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Ultrastructural study of peripheral lymphocytes and polymorphonuclear leukocytes in children with lysosomal enzymopathies and hyperlipoproteinemia

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On the basis of electronmicroscopic examinations of the peripheral lymphocytes and polymorphonuclear leukocytes (PMNL) in mucopolysaccharidosis of types I and II in Gaucher and Nieman-Pick diseases, in metachromatic leukodystrophy and in hyperlipoproteinemia, the ultrastructural characteristics are described.

Pathological findings with vacuoles formations were observed in Gaucher disease and in metachromatic leukodystrophy against the preliminary literature.

The ultrastructural pathological changes are reported from the first ultrastructural PMNL examinations in hyperlipoproteinemias.

Electronmicroscopic analysis of the leukocytes is considered to give information equivalent in value to that from liver biopsy studies, but is advantageous in view of its non-invasive nature.

During the past 10 years, numerous efforts have been made to examine the most varied biopsy material with a view to the morphologic diagnosis of lysosomal enzymopathies. Goebel et al [5] proposed muscle biopsy examination in neuronal ceroid-lipofuscinosis and also ultrastructural examination of the peripheral nerve biopsy tissue [5a]. Haynes et al [6] considered electronmicroscopic analysis of a skin biopsy sample and of the lymphocytes of the peripheral blood to be informative in the above disease. Fingerprint-like ultrastructural formations of the lymphocytes were reported by Bauman and Markesbery [2] to be pathognosticin juvenile amaurotic idiocy (neuronal ceroid lipofuscinosis) (NCL). Aula et al. [1] described the phenomenon of cytoplasmatic vacuolization of the T and B lymphocytes in lysosomal diseases.

In I-cell disease, Rapola et al [10] reported ultrastructural cytoplasmatic inclusions of the lymphocytes filled with polymorphous material; they emphasized that other cytoplasmatic organelles showed no pathologic changes. The observed inclusions corresponded with the fibroblast inclusions. They were rounded formations with a diameter of 60–200 nm, with a light centre; they were bordered by membrane and were filled with

TABLE I

Values of enzyme activities of patients suffering from lysosomal enzymopathies

Leukocytehomogenisate enzyme activity μmol/g protein/min	MPS I P. A. ♀ 7 y.	%	MPS I T. G. ♂ 5 y.	%	MPS I Sz. I. ♂ 11 y.	%	MPS I H. K. ♂ 3.5 y.	%	MPS I K. E. ♀ 7 y.	%	MPS II K. G. ♂ 6 y.	%	Control Sz. G. ♂ 5 y.	100%
Alfa-L-iduronidase	0.35	5.9	0.68	11.4	0	0	0.2		0.1		2.7		5.94	
Alfa-N-acetyl-glucosaminidase	187.0	90.6	164.0	79.4									206.5	
Beta-D-galactosid-ase	15.4	108.5	8.29	58.4									14.2	
Alfa-L-fucosidase	1.45	53.1	0.55	20.1									2.73	
Alfa-D-mannosid-ase	2.57	75.4	1.68	49.3									3.41	
Beta-D-glucosidase					0.66	300							0.22	
N-acetyl-beta-D-glucosaminidase					9.7	144.8							6.7	
Beta-glucosidase											4.49			
Serum arylsulphat-ase-A μmol/ml/4h	27.2	41.6											40.0	

TABLE II

Values of lysosomal enzyme activities from leukocyte homogenisate

Dg: Gaucher disease				
Beta-glucosidase μmol/g protein/h	Sz. Zs. ♂ 3.2	7 y. 22%	Controls 14.4	(n = 12) 100%
Dg: Metachromatic leukodystrophy				
Serum arylsulphatase A μmol/ml/h	G. I. ♂ 0.8	3 y.	Control 40.0	100%
Dg: Niemann–Pick sphingomyelinosis				
Sphingomyelinase μM/g/min	F. É. ♀ 30	3 y.	Controls 120–190	(n = 10) 100%

