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Membranous lipodystrophy-like changes in ischemic necrosis of the legs*

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Summary. Subcutaneous fat from 3 patients with ischemic necrosis of the legs due to arteriosclerotic obstruction were examined histologically and ultrastructurally. Markedly convoluted membranocystic changes were found in all 3 cases. The light and electron microscopic findings of the membranocystic lesions are very similar to those of fat tissue changes in membranous lipodystrophy. Bone lesions and mental disturbance which suggest membranous lipodystrophy, however, were absent in these cases. It is concluded from these results that the membranocystic changes characteristic of membranous lipodystrophy can be produced by circulatory disturbance and the lesions are one of the non-specific changes of adipose tissue.

Key words: Membranous lipodystrophy – Fat tissue – Ischemic necrosis – Ultrastructure

A new disease has recently been recognized in Japan in which a peculiar dystrophy of adipose tissue of the long bones and soft tissues occurs. The lesion is characterized by convoluted membranocystic changes and the disease is associated with sudanophilic leukodystrophy (Nasu et al. 1973). The same disease has been reported independently by Finnish doctors (Järvi et al. 1968) and described under the title of osteodysplasia polycystica hereditaria combined with sclerosing leukoencephalopathy (Hakola et al. 1970). The bone lesion has also been named lipomembranous polycystic osteodysplasia (Hakola 1972). Although the disease has been believed to be a hereditary disorder of lipid metabolism (Nasu et al. 1973; Wood 1978), we have found similar membranocystic lesions of the subcutaneous fat tissue in the cases of ischemic necrosis of the legs. Light and electron microscopic findings of the fat tissue changes in 3 patients are presented in this report.

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Case report

Case 1

A 76-year-old man with a history of diabetes mellitus for more than 20 years underwent amputation of his left leg due to necrosis of the foot. Histopathological examination of the anterior and posterior tibial arteries and of the peroneal artery revealed atherosclerotic obstruction. Neither bone lesion nor mental disturbance was found in this patient.

Case 2

A 61-year-old woman had been treated for diabetes mellitus for two years before amputation of her left leg due to necrosis. An attack of cerebral infarction and left hemiplegia developed 3 months before amputation. Histological examination of the amputation specimen revealed marked intimal fibroelastosis with thrombosis of the left anterior tibial artery and a diagnosis of arteriosclerotic obstruction was made. Neither bone lesion nor mental disturbance was present in this case.

Case 3

A 67-year-old female without history of diabetes mellitus noticed cyanosis and numbness in her right leg one year and 5 months before amputation of her foot due to necrosis. Her right dorsal artery of the foot was not palpable a month before amputation. In spite of treatment with vasodilatator, pain and necrosis of her foot increased necessitating amputation. Histologically, the dorsalis pedis artery and other small arteries of the foot showed intimal fibroelastosis with organized thrombi and a diagnosis of arteriosclerotic obstruction was made. Neither bone lesion nor mental disturbance could be detected.

Materials and methods

Several blocks of the subcutaneous fat tissues near the site of ischemic necrosis of the legs were fixed in 10% formalin and embedded in paraffin. Sections of the fat tissues were stained with hematoxylin and eosin, alcian blue, periodic acid-Shiiff (PAS), silver impregnation, luxol fast blue, Azan-Mallory stain and resorcinol fuchsin stain for elastin, and were examined in the light microscope. Frozen sections of the subcutaneous fat tissues were also stained with Sudan III.

Small slices of the same subcutaneous fat tissues for electron microscopy were obtained from Case 1 and Case 3 after routine formalin fixation. They were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer at 4° C, post-fixed in 1% OsO_4 and embedded in Epon 812 or in Araldite. Ultrathin sections were stained with uranyl acetate and lead, and examined in a Hitachi HU-12 or a JOEL 100 C electron microscope.

Fig. 1. Fully developed membranocystic change showing markedly convoluted appearance observed in Case 1. A cell nucleus can be seen in the middle of the lesion which is closely surrounded by fat cells. H.E. × 600

Fig. 2. Many membranocystic changes are present in fibrosis in adipose tissue from Case 1. H.E. × 280

