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An Ultrastructural Study in a Case of Wolman's Disease*

(Clinical, Biochemical, Light and Electron Microscopic Study)

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Wolman's disease usually becomes clinically evident in the first weeks of life and is characterized by gastrointestinal symptoms, failure to thrive, hepatosplenomegaly, steathorrhea, calcification and enlargement of adrenals.[1] The greatly increased amounts of cholesterol and neutral fat in this lipidosis were first shown by Wolman' et. al. in the liver, spleen and adrenals.[2] Recently a total deficiency of acid lipase activity directed toward the hydrolysis of triglycerides and cholesterol esters, has been demonstrated in this disease.[3]

Since ultrastructural studies could be carried out only twice,[4, 5] in Wolman's Disease, liver and spleen biopsy materials results should be given in detail.

Materials and Methods

Liver and spleen needle biopsy materials were obtained from 45 day old girl in whom Wolman's disease was diagnosed by clinical, laboratory and chemical analysis,[5] and they were fixed in phosphate buffered 2,5 % of gluteraldehyde and 1 % of osmiumfetroxyde,[6, 7, 8] dehydrated

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through increasing concentrations of ethyl alcohol.[9] Thick and thin sections were taken from the araldite embedded tissues using LKB-Ultratome III.[10] 1 % toluidin blue stained thick sections were examined under the light microscope, lead citrate,[11] and uranly acetate[12] contrasted ultrathin sections were examined in a Carl Zeiss 9 A type electron microscope. Biometric measurements were employed according to Weible.[13]

Results

A. Liver: Under the light microscope, connective tissue in the portal areas and also areas between the parenchymal cells seemed to be increased. Plenty of vacuoles spread out in the connective tissue, some of them fused with each other, were seen under higher magnification.

In the low power electron micrographs, lipid inclusions of various electron densities were observed in the cytoplasm of some of the parenchymatic cells (Figure 1). Some parenchymatic cells also showed organel free degenerated areas (Figure 1). the cytoplasm especially nuclei, mitochondria, rough and smooth endoplasmic reticulum were prominent (Figures 1, 2, 3).

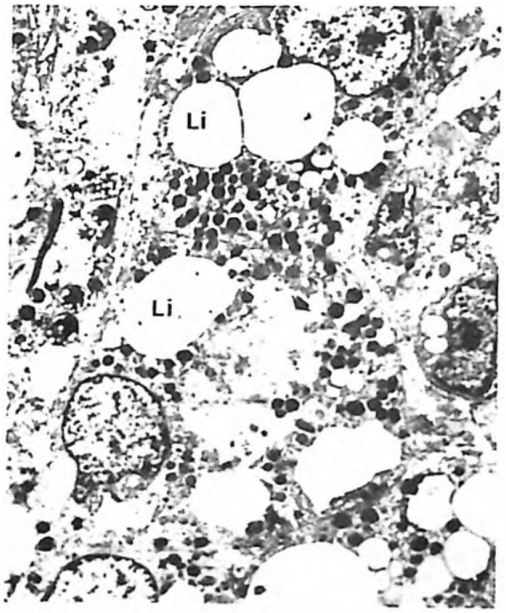

Figure 1

Low power electron micrograph of liver parenchymal cells. Degenarated areas (arrow) and lipid droplets (Li) are seen in the cytoplasm X 5000

