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Studies on Subcutaneous Fat Necrosis of the Newborn*

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Summary. Biopsy specimens from the skin and subcutaneous fat tissue of four cases with neonatal subcutaneous fat necrosis were made and investigated by light and electron microscopy at 2, 4, and 6 weeks, and 5 months (Case 2) from the onset of the disease. Three stages of ultrastructural change of fat cells were observed. The evolution of crystal formation in the fat cells was seen and phagocytosis of crystals and fat droplets by macrophages and foreign-body giant cells was also noted. In the light microscope accumulation of calcium concretions in the spaces between and inside the fat cells was found. In the electron microscope we detected foci of highly electron-dense granules, which were similar in distribution and structure to calcium salts stained with the von Kossa method. Changes in small and medium size blood vessels were observed.

Key words: Electron microscopy of fat necrosis newborn – Crystallization of fat cell – Calcium deposition – Blood vessels.

Adipose tissue is extremely fragile and delicate. Irritants such as physical, chemical, infectious, toxic, allergic, and metabolic agents may induce necrosis of the fat cells (Blanc, 1951; Michelson, 1957).

The most important causative aetiological factors of the development of Adiponecrosis subcutanea neonatorum (ASN) are obstetric trauma (McDonald, 1955; Jabłońska, 1963), anoxaemia from asphyxia (Holzel, 1960) and hypothermia (Duhn et al., 1968). On the other hand, extensive fat necrosis has been reported in infants born by caesarian section without trauma or asphyxia (Ivy and Howard, 1953). In great many infants the cause of the fat necrosis remains mysterious.

Some authors (McIntosh et al., 1939; Noojin et al., 1949; Doose and Scheffner, 1959; Prokš, 1964; Horsfield and Yardley, 1965; Montgomery, 1967) suggest

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that the disease may be due to a delay in the maturation of fat, which leads to the production of fat with an unusually low oleic acid content so that it solidifies more easily than normal.

ASN occurs in apparently healthy, normal weight, full-term infants between 2 and 20 days after birth. The lesions are characterized by a bluish-red discoloration of the skin and sharply demarcated areas of induration in the subcutaneous tissue of the buttocks, thighs, shoulders, back, cheeks, and arms. They have a non-pitting, wooden-to-stony consistency. Softening and absorption of the indurated areas is usually complete in 3 or 4 months, but the larger plaques may become impregnated with calcium (Martin and Steven, 1957; Wesener, 1957; Michael et al.; 1962), when their slower resolution may be accompanied by hypercalcemia (Flory, 1948; Barltrop, 1963). The condition does not affect the general health of the infant but is not always a benign disease (Flory, 1948; Montgomery, 1967; Soepraptijah and DeMonchy, 1970; Pasyk, et al., 1973).

Histological studies of indurated areas has shown a varying degree of necrosis of the subcutaneous fat tissue, and a granulomatous infiltration containing lymphocytes, fibroblasts, macrophages, and foreign-body giant cells, in which needle-shaped crystals of fatty acid may be present. In older lesions there are calcifications. Light microscopic investigations have been carried out on this disease (Fox, 1933; Flory, 1948; Martin and Steven, 1957; Wesener, 1957; Lever, 1961; Marks, 1962; Craig, 1965; Montgomery, 1967; Duhn et al., 1968), but the ultrastructural features of ASN have not been described.

The purpose of this work was to carry out light and electron microscopic studies of the lesions of ASN and to attempt to answer the following questions:

1. Are the epidermis and dermis involved in the pathological process?
2. What changes occur in the fine structure of the fat cells in ASN?
3. Can the stage of necrosis of the fat cells be observed?
4. Can the evolution of crystal formation in the fat cell be seen?
5. Are the fat cells mature?
6. What is the role of granulomatous infiltration in this disease, and how long is it maintained?
7. Are the blood vessels changed?
8. Where are the calcium deposits?

Materials and Methods

Clinical Data

For this study, biopsy speciments of the indurated areas of four infants with ASN were used[1]. During laparatomy biopsy specimens of normal subcutaneous fat from a 3 week old infant were obtained, for the purpose of comparison.

Case 1. A female infant, born after an uneventful gestation of 9,5 months. There was slight anoxaemia at birth, a birth weight of 3150 g, length 54 cm. On the second day of life indurations in subcutaneous tissue were observed on the back. During the next few days new lesions developed, becoming confluent and assuming a bluish-red colour. At age 2 weeks the infant was referred for clinical

[1] Written consent to study the infants was given by the mothers prior obtaining the fat biopsy

treatment. Examination showed that the whole back and lumbar area was involved by hard, bluish-red indurations of subcutaneous tissue. Biopsy specimens from the back were obtained for light and electron microscopic study.