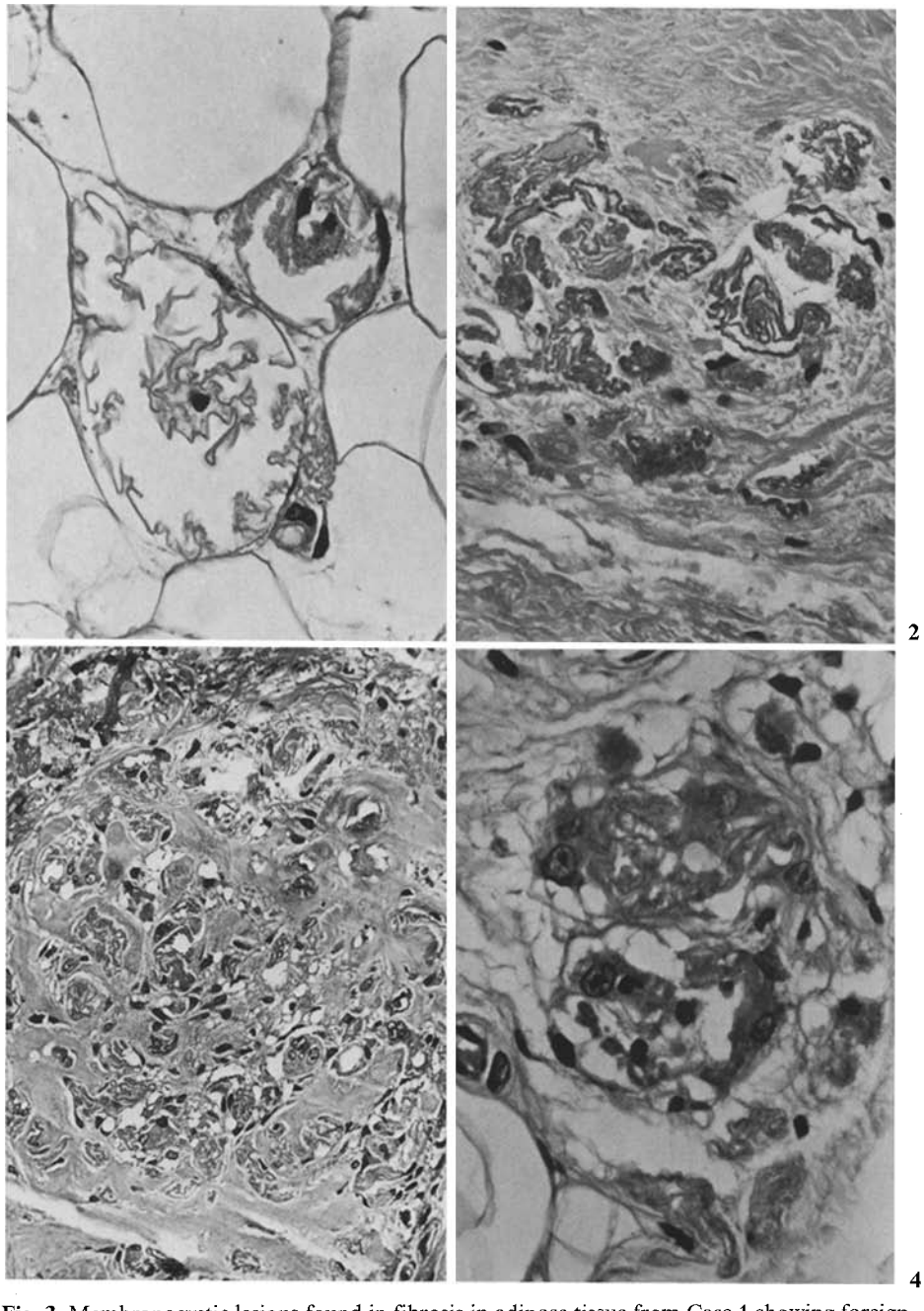

Fig. 3. Membranocystic lesions found in fibrosis in adipose tissue from Case 1 showing foreign body giant cell reaction. H.E. × 280

Fig. 4. Slightly basophilic, partly membranous and partly amorphous substance can be seen in Case 3. The substance is associated with a few macrophage-like cells. H.E. × 580