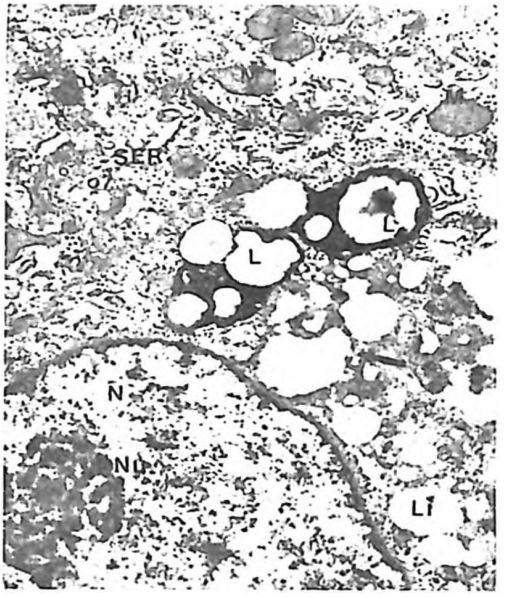

Figure 2

Detail of a liver parenchymal cell. N; Nucleus; Nu: Nucleolus; M: Mitochondria; SER, Smooth Endoplasmic Reticulum; L: Secondary Lysosome; Li: Lipid Droplet and degenerated areas (arrow) X 1800

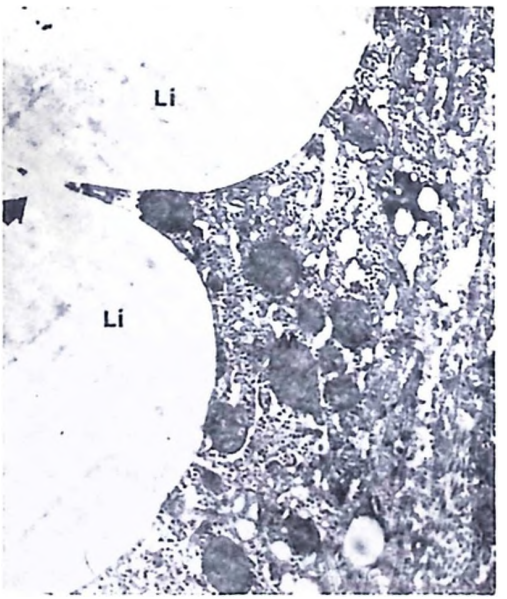

Figure 3

Mitochondrions have shown irregular crystal patern (M) and a connection is seen between two lipid droplets (Li). X 18000

With the higher magnification steps of electron microscope detailed structure of parenchymal cells was seen. The heterochromatin inclined to be aggregated around the periphery of the nucleus. This is considered as a normal structure (Figures 1, 2). Dilated vesicules of smooth surfaced endoplasmic reticulum were scatterred all over the cytoplasm (Figure 2, 3) but Golgi complex seemed normal (Figure 2). Mitochondrions of various sizes (0,2-0,8 micron diameter) and different electron densities were scatterred all over the cytoplasm (Figures 2, 3). The cristee of mitochondria were curved irregularly (Figures 2, 3). At places where outher limiting membrane of mitochondria was discontinious, mitochondrial matrix seemed to be extruded to the cytoplasm (Figure 2, 3). Lysosomes were seen in the vicinity of lipid inclusions (Figure 2). Among the smooth surfaced endoplasmic reticulum tubules and mitochondrions, alfa and beta glycogen particles were observed (Figure 2, 3). Lipid inclusions of various sizes (0,1-6 micron diameter) and electron densities covered most of the cytoplasm (Figures 1, 2, 3). Some of them were fused with each other (Figure 3 arrow). There were also secondary lysosomes containing several lipid droplets (Figure 2). Electron density of the lipid inclusions varried. Some of them had electron dense lameller structure embedded in a less elecron dense matrix (Figure 3).

Like the parenchymal liver cells; Kupffer cells also contained lipid inclusions of various size and electron densities (Figure 4). In addition to this they also contained clefts of cholesterol crystal (Figure 4).

B. Spleen: Under the light microscope foam cells and lymphocytes were observed. All the blood vessels and sinusoids were full of foam cells.

In low power electron micrographs small and medium sized lymphocytes, macrophages, plasmocytes and erythrocytes were seen (Figure 5). Using higher magnifications lymphocytes could be divided into two groups (Figure 4).

