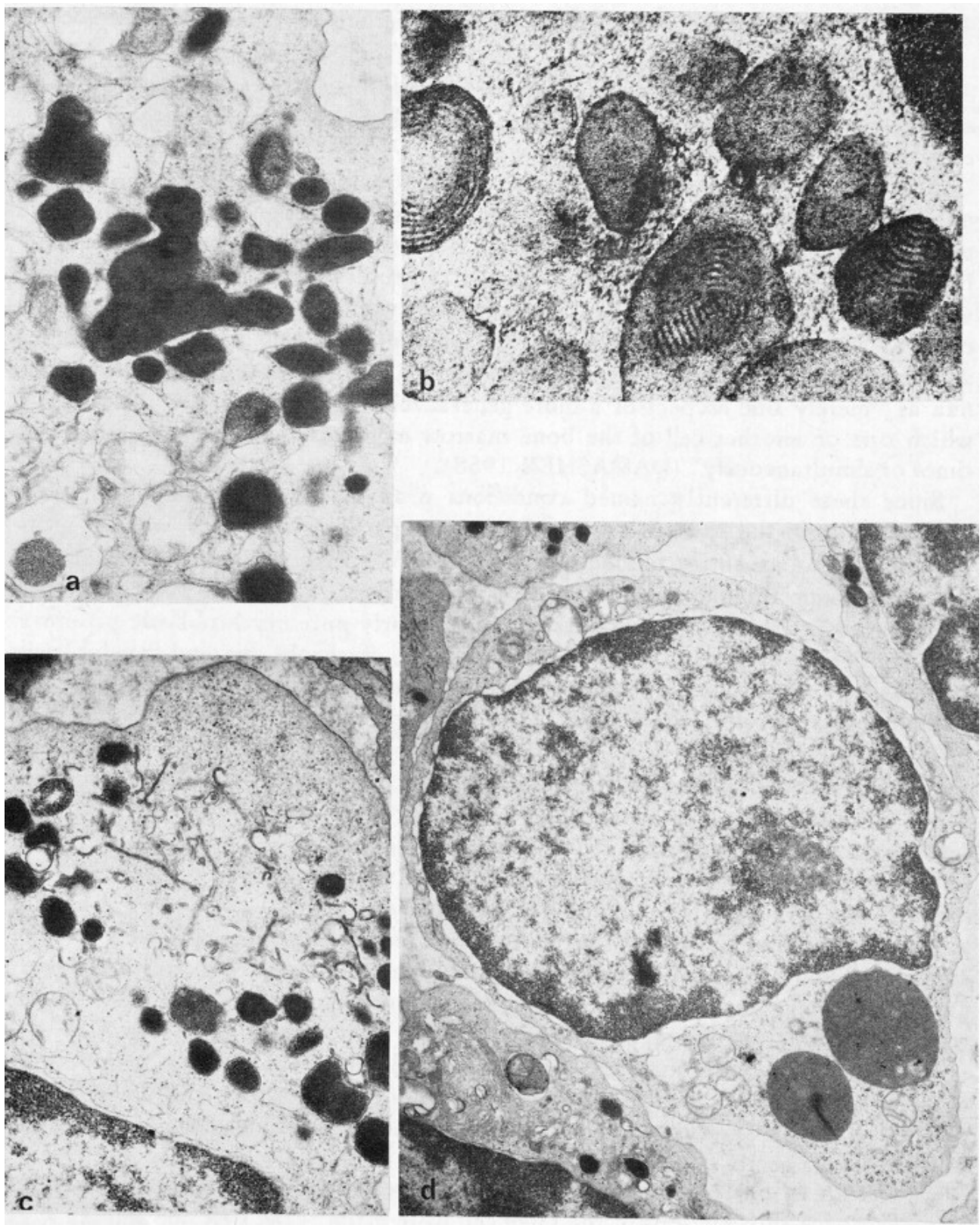e: localized to granules by electron microscopic methods

Fig. 2: Leukemic changes of prim. and sec. granules a) promyelocyte; confluent primary granules
b) laminar structures in secondary granules c) comma-like primary graunles d) enlarged primary
granules.