TABLE III

Serum lipid and lipoprotein parameters in HLP cases

Sign	Cholesterol mmol/l	Triglyceride mmol/l	Beta-lipoprotein g/l	HDL-Ch mmol/l	Type of HLP
B. Z. ♂ 6 y.	7.2	2.84	12.95	0.21	II b
D. E. ♀ 11 y.	8.5	2.00	16.80	0.51	II a
B. K. ♂ 7.5 y.	7.1	1.90	9.20	0.61	II a
Normal values	<6.5	<1.8	<8.0	1.5	—

amorphous material. Lazarus et al [9] described lamellar membranous lymphocyte inclusions in Niemann-Pick disease, similarly as found by Heyne et al [7] in NCL and GM_1 gangliosidosis.

Our present report relates to 6 children with mucopolysaccharidosis (5 of MPS type I and 1 of MPS type II), one child suffering from Gaucher disease, each from Niemann-Pick sphingomyelinosis, from metachromatic leukodystrophy and 3 children from hyperlipoproteinemia of Fredrickson type II.

Ultrastructural examinations were carried out on the peripheral blood lymphocytes and on the PMNL in HLP.

Data on the examined cases are presented in Tables I and II, which give the clinical diagnoses, the enzyme activities and the serum lipid and lipoprotein parameters in the HLP cases.

Method

The lymphocytes and the PNML were isolated with the Ficoll–Hypaque method fixed in buffered glutaraldehyde, washed in the same buffer, and postfixed in OsO_4. After a dehydration process, the blocks were embedded in Araldite. The ultrathin sections were examined in Tesla 3550 and a Zeiss EM 9S2 electronmicroscope. The methodology relating to the enzyme examinations is described in references [3, 4].