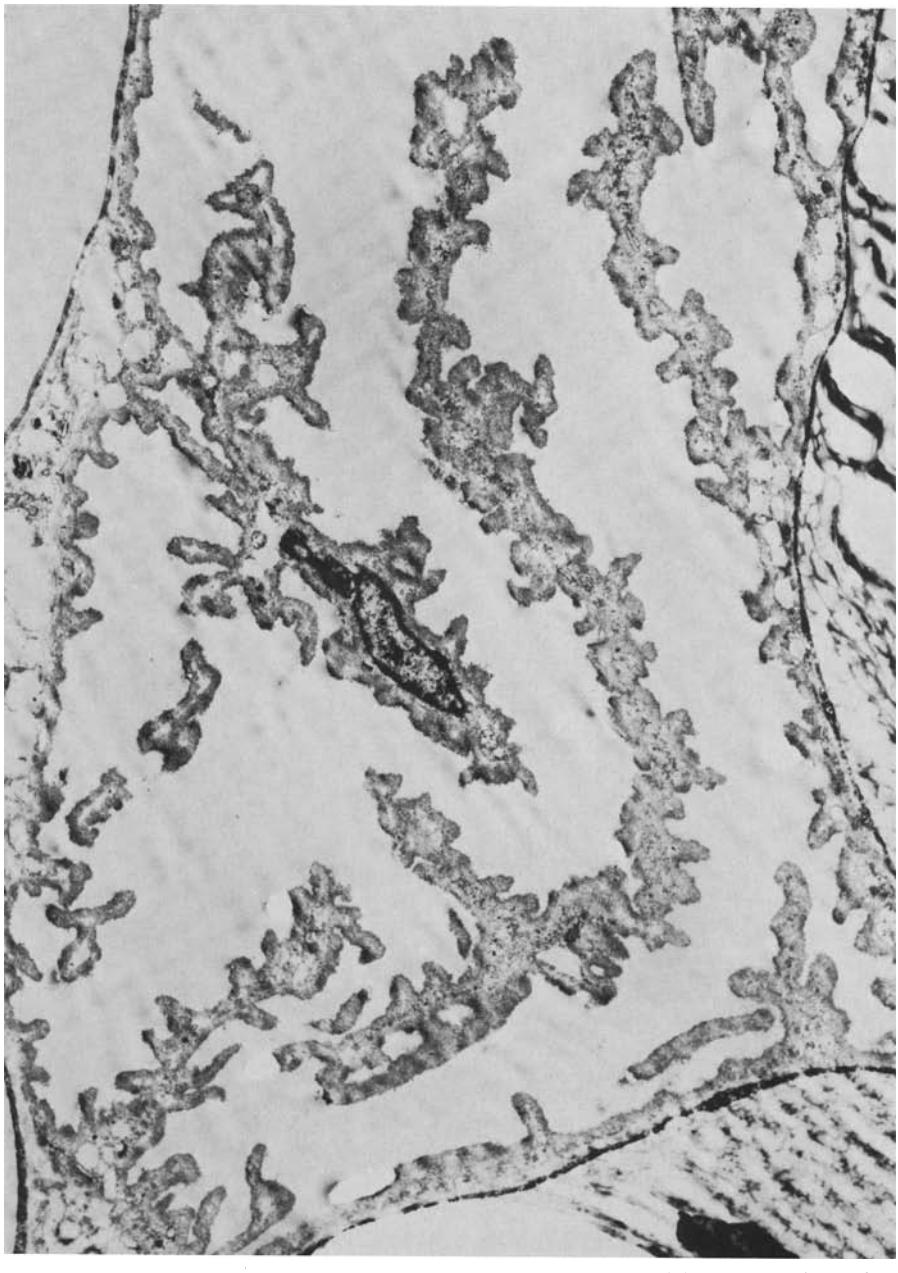

Fig. 5. Ultrastructure of a fully developed membranocystic change which corresponds to Fig. 1. The lesion is closely surrounded by fat cells and a cell nucleus can be seen in the middle of the figure. ×7,200

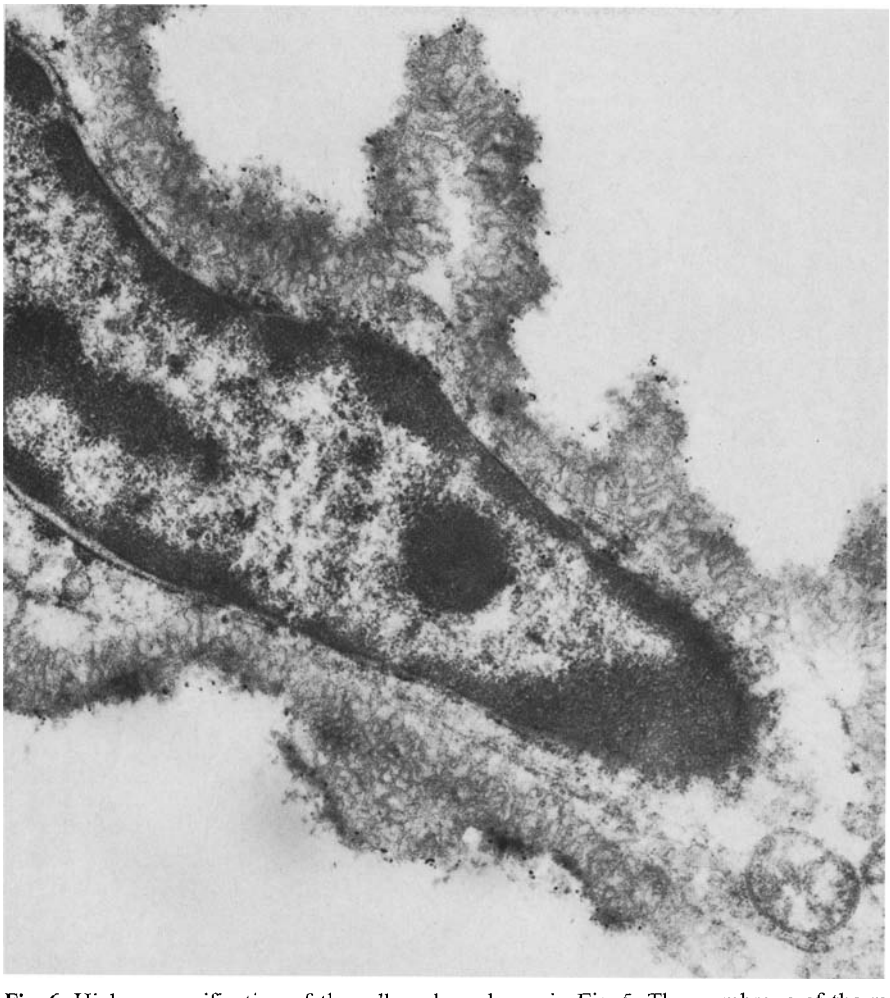

Fig. 6. Higher magnification of the cell nucleus shown in Fig. 5. The membrane of the membranocystic change is about 0.5 micron in thickness and composed of minute tubular structure with width of about 0.05 micron. No cell membrane can be seen between the nucleus and the membrane of the membranocystic lesion. × 24,000

Results

Light microscopy

The subcutaneous fat tissue of the leg in Cases 1 and 2 and of the foot in Case 3 near the site of necrosis showed foci of markedly convoluted membranocystic changes. The membranocystic lesions measured from 10 to 20 micron in diameter containing material positive for Sudan III. They were scattered among normal appearing fat cells in Cases 1, 2 and 3 (Fig. 1) or found in fibrosis of adipose tissue in Cases 1 and 3 (Fig. 2). A nucleus was detectable in some of the lesions (Fig. 1). Foreign body giant cell reac-

Fig. 7. A well developed membranocystic lesion in fibrosis of adipose tissue containing electron dense material similar to that of the fat droplet of a fat cell at the right lower part of the figure. ×4,300