4. Transitional forms between leukemias of the granulocytic and the monocytic series can be observed.

We now will consider the *malignancies of erythropoietic cells*. Here we expect characteristic morphologic findings depending on hemoglobin-synthesis and nuclear expulsion.

A condition characterized by a generalized malignant proliferation of the nucleated red blood cells resembling leukemia, was first recognized as a clinical and pathological entity by Di GUGLIELMO in 1923; he termed it *"acute erythremia"*. In 1917 this author had described a mixed erythroblastic-leukocytic proliferation, which he called *"erythroleukemia"*. As the years went on, an increasing number of cases of apparently "pure" erythroblastic disorder gave way to erythroleukemia, and finally to myeloblastic leukemias. Thus, DAMASHEK thought of erythroleukemia as "merely one ascpect of a more generalized myelo-proliferative disorder, in which one or another cell of the bone marrow might participate either at various times or simultaneously" (DAMASHEK 1958).

Since these differently named conditions probably represent only transistory clinical stages in the natural history of the same pathological entity, we have grouped them into one entity termed *"DI GUGLIELMO'S-SYNDROME"*. The clinical and cytochemic findings of 7 patients suffering from Di Guglielmo's-Syndrome are summarized in table3. In 4 patients we saw a nearly pure erythroblastic proliferation, in 3 patients mixed proliferation of both, erythroblastic and myeloblastic elements. In all patients anemia and thrombopenia; in only 3 patients more than 10.000 nucleated cells [HUHN e. a. 1973]. The type of erythroblasts predominating in one patient reached a very *different degree of differentiation*: in one patient there were quite undifferentiated blasts, and only cytochemic and electron micros-copic methods made sure that they belonged to the erythropoietic series. In a second patient, the malignant cells appeared quite polymorphous, extending from undifferentiated blasts to atypical normoblasts. In the remaining patients atypical normoblasts predominated in blood and bone marrow.

Only occasionally, *deposits of Prussian blue-positive material* were clearly augmented and numerous "ringed sideroblasts" could be seen. By electron micros-copy, cytosomes and especially mitochondria containing ferritin and hemosiderin could be demonstrated in all patients, and even so in proerythroblasts (fig. 3).

PAS-positive material in proerythroblasts was deposited in the form of spots and granules, in normoblasts diffusely. PAS-positive deposits do not prove the diagnosis of Di Guglielmo's-Syndrome. They can be demonstrated in sideroblastic anemias as well as in thalassemia.

The *nature of the PAS-positive material* was investigated by electron microscopy: two successive thin sections of the same cell were made — the first one stained with PAS and checked by light microscopy, the second one stained with lead hydroxide or silver methenamine and checked by electron microscopy. In most cases, the PAS-positive material consisted of typical glycogen particles, either localized or diffusely distributed. In some cells, however, the PAS-positive granules appeared empty in lead-stained electron microscopic sections and stained weakly with the silver methenamine (fig. 4). We also got very similar results in lymphoblastic leuke-mia, but never in normal blood cells. Summarized, the PAS-positive material de-

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Table 3. Findings (blood, bone marrow) and course in 7 patients with Di Guglielmo-Syndrome.