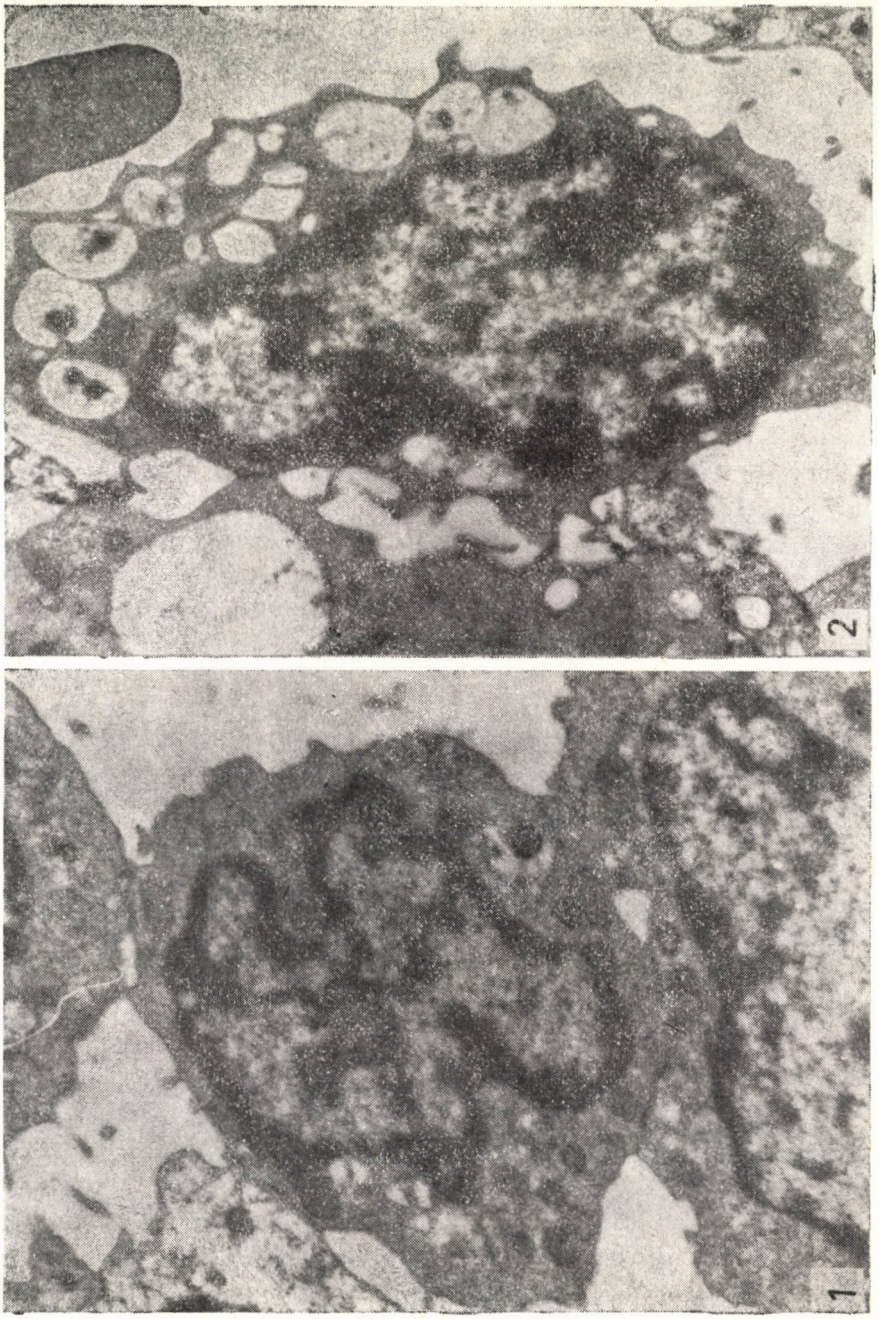
Results

A common pathological change in the lymphocytes 5 of the MPS I patients and 1 of the MPS II patient was the high degree of vacuolization. The number of vacuoles varied between 5 and 18 in the perinuclear cytoplasmatic region or in the full width of the cytoplasm. The variable diameter of the vacuoles or inclusions should be noted (0.2–1.8 μm). Aggregated osmiophilic substance or striped, reticular, granular, vesicular, amorphous material could be seen centrally in many vacuoles. The individual vacuoles were surrounded by a limiting membrane; they were round or oval in shape, most of them having a diameter of ca. 0.5 μm. The proportion of vacuolized lymphocytes was very high, at 20–25% (Figs 1–4).