In first group of lymphocytes cytoplasm was filled with ribosomes, polysomes and their electron density was higher than the others (immunoblast or pyrinophyl cell) (Figure 4). Second group of lymphocytes had low electron density cytoplasmic matrix; and their organelles were easily distinguished (Figure 5). Among the lymphocyte groups, plasmocytes were also observed (Figure 5). Cytoplasms of plasmocytes had well developed rough surfaced endoplasmic reticulum; filled with electron dense material (Figure 5). The electron density of the mitochondrial matrix was less than that of the cytoplasm (Figure 5). Another recognised cell type was macrophage (Figures 6, 7). Some of them were inactive (Figure 6), and the others were active (Figure 6, 7). These active cells

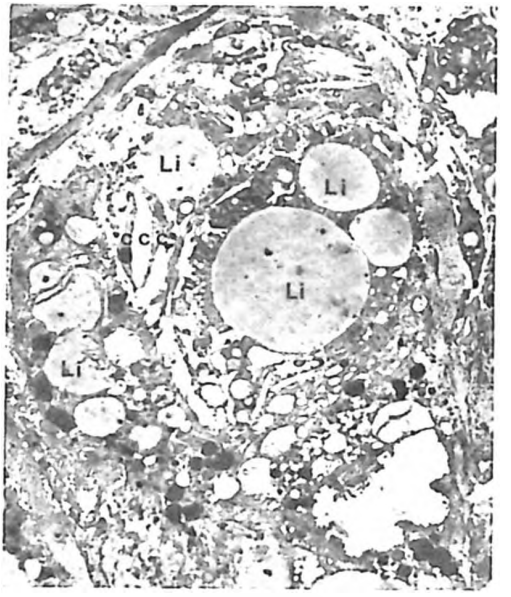

Figure 4
A characteristic view of Kupffer cell in Wolman's Disease. Li. Lipid Droplets; CCC: Cholesterol Crystal Cleft. X 18000

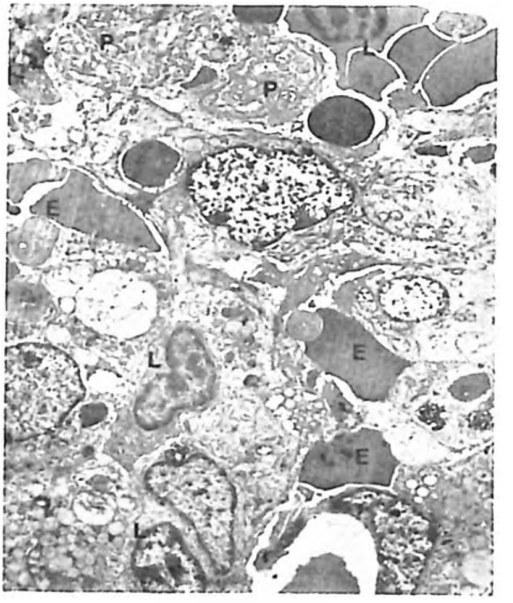

Figure 5
A panoramic view of spleen. Active and inactive macrophages, plasmocytes (P), Lymphocytes (L) and erythrocytes (E) are seen X 5000

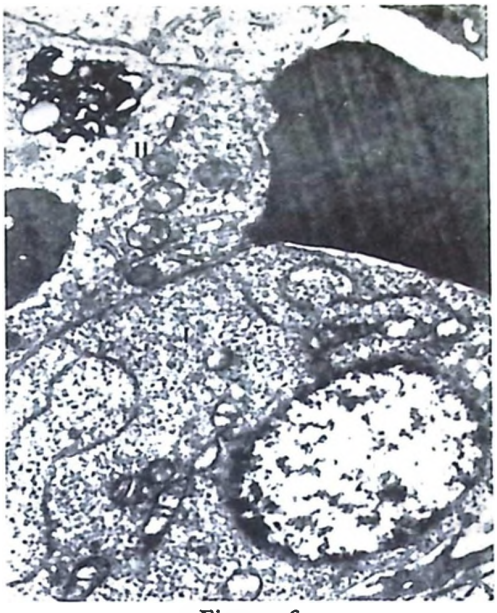

Figure 6
Inactive (I) and active (II) macrophages cytoplasms can be seen in electron micrograph X 18000