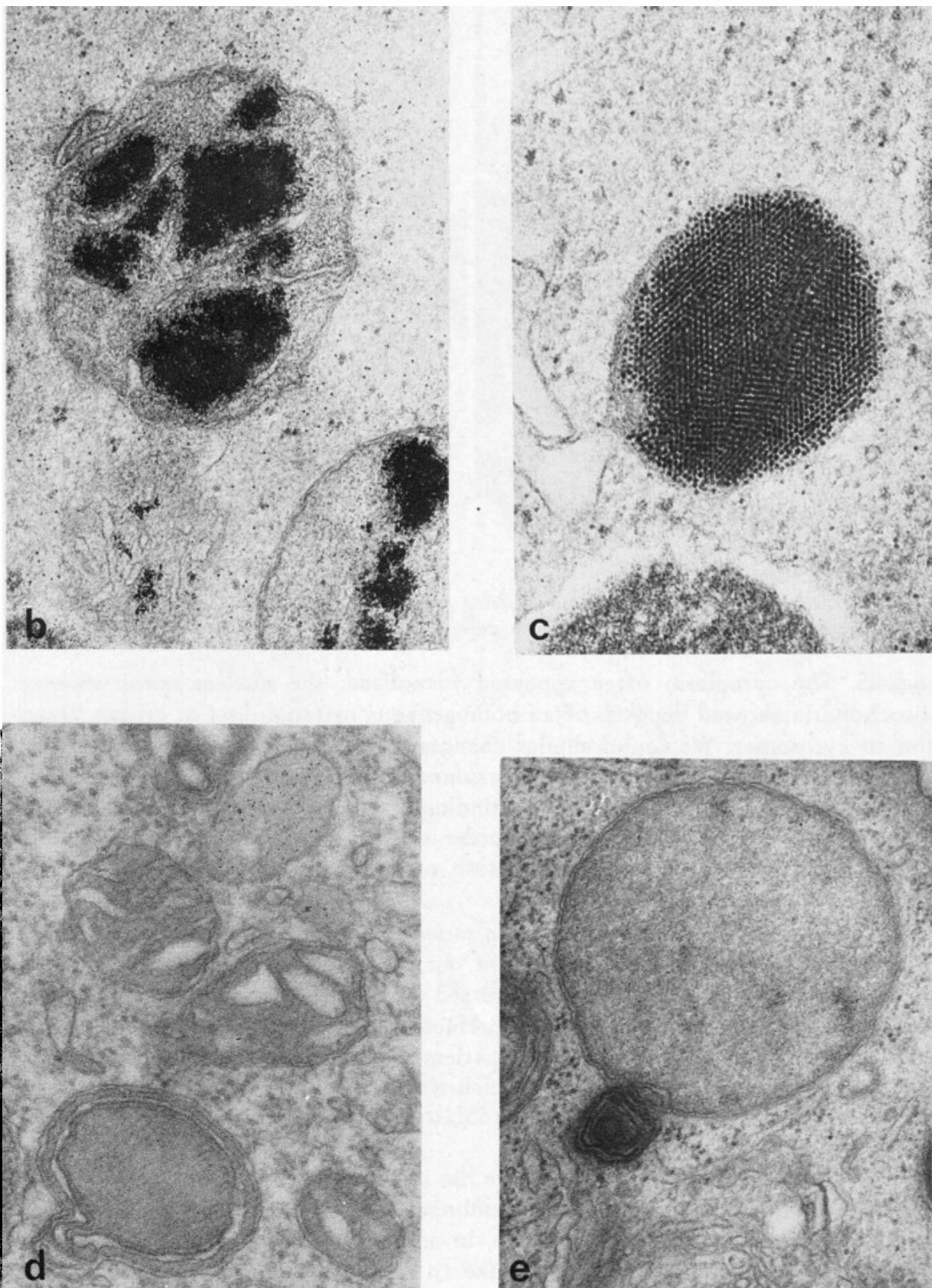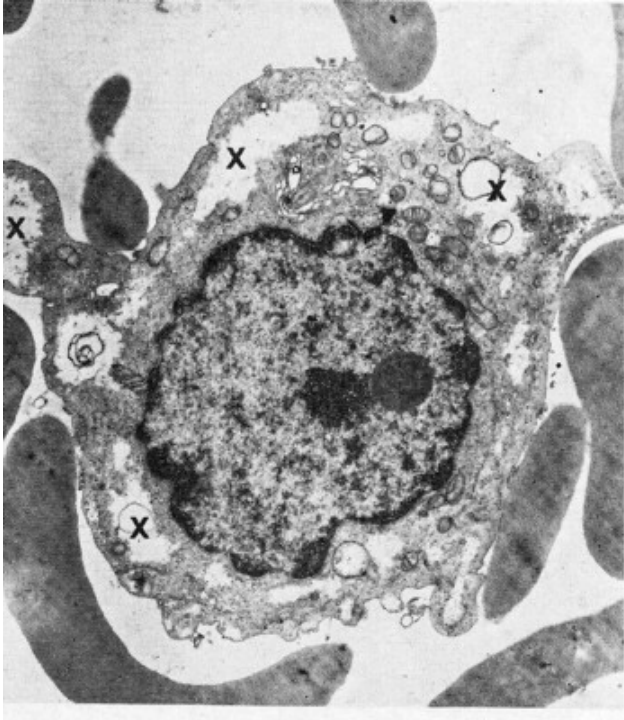|	patients age sex	hb (g%)	platelets (/mm³)	nucleated (/mm³)	erythrobl. (/mm³)	myelobl. (/mm³)	erythrobl. (% of nucleated)	myelobl.	diagnosis - death (months)	therapy	cause of
				blood			bone marrow		course		
"acute ery-thre-mia"	K.S. 43 a ♂	4.9	7.000	2.300	69	0	96 %	0	1.5	ARA-C Predn.	intest. hemorrh.
	H.S. 30 a ♀	8.6	69.000	3.600	0	0	75 %	·1 %	6	ARA-C Predn.	intest. hemorrh.
	K.F. 66 a ♂	9.6	28.000	18.400	770	0	60 %	10 %	4	Busul. fan	anemia
	J.W. 68 a ♂	6.7	12.000	4.600	2.800	92	90 %	2 %	2	ARA-C Predn.	intest. hemorrh.
"ery-thro-leuk-emia"	A.R. 52 a ♂	1.8	13.000	47.700	7.600	32.900	30 %	66 %	1	ARA-C Predn.	intest. hemorrh.
	K.C. 40 a ♂	3.8	89.000	4.100	1.940	820	20 %	75 %	4.5	ARA-C DNR VCR 6-MP Predn.	pneumo-nia (Soor)
	A.S. 48 a ♀	8.6	88.000	15.000	6.000	4.500	40 %	58 %	0.5		Sepsis