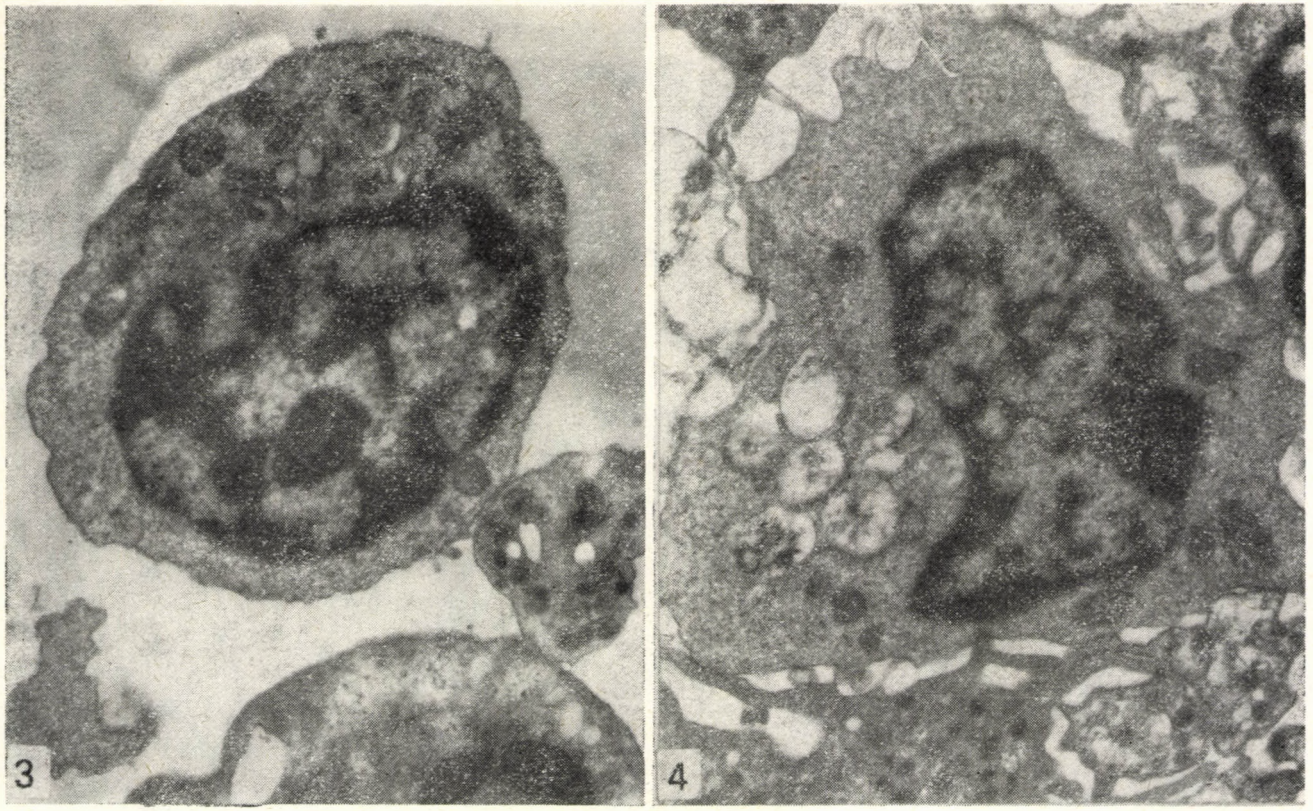
In our Gaucher disease patient (Sz. Zs. 7 y.) the lymphocytes and the PMNL displayed pathological vacuolization and inclusion formation intracytoplasmatically with a limiting membrane, and with fine granular and striped amorphous material accumulated (Fig. 5).

A number of rough myelin-like structures were detected in the cytoplasm of the lymphocytes; in the neutrophilic granulocytes, the intracytoplasmatic inclusions varied greatly in diameter (Fig. 6).

In Niemann-Pick sphingomyelinosis, the pathological change of the lymphocytes was manifested in the intracytoplasmatic large number of membranous lipid inclusions and lipid droplets, and electro-dense granular inclusions (Fig. 7). The cytoplasm of the lymphocytes of our MLD patient (G. I. 3 y.) was packed with partly empty, variable-diameter, and partly big multicystic vacuoles, with striped



Figs 1–2. (1) Control lymphocyte. The nucleus and the other cytoplasmatic organelles appear normal (×13 000).
(2) P.A. Dg: MPS type I (×15 000)



FIGS 3–4. Lymphocytes of MPS cases. (3) K.G. Dg: MPS type II. (×13 000). (4) T.G. Dg: MPS type I. (×13 000) membrane limited lymphocytic vacuoles tend to assemble in groups and contains membranous or other non-specific structures