Case 2. A male, full-term infant, normal delivery, birth weight 4200 g, length 59 cm; symptoms of cerebral haemorrhage and maceration of the umbilical cord were present. At 12 days indurations appeared on the right shoulder, trunk and buttocks; these spread to the arms and thighs. In the 6th week post partum the infant was hospitalized in the Institute of Pediatrics. At this time there were on the cheeks, back, buttocks, arms, and thighs numerous, deep, hard as wood, nodular infiltrations from 3 to 5 cm in diameter, in some places deeply fixed. They were non-pitting. The skin in the infiltrated areas was bluish-red and was not able to form folds. Biopsy specimens from the back lesions were obtained at age 6 weeks, for both light and electron microscopic study. The infant died at the age of 5 months as the result of pneumonia and acute circulatory insufficiency. During autopsy specimens were obtained, from various sites, of clinically normal skin and subcutaneous fat tissue for histopathological examination.

Case 3. A male infant born by caesarean section with a weight of 4550 g, and length 58 cm. After birth the Apgar Score was 5. On the second day bluish-red indurations appeared on the dorsum and abdomen. The child was admitted for clinical treatment at 2 weeks of life with new indurations on the buttocks and the cheeks. At 6 weeks biopsy specimens were made from the lesions on the back.

Case 4. A male infant was delivered vaginally after rapid labour, with a birth weight of 3580 g, length 53 cm. During a period of 2 weeks the child had symptoms of CNS injury. After this time areas of induration were noted on the back and the chest. By the age of 4 weeks, the patient was referred to our Institute. Biopsy specimens were obtained from the right back area at 6 weeks for both light and electron microscopy.

Morphological Techniques

Samples of skin and subcutaneous adipose tissue of the indurated area were taken under general anaesthesia. For light microscopy some of the biopsy specimens were fixed in buffered 10% formalin and the rest was frozen. Sections were prepared by paraffin embedding and were stained using the hematoxylin and eosin technique, and the van Gieson and the von Kossa methods. The frozen sections were cut using a Frigistor and viewed after staining with Sudan III, Sudan-colorant, or Nile blue sulphate. Unstained sections were examined in the phase contrast microscope.

For electron microscopy, tissues were divided into 1 mm cubes, placed immediately in 3% buffered glutaraldehyde with 0.1 M cacodylate (pH 7.4) in 4° C and left overnight. After this they were rinsed in 7% saccharose which was buffered with 0.1 M cacodylate (4 times for 30 min) and then placed for two hours in 2% OsO_4 buffered with 0.1 m cacodylate, pH 7.4. They were dehydrated in graded series of alcohols and propylene oxide, and embedded in Epon 812 (Luft, 1961). In the 2nd case the tissues were fixed for four hours in 2% OsO_4 buffered with 0.1 M cacodylate (pH 7.4), dehydrated and embedded in Epon 812.

For light microscopy thick sections (0.5–1 micron) were made from the epon blocks and stained with hematoxylin and basic fuchsin or 1% methylen blue and Azur II; they were used for selection of areas to be cut for electron microscopy. Thick sections were also viewed with a Leitz Orthoplan light microscope equipped with a Nomarski differential interference contrast (DIC) accessory for transmitted light. Micrographs were taken with both conventional and DIC systems.

Ultrathin sections were cut with a glass knife on LKB ultrotome III, and double-stained with uranyl acetate and Reynold's lead citrate, then examined in a Philips EM 300 electron microscope at 80 kV.

Results

Normal Subcutaneous Adipose Tissue of a 3 Week Old Infant

The fat cells of the subcutaneous adipose tissue were round in shape, and about 100 μ in diameter. The central lipid droplet was seen as a uniform,