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a

Fig. 3 b—e: Transformation of mitochondria to cytosomes in all kind of leukemia

b—c: Di Guglielmo's syndrome; deposits of homogenous material, of ferruginous micelles, of ferritin in mitochondria

d—e: lymphoblastic leukemia, deposits of homogenous material in mitochondria.

quence of the disturbed mitochondrial function. Vacuolisation with widening of perinuclear space may be of special importance in erythroblasts: The normal performance of nuclear expulsion will be impossible in such a cell.

In the preceding part we tried to demonstrate the predominance of characteristic abnormalities of the granules in myeloic leukemias and of hemoglobin synthesis and nuclear expulsion in malignancies of red cell precursors. In *all forms of leukemias*, especially in lymphoblastic leukemia, we may observe more or less frequently:

1. Deposits of a PAS-positive material, which may appear electron microscopically as 200–300 Å large glycogen particles or as "empty" irregular spots not stainable with lead-hydroxide.
2. Furthermore we see characteristic abnormalities of mitochondria (fig. 3): depositis of a homogeneous or finely granular material, loss of cristae and transition to cytosomes.

For the better diagnosis and classification of leukemias of the *lymphoreticular system* the light and electron microscopic demonstration of membrane-antigens and membrane-immunoglobulin-receptors by combined *immuno-histochemical methods* will be of increasing importance.