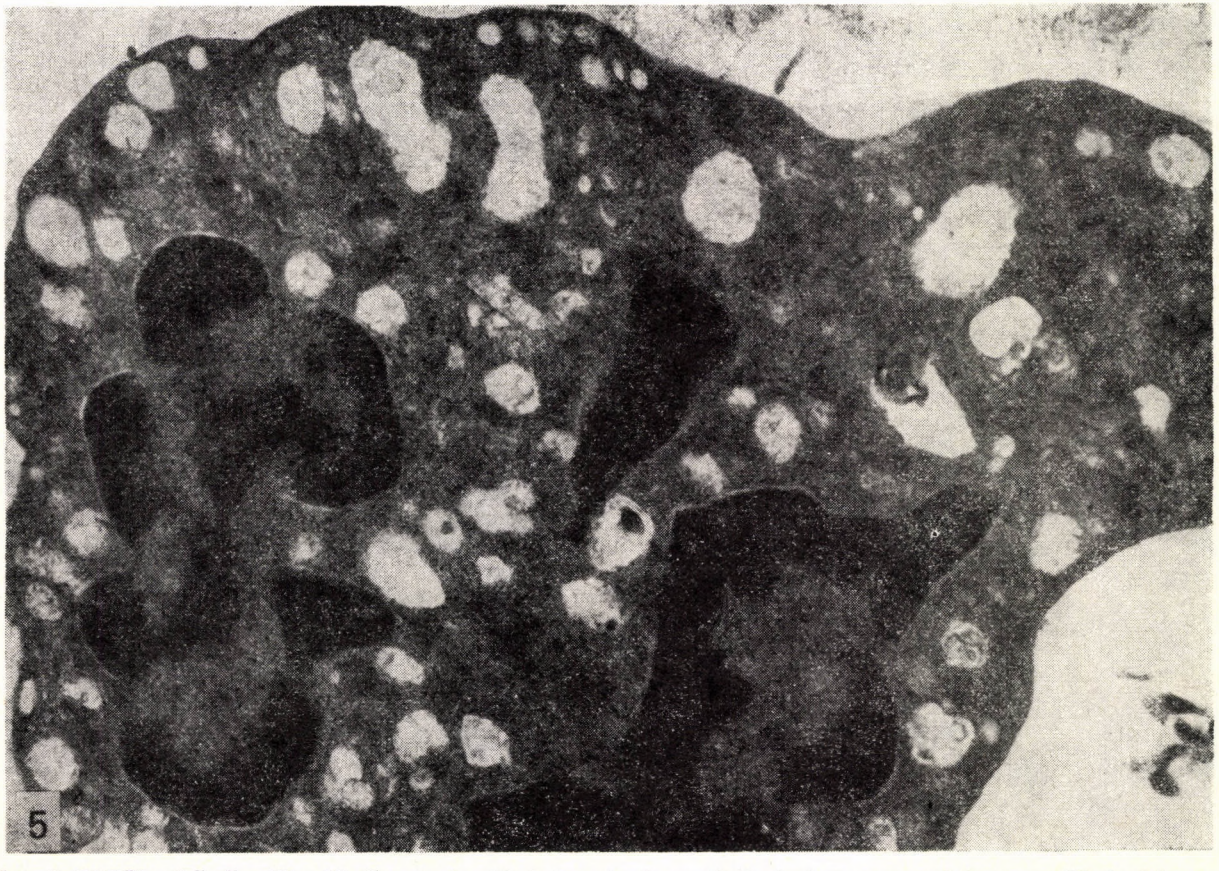
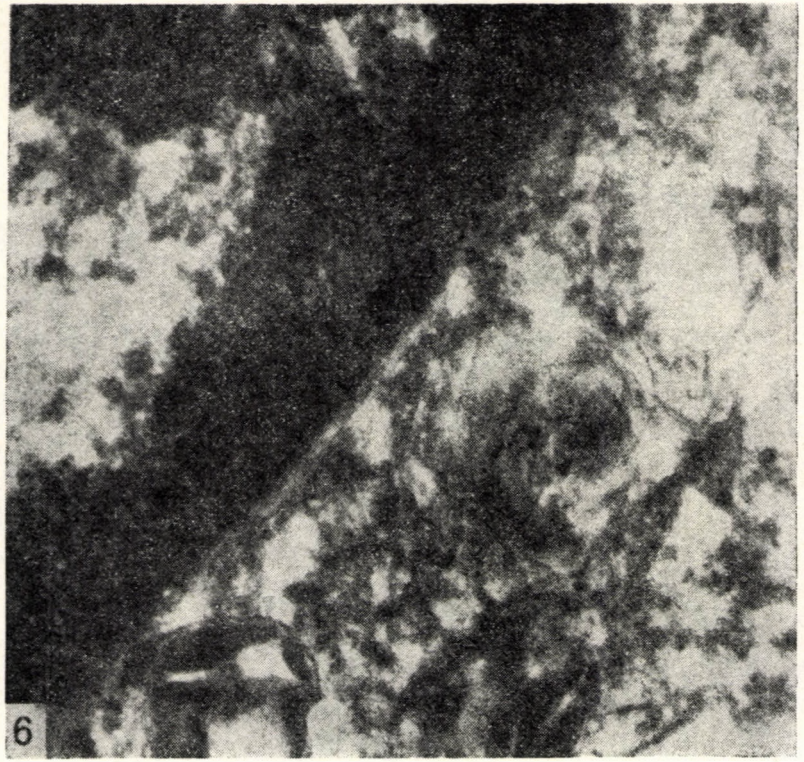