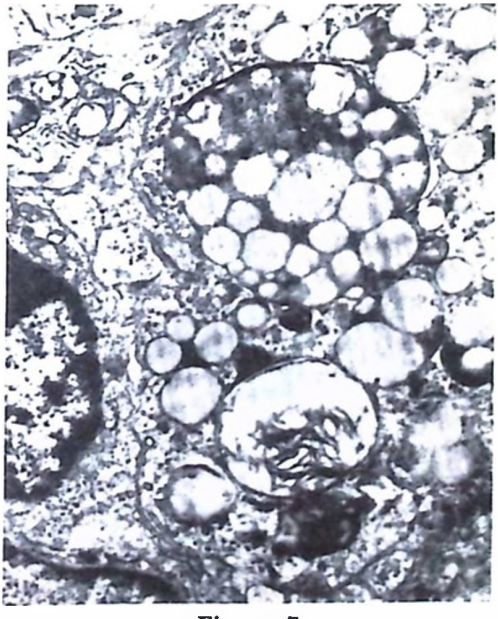

Figure 7
An electron micrograph of a active macrophages. Electron dense material can be seen at the periphery of the lipid droplets. X 18000

had lipid inclusions of various lengths (0,1-2 micron) diameter and electron densities. There were electron dense granules very close to the lipid droplets (Figure 7). Secondary lysosomes were also noticed in the activated macrophage cytoplasm (Figure 7). There were degenerated macrophages in the sinusoids.

Discussion

The morphologic criteria of the cholesterol strorage in this lipidosis are cholesterol crystal clefts in the macrophages.[5] Several investigators found macrophages which were filled by cholesterol crystal clefts and lipid inclusions in spleen, adrenal gland and intestinal mucosa.[4, 5, 15] In our case we also found various size and density lipid inclusions of electron in the macrophages of spleen and liver. The term of "foam cell" instead of macrophages is preferred, in lipidosis by some authors.[14, 15]

In some of the foam cells caryolytic nuclei were observed and cell membranes were discontinuous, as has also been mentioned by Lough et. al.[5] There was no inflammatory reaction in the surrounding tissues. Cholesterol and its esters are seen as empty clefts in the electron microscope, and named "cholesterol crystal clefts."[5] We observed morphological similarities between these clefts and those of aterosclerotique plaques which were described by Ghidoni et. al.[16] in the eletron microscope, which is another criteria of the cholesterol phagocytosis by the Kupffer cells. We also observed lipid inclusions of various electron densities in the liver parenchymal cells, which were mentioned previously.[5] Cholesterol crystal clefts were not seen in liver parenchymal cells as indicated in this disease.[5]

Although foam cells, in other kinds of lipid storage diseases, could be seen but one may easily distinguish Wolman's disease by seeing cholesterol crystal clefts inside of the macrophages[5, 15] which is the advantage of investigation by electron microscopy..

Various hypothesis for the etiology of the disease may be divided into two groups; production excess or destruction failure. According to Sloviter[17] synthesis of lipids especially triglycerides is increased. However, following Patrick and Lake[3, 9] findings that lysosomal lipase is diminished, it is clear that the failure of catabolism of cholesterol esters and triglycerides are main steps of this disease. The excess lipids and cholesterol esters are phagocytossed by reticuloendothelial cells all over the body organs this cause the enlargemet of them.

Summary

The ultrastructural studies of liver and spleen in a case of Wolman's disease are reported. Lipid accumulation in parenchymal cells, and cholesterol crystal clefts in Kupffer cells were observed in the liver. Lipid deposited macrophages (foam cells) were observed in the spleen.

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