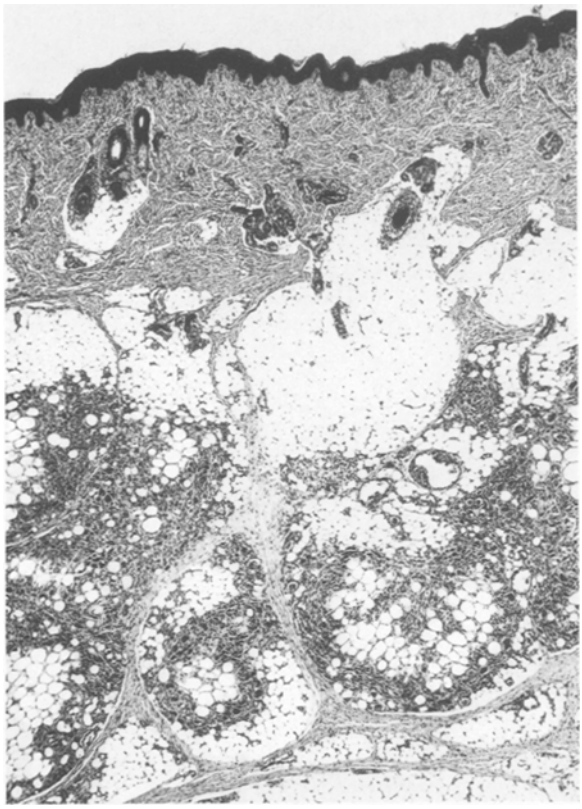

Fig. 1. Necrosis of the deeper layers of the fat lobules and a foreign-body giant cell type granulomatous reaction. Large fat cells with needle-shaped clefts. Distinct dilatation of the blood vessels of the deep plexus. The veins of the interlobular spaces are wide, arterioles have thickened walls and a narrowed lumen. Paraffin embedded sections. (Case 2, H+E, ×40)

moderately electron-dense material, surrounded by a thin ring of cytoplasm, which in the region of the nucleus was slightly thicker; the cell resemble a ring. The border line between the central lipid droplet and the cytoplasm appeared smooth. Inside the cytoplasm, especially around the area of the nucleus, typical cellular organelles were found. The fat cells were surrounded by a delicate net of collagen fibres. The blood vessels laying between the fat cells appeared to be normal.

Adiponecrosis Subcutanea Neonatorum

I. Light Microscopy

In paraffin sections, the epidermis appeared normal. In the dermis there was slight edema of the stroma and a some infiltration of mononuclear cells around