FIG. 5. PMNL of Sz.Sz. (Dg: M. Gaucher) with intracytoplasmatic inclusions, some of them are filled with curvilinear profiles (×20 000)



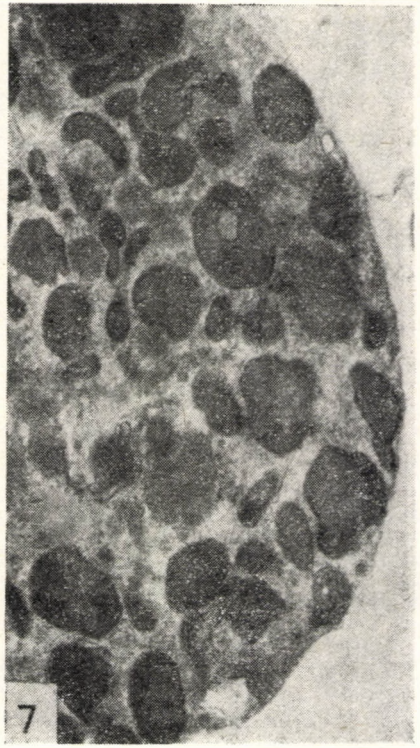


FIG. 6. Lymphocyte of Sz.Zs. (Dg: M. Gaucher) with myelinlike structures (×70 000)
FIG. 7. Lymphocyte of F. É. Dg: Niemann-Pick sphingomylinosis (×10 000)