At the end of my explanation this shall be demonstrated with the example of *hairy-cell-leukemia* (investigations in cooperation with Dr. H. Asamer, Innsbruck).
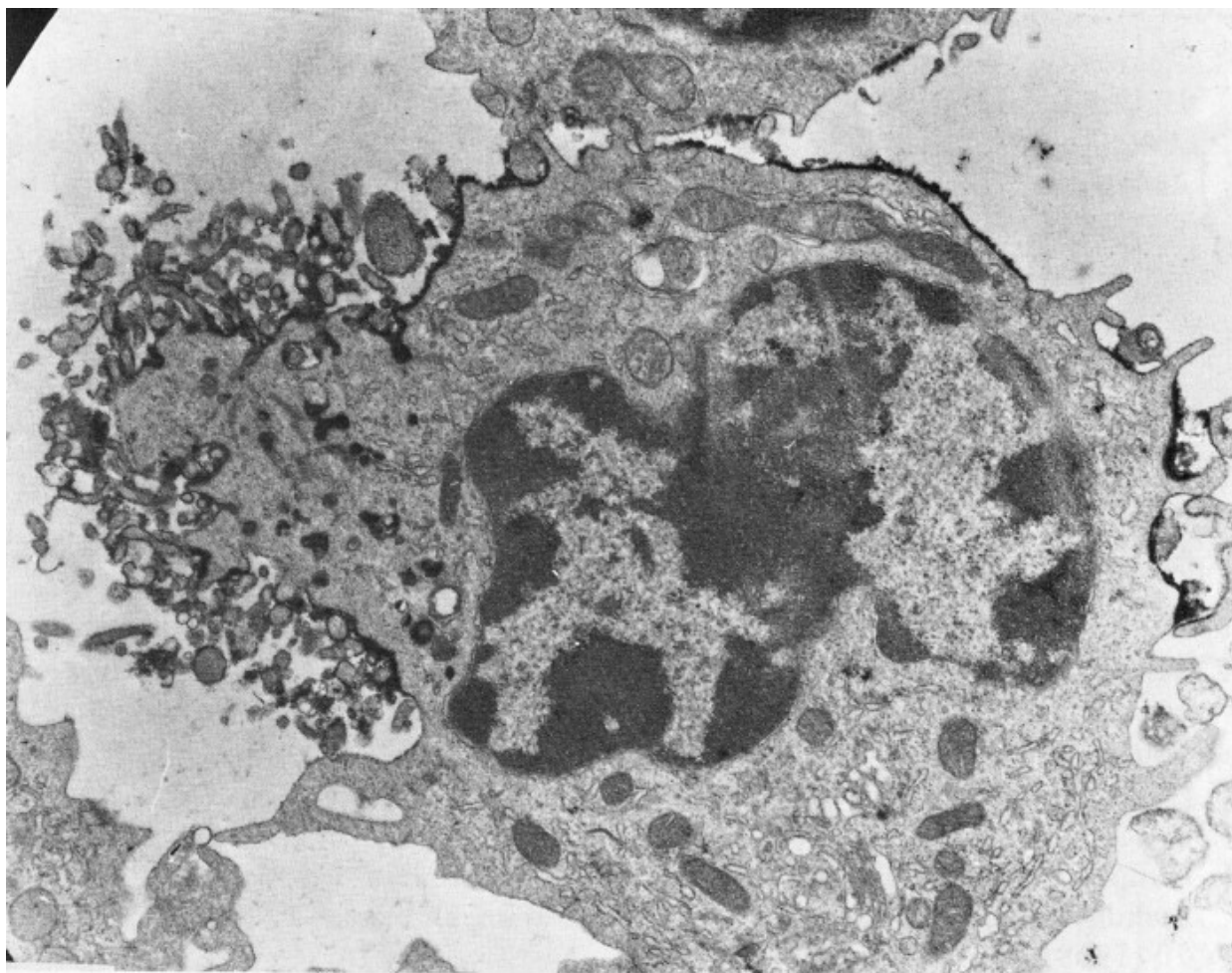

Fig. 5: Hairy cell; demonstation of K-immunoglobulinreceptors by perocidase-labelled antibody.

The leukemic cells of this rare malignancy are characterized by numerous microvilli-like protrusions of the cell membrane. In addition, the cells contain very many small vesicles, few ribosomes or ergastoplasm, small granules and prominent bundles of fibrills. Perinuclear space, endoplasmic reticulum and granules exhibit distinct activity of acid phosphatase, but not of peroxidase. After incubation with ferritin only small quantities are phagocytized and deposited in vacuols or cytosomes. All these findings indicate the lymphocytic but not the monocytic or histiocytic nature of the "hairy cell". This was confirmed by the demonstration of immunoglobulin-receptors of the cell membrane. By fluorescence microscopy (Dr. Asamer) and by an indirect method using peroxidase-coupled immunoglobulins, immunoglobulin receptors of the IgG-K-type could be demonstrated (fig. 5). After these findings, the "hairy cell" seems to be a special form of B-lymphocyte.

Summarizing, we conclude: electron microscopic and cytochemic investigations can facilitate diagnosis and exact classification of leukemias in certain cases. In addition, by these means the understanding of the normal and the pathologically altered functions of the cell and of their organelles is improved. Conclusions concerning the pathogenesis or even aetiology of leukemia are speculative up to now.

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