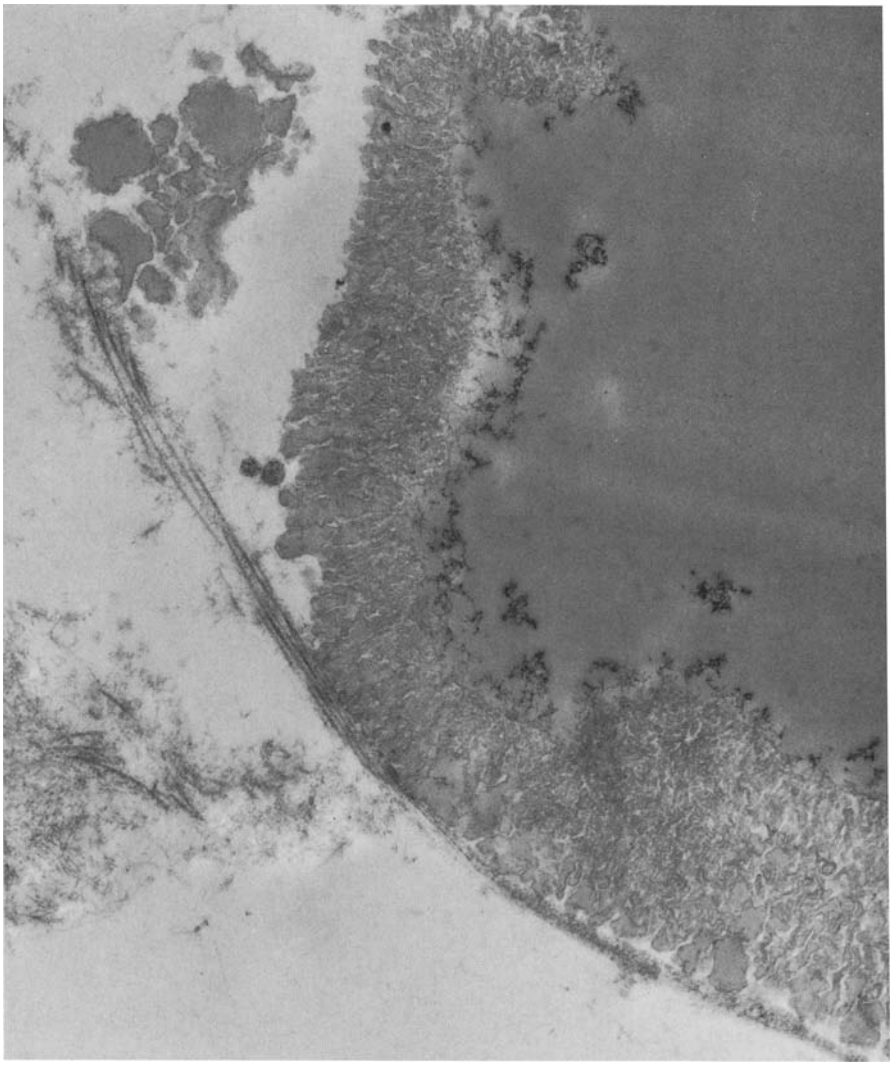

Fig. 8. The membrane of a membranocystic change in fibrosis. The membrane is 0.6–2.0 micron in thickness and composed of minute tubular structures with width of 0.03–0.3 micron. The same osmiophilic material to that of fat droplets can be seen not only in the cyst of the membranocystic change but also in the minute tubular structures. A trilaminar structure of the unit membrane is not detectable. A few small membrane bound lipid droplets can be seen at the left upper corner suggesting dilated tubular structures of the membrane of the membranocystic change. × 16,800

tion could be seen around the membranocystic changes in Case 1 (Fig. 3). The thickness of the membrane of the membrano-cystic changes varied from lesion to lesion (Figs. 1 and 2). The membrane was PAS positive and was stained red by Azan-Mallory staining, blue by luxol fast blue and black by silver impregnation, but was negative for resorcinol fuchsin staining for elastin and for alcian blue staining for mucin. Slightly basophilic, partly