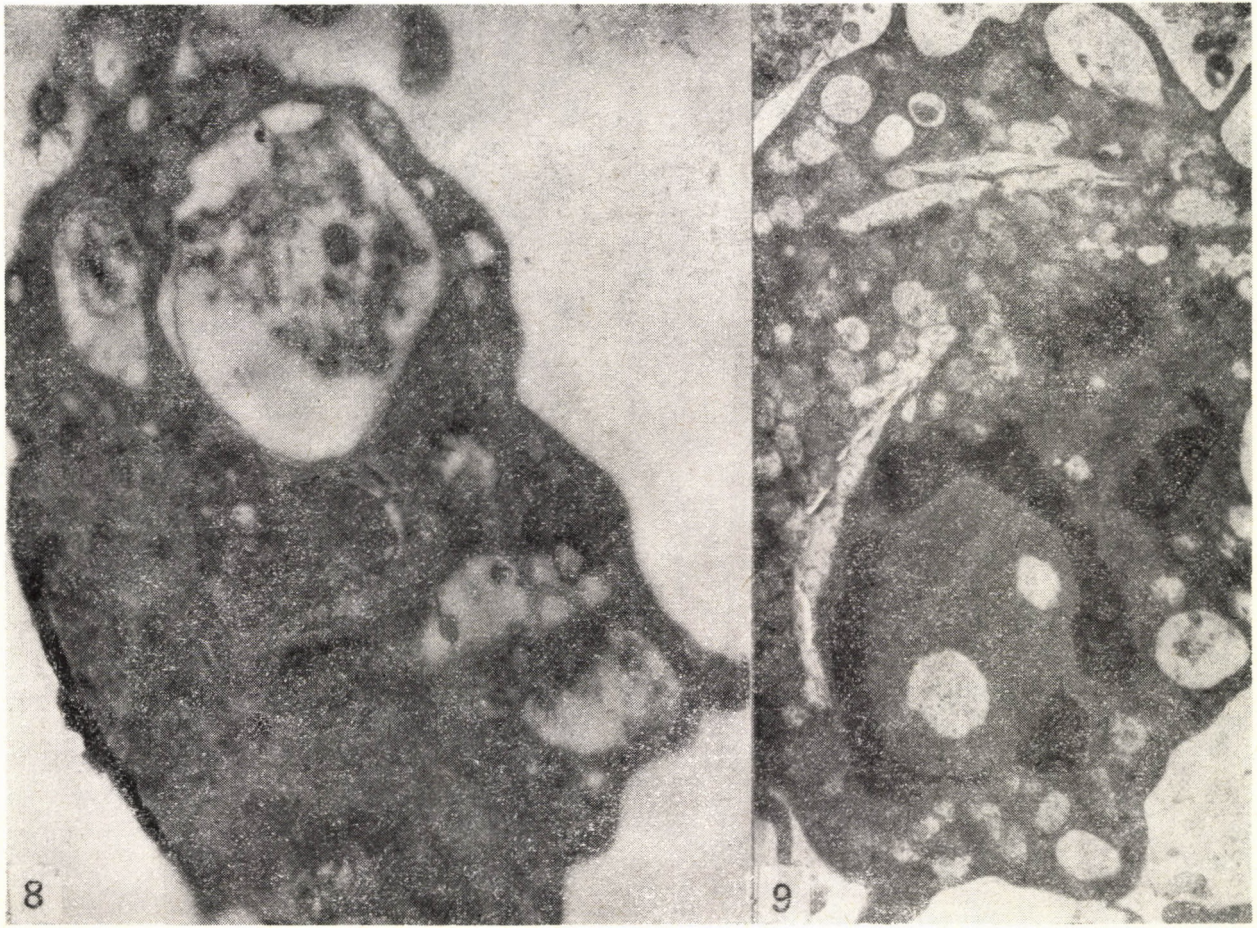


FIG. 8. Lymphocyte of G.I. Dg: Metachromatic leukodystrophy (×17 500)
FIG. 9. PMNL of B.Z. (Dg: HLP type IIb) with cholesterolcrystals (×10 000)