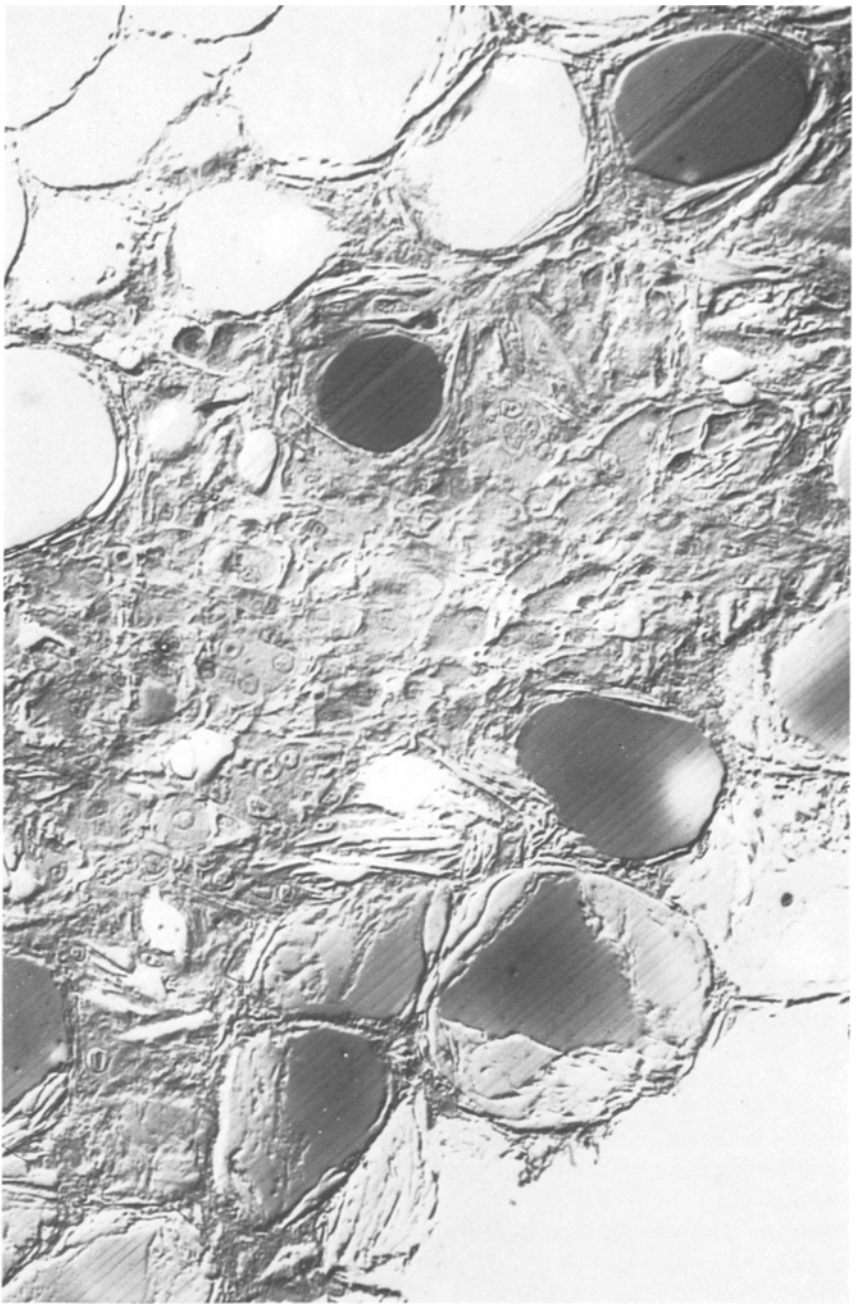

Fig. 2. Different shapes, sizes and staining of the fat cells. Breaking or destruction of the fat cell membrane. Fragmentation of the central lipid droplet into separate spaces and formation of needle-shaped clefts around the periphery of the fat cells in the form of rosettes or wheel-like arrangements. The needle-shaped clefts and lipid droplets lie deep in the granulomatous infiltration. Microphotography using differential interference contrast microscopy with Nomarski equipment. (Hematoxylin and basic fuchsin-stained epoxy section, ×280)

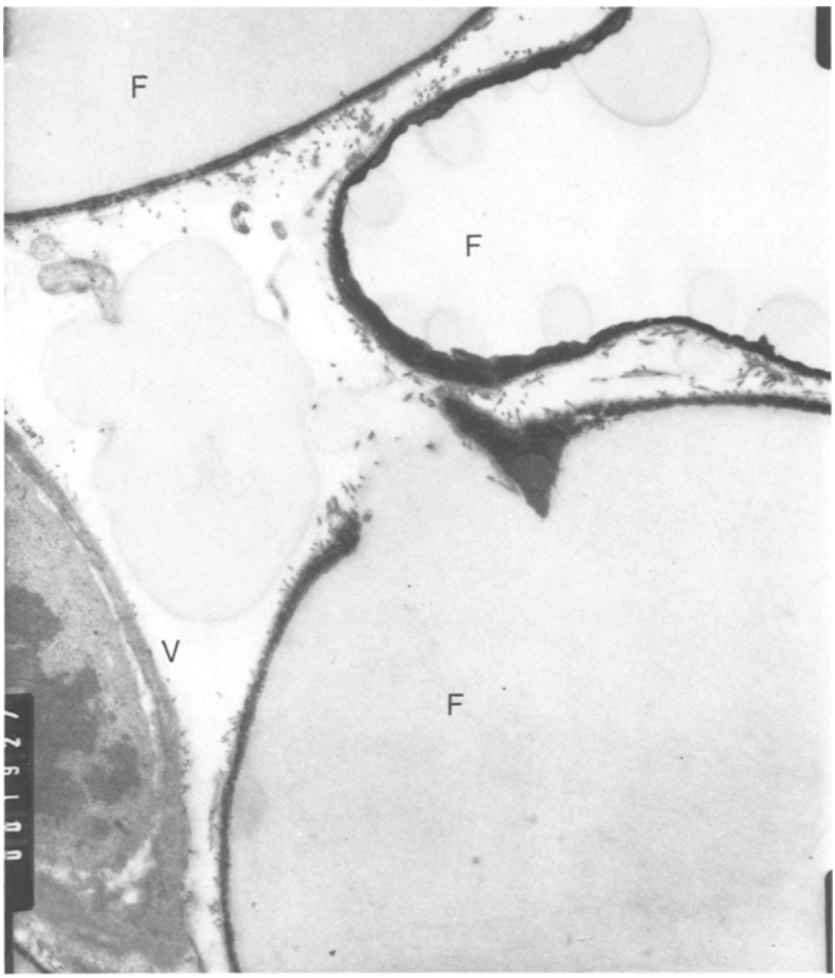

Fig. 3. Parts of three fat cells (*F*) of which one is ruptured. The lipid is extracellular. The non-osmiophilic, collapsed cell contains small lipid droplets in peripheral area. Note wide spaces between fat cells with loosely distributed collagen. Part of a capillary vessel (*V*) (×16,800)