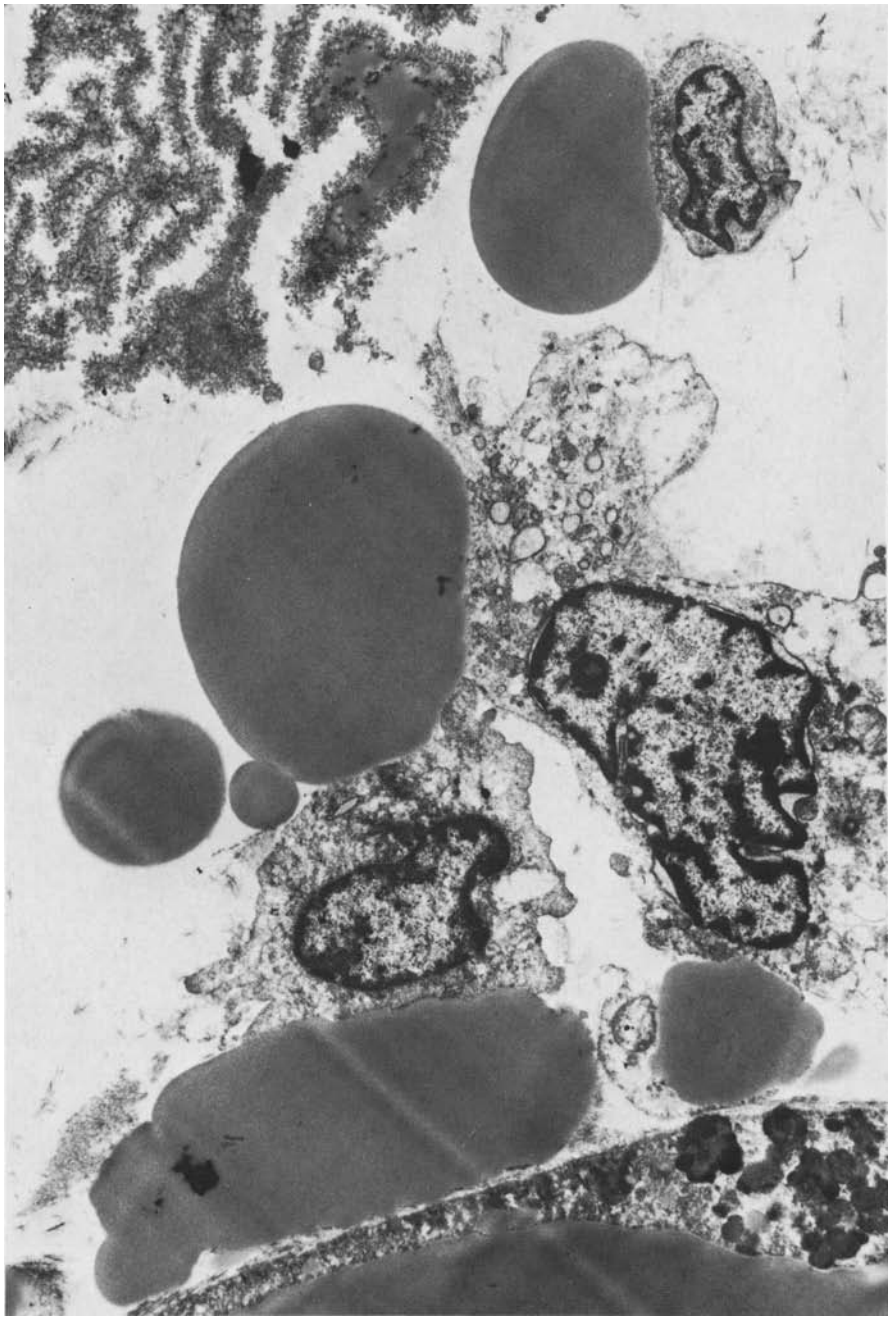

Fig. 9. A few free fat droplets without any membrane are seen in close contact with macrophages. A membranocystic lesion and a fat cell can also be seen at the left upper corner and the right lower part of the figure, respectively. ×7,200

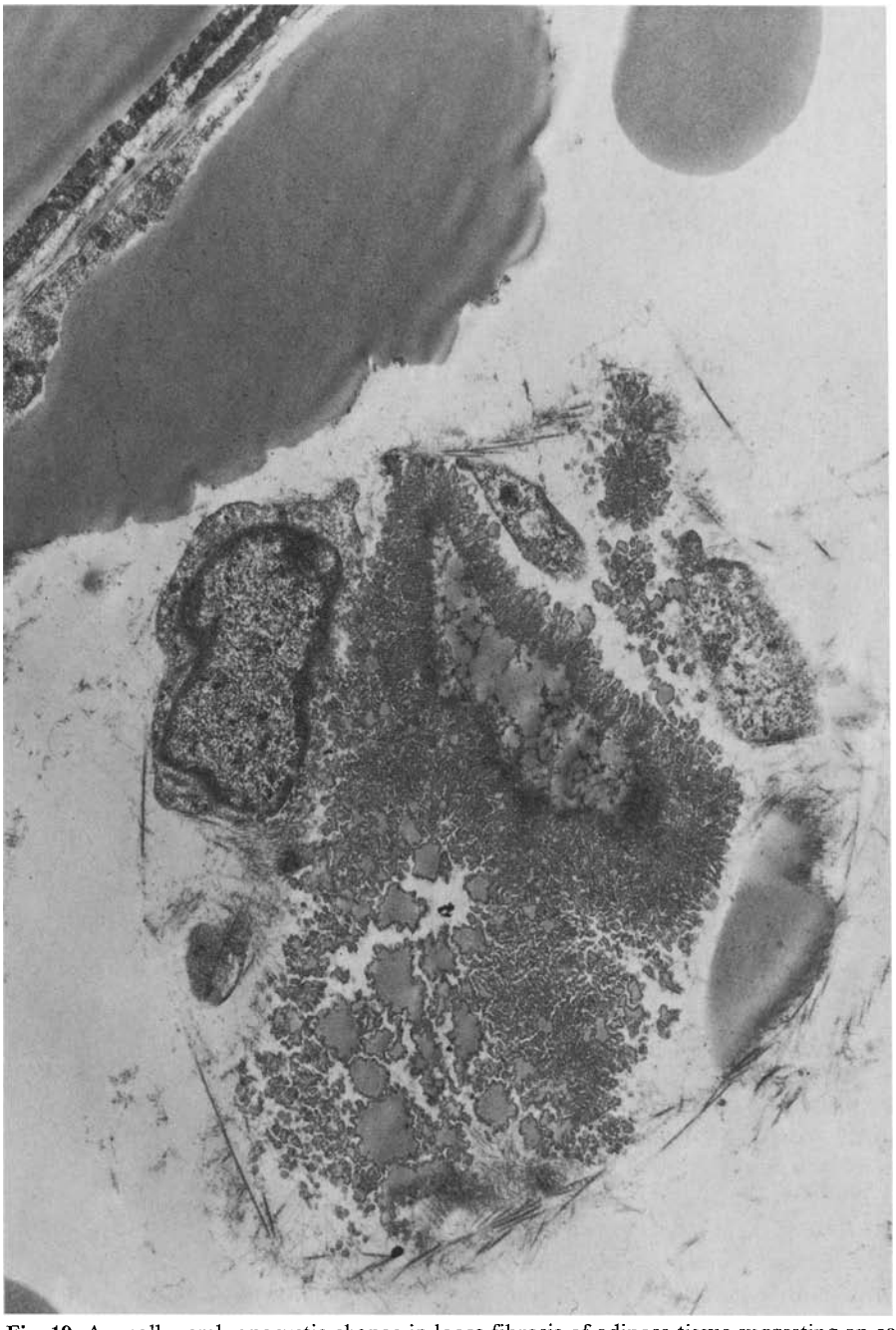

Fig. 10. A small membranocystic change in loose fibrosis of adipose tissue suggesting an early stage of the lesion. Dilated tubular structures containing osmiophilic lipid are seen at the lower part of the figure. A disintegrating fat cell can be seen at the left upper corner. Two free fat droplets are also seen at the left upper and lower part of the figure. ×11,500