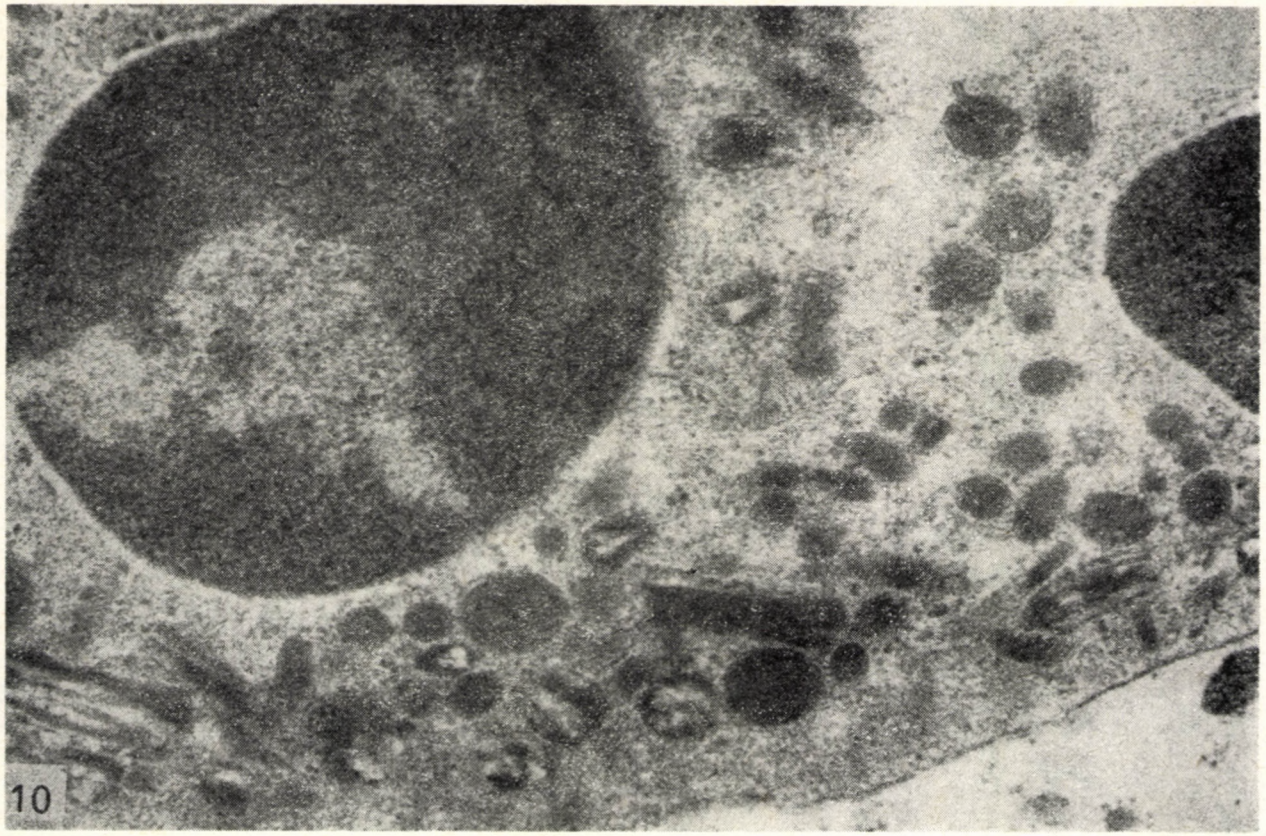


Fig. 10. PMNL of B.K. (Dg: HLP type IIa) with cholesterolcrystals and intracytoplasmatic vacuoles (×45 000)

or granular amorphous deposits (Fig. 8).

In the HLP cases, the most specific change is considered to be the meedle-shaped accumulation of PMNL, corresponding to an intracytoplasmatic, separated cholesterol crystal; in addition, very variable-diameter vacuolization could be seen, corresponding to the phagocytized stored lipid or lipoproteins, with a fine granular striped amorphous content and with bizarre, inclusion-like membranous lipid structures (Figs 9–10). Of the lymphocyte changes, the long striped myelin bodies and the amorphous material stored in the vacuoles surrounded by intracytoplasmatic membrane, presumably representing lipoproteins.

Discussion

Ikeda et al [8] divided the lysosomal disorders into the following three groups, group 1: pathological findings with specific inclusions: each type of NCL, presumed mucolipidosis of type IV, glycogenosis of type II; 2: pathological findings with vacuoles: MPS of type I—II, IIIA, IIIB, IV, VIA, VIB, GM_1 gangliosidosis; 3: apparently no pathological findings: juvenile and adult MLD, mucolipidosis of type III, GM_2 gangliosidosis, Gaucher disease.

On the basis of the pathological changes we observed in Gaucher disease and juvenile type of MLD in our present studies, we do not agree that these should be included in group 3 of the classification of Ikeda et al [8]; we suggest instead that these two diseases be classified into group 2.

In agreement with Ikeda et al [8], we consider ultrastructural examination of the peripheral lymphocytes and also the PMNL to be important and informative in the cases of lysosomal enzymopathies. It should be noted that liver biopsy material was also subjected to ultrastructural examinations in our cases. In our view, the morphological findings on the peripheral leukocytes are equal in value to the ultrastructural information on the liver biopsy material and, as a non-invasive technique, can advantageously replace the biopsy.

Naturally, the supplementary specific lysosomal enzymatic activity and clinical examinations must still be performed if a diagnosis of the lysosomal diseases is to be made.

It should be stressed that the above ultrastructural examination does not permit differentiation between MPS of type I (Hurler disease) and MPS of type II (Hunter disease).

We have encountered no publications on similar ultrastructural examinations of peripheral leukocytes in hyperlipoproteinemic cases.

We wish to draw attention to the possibility of the ultrastructural examination of peripheral lymphocytes and PMNL which provides rapid informative data of diagnostic value in connection with lysosomal enzymopathies and (for the first time) HLP.

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