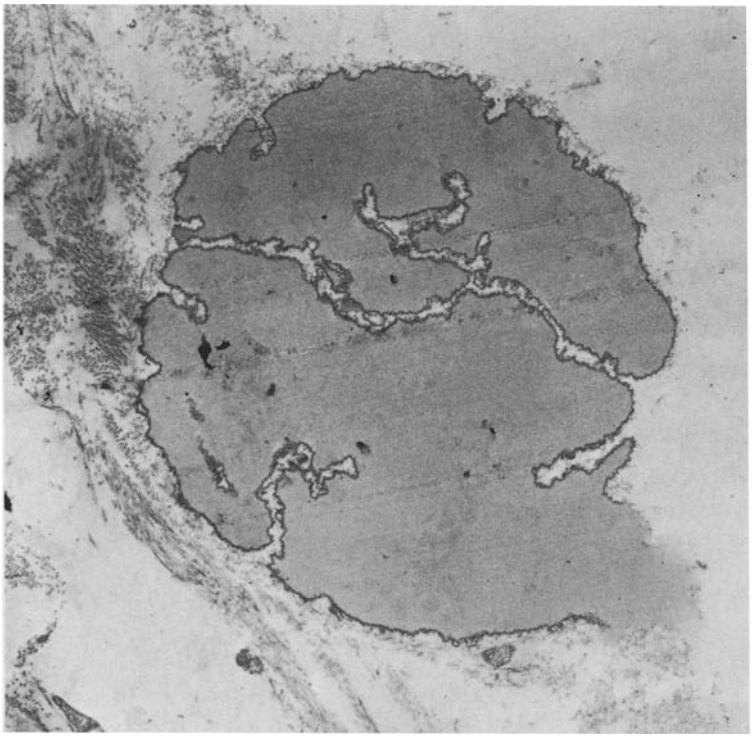

Fig. 11. Fat droplets covered by a thin membrane without tubular structure in fibrosis. ×9,500

membranous and partly amorphous substance was found together with the membranocystic changes in Case 3. The lesion was surrounded by a few macrophage-like cells suggesting an early stage of membranocystic changes (Fig. 4).

Electron microscopy

Several types of membranocystic changes were observed in the electron microscope. Well developed lesions showed marked undulation of the membrane containing a similar substance to the fat droplets of fat cells. A cell nucleus could be seen in some of the well developed lesions closely surrounded by fat cells as seen in the light microscope (Fig. 5). Under higher magnification, the membrane of the membranocystic change was about 0.5 micron in thickness and composed of minute tubular structure with a width of about 0.05 micron and an arrangement perpendicular to the inner surface of the cystic space. No plasma membrane could be seen between the cytoplasm surrounding the nucleus and the membrane of the membranocystic change (Fig. 6). Well developed membranocystic lesions were also found in fibrosis of adipose tissues together with fat cells (Fig. 7). Under higher magnification, the membranes of these lesions were

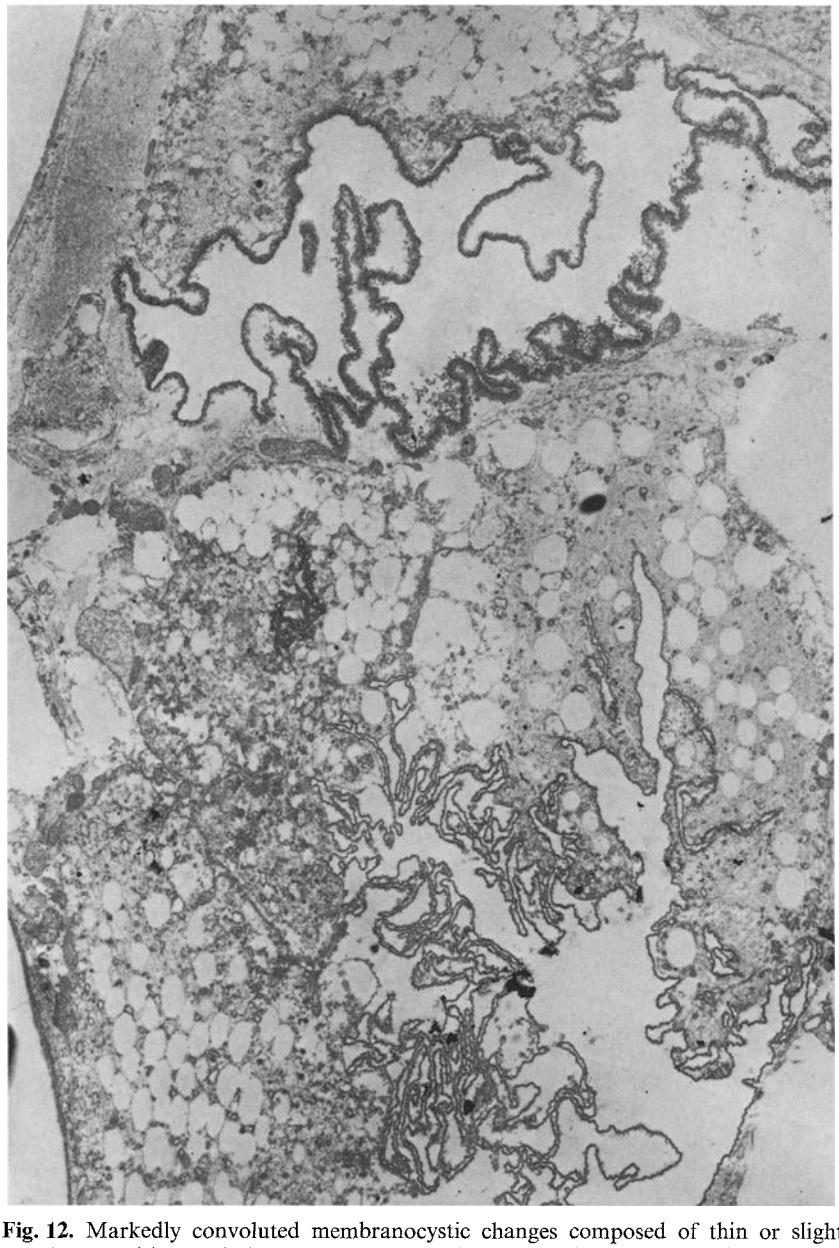

Fig. 12. Markedly convoluted membranocystic changes composed of thin or slightly thick membranes without tubular structure are seen in the cytoplasm of macrophages which contain many small fat droplets. ×4,800

0.6–2.0 micron in thickness and composed of perpendicularly projecting microvilli or minute tubular structures with width of 0.03 to 0.3 micron. The microvilli or crypts of the membrane contained the same electron dense material to that of fat droplets and showed communication with the cysts

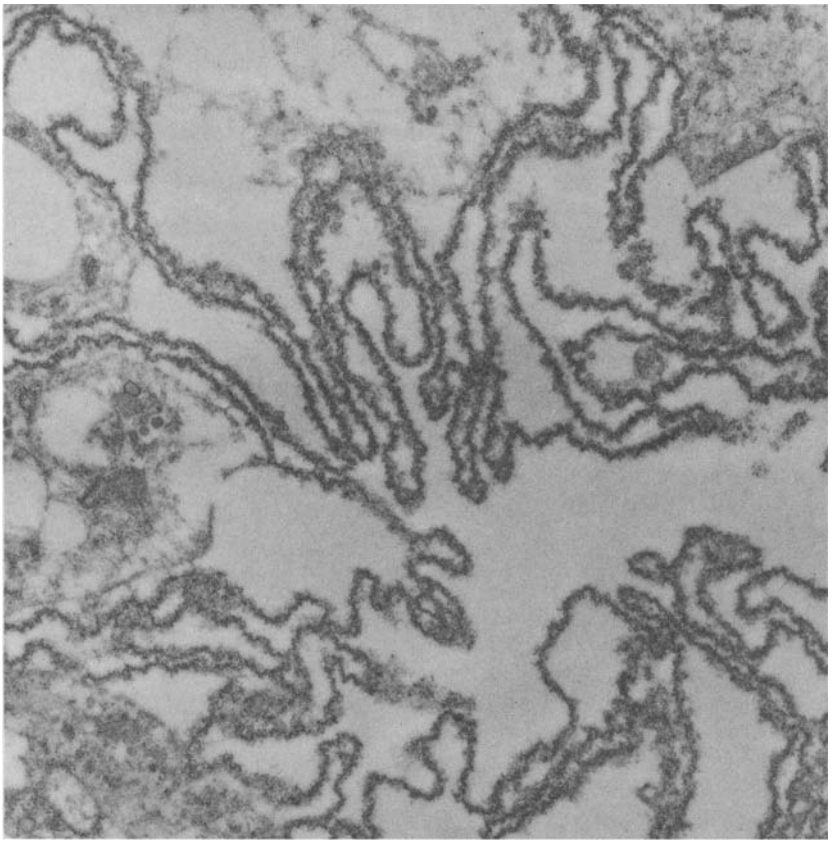

Fig. 13. Higher magnification of the markedly convoluted membranocystic change composed of thinner membranes in the macrophage shown in Fig. 12. × 29,300

of the membranocystic changes (Fig. 8). A definite trilaminar structure for the unit membrane was not detected in the membranes that composed the microvilli or crypts. A few free fat droplets without any covering membrane were seen in close contact with macrophages (Fig. 9). A small membranocystic change with associated minute tubular structures was found together with free fat droplets and a disintegrating fat cell in the loose fibrosis of the fat tissue (Fig. 10). The dilated tubular structure or crypts had the same osmiophilic amorphous material to fat droplets and the content of the cysts of the small membranocystic change. A few fat droplets covered by a thin membrane without tubular structures were also found in fibrosis (Fig. 11). The small membranocystic change shown in Fig. 10 seemed to be an intermediate stage between the well developed lesions shown in Figs. 5 and 7 and these fat droplets covered by a thin membrane shown in Fig. 11. Markedly convoluted membranocystic changes composed of thin or slightly thicker membrane without tubular structure were found in the cytoplasm of macrophages which also contained numerous small fat droplets (Figs. 12

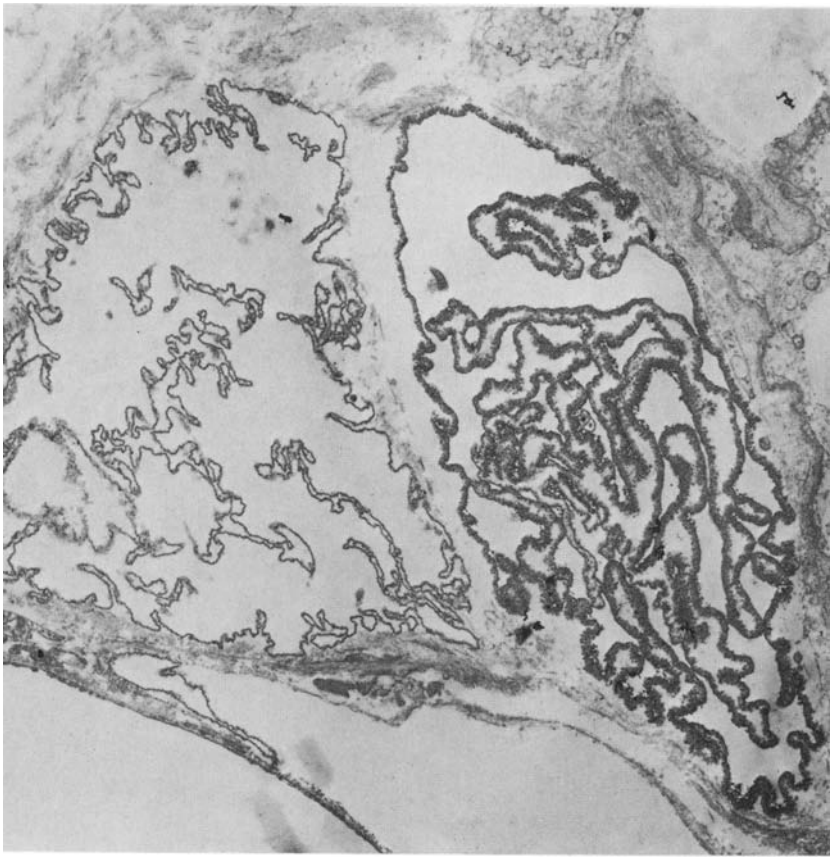

Fig. 14. Markedly convoluted membranocystic lesions in fibrosis showing both thin and slightly thick membranes similar to that of Fig. 12 but without any association with macrophages. No tubular structure can be seen in the membrane. ×6,100

and 13). Similar membranocystic changes showing both thin and slightly thick membranes without tubular structures were also found in fibrosis without any association with macrophages (Fig. 14).

Discussion

The light and electron microscopic findings in membranocystic changes found in our 3 cases of ischemic necrosis of the legs coincide well with those of fat tissue changes in membranous lipodystrophy (Nasu et al. 1973; Yagishita et al. 1976; Akai et al. 1977; Nasu 1978; Wood 1978). Two types of membranocystic lesions have been observed in the electron microscope in this study; one with a thick membrane showing minute tubular structures or perpendicularly projecting microvilli and the other with a thin membrane without microvillous projection or tubular structures. These two types of lesions have already been described in the bone marrow fat of membranous

lipodystrophy (Nasu et al. 1973; Wood 1978). The membranocystic lesions with a thin membrane without tubular structures seem to be an early stage of the lesion compared with the changes with thick membrane showing perpendicularly projecting microvilli, and an intermediate stage between the two could be observed in the present cases. The ultrastructural evidence of disintegrating fat cells, free fat droplets closely associated with macrophages and membranocystic lesions in the cytoplasm of macrophages demonstrated in the present study suggests that free fat droplets released from degenerated fat cells are processed by macrophages to produce membranocystic changes. Similar intracellular membranocystic lesions have been reported to be present in the cytoplasm of those mesenchymal cells (Yagishita et al. 1976) that are quite similar to our macrophages in ultrastructural appearance. In the present cases, a cell nucleus was seen in some of the well developed membranocystic lesions, and no cell membrane was detectable between the nucleus and the membrane of the membranocystic lesions. This may indicate that some of the well developed lesions are still in the cytoplasm of macrophages.

Membranous lipodystrophy is the name previously given by Nasu et al. (1973) to a rare disease in which cyst-like lesions of fat including that of long bones occur together with sudanophilic leukoencephalopathy. The same disease has been independently reported by Finnish doctors (Järvi et al. 1968; Hakola et al. 1970; Järvi 1970; Sourander 1970; Hakola 1972) and described under the title of osteodysplasia polycystica hereditaria combined with sclerosing leukoencephalopathy, or a new hereditary disease characterized by progressive dementia and lipomembranous polycystic osteodysplasia. Although it has been considered that remarkable complexity of the membranocystic changes of membranous lipodystrophy exceeds the reaction of normal fat cells and may be more consistent with a concept of an inborn error of metabolism of adipose tissue (Wood 1978), the same complex lesions were found in our cases of ischemic necrosis of the legs. The bone lesions and mental disturbance that suggest membranous lipodystrophy were absent in the cases reported here; therefore we consider that the membranocystic changes found in our cases are caused by ischemia due to arteriosclerotic obstruction. The similar histological features of membranocystic lesions of fat have been reported in several other conditions than membranous lipodystrophy such as lupus erythematosus profundus (Arnold 1956), fat tissue granuloma (Abrikossoff 1929; Horie 1954) and experimental ischemic changes of bone marrow (Rutishauser et al. 1960). A light and electron microscopic examination of the bone marrow fat tissue in experimental induction of myelofibrosis by saponin has also disclosed the same membranocystic lesions (Shimamine 1976; Nasu 1978). We draw the conclusion from these results that the membranocystic changes characteristic of membranous lipodystrophy can be produced by circulatory disturbance and the lesion is one of non-specific changes in adipose tissue.

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