small blood vessels and epidermal appendages. The blood vessels close to the subcutaneous tissue were dilated. The band of superficial subcutaneous fat tissue was normal (Case 2 and 4). However, in the deeper layer of the subcutaneous fat the cells showed an increase in size (140–150 μ). They exhibited irregular round shapes, and various degrees of necrosis. Widespread necrosis of the fat lobules, and a massive granulomatous reaction were present, both in the central part and in the periphery of the lobules. The granulomatous tissue contained foreign-body giant cells, macrophages, lymphocytes and fibroblasts. In both the necrotic fat cells and foreign-body giant cells, empty, needle-shaped clefts about 50 μ × 2 μ were observed. In the central parts of some fat lobules affected by necrosis, areas of calcium deposits in the spaces between and inside the

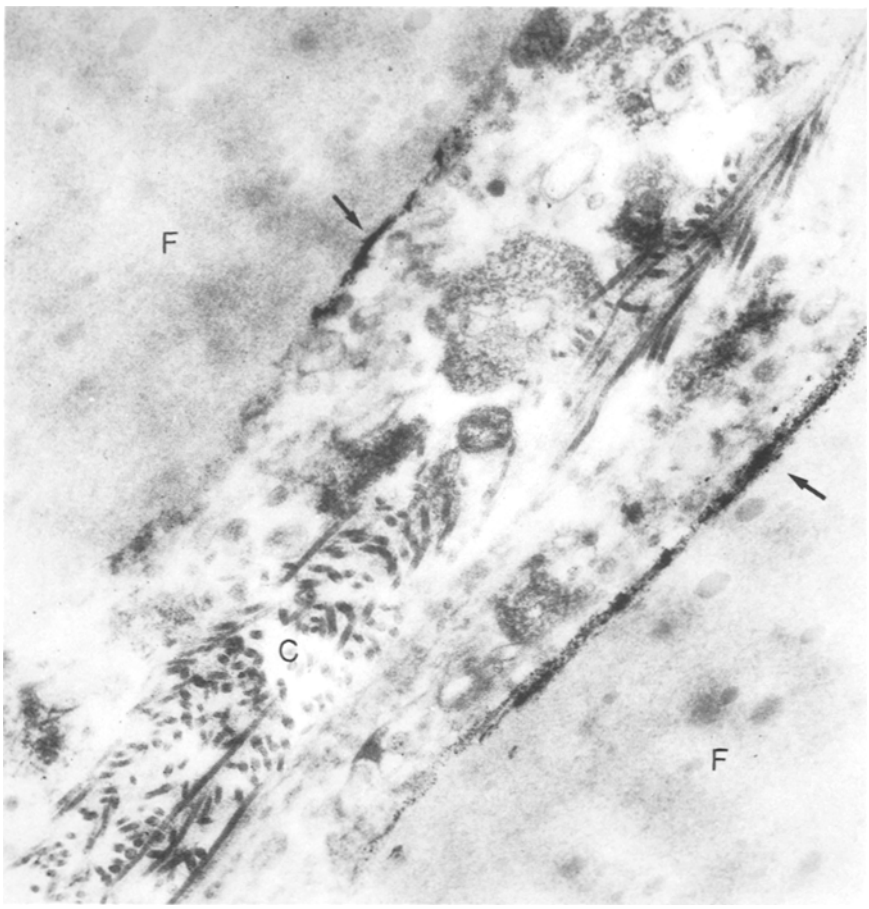

Fig. 4. Parts of two fat cells (*F*) with obliteration of structures in the cytoplasm, an interrupted border line between the central lipid droplet and cytoplasm, and lack of cellular membranes. Highly electron-dense granules (arrows) are seen at the margin of the central lipid droplets. Note non-uniformly electron-dense material in the central lipid droplets. Collagen fibres (*C*) (×35,100)

fat cells were found. In many places the interlobular septum was obliterated by granulomatous infiltrations. The interlobular veins were dilated; arterioles had thickened walls (Fig. 1).

In unstained frozen sections the fat cells contained bundles of birefringent crystals which were also present within the foreign-body giant cells and dispersed among the granulomatous infiltration.

The thin epon-embedded sections, stained with hematoxylin and basic fuchsin and viewed by differential interference contrast microscopy (Nomarski equipment), showed differences in cell size and shape. In some fat cells the central lipid droplets stained uniformly but in others stained irregularly or not at all. In many cells the lipid droplets exhibited fragmentation into separate, differently stained spaces. The membranes of some fat cells were broken or indistinct. Needle-like clefts or spaces around the periphery of the fat cells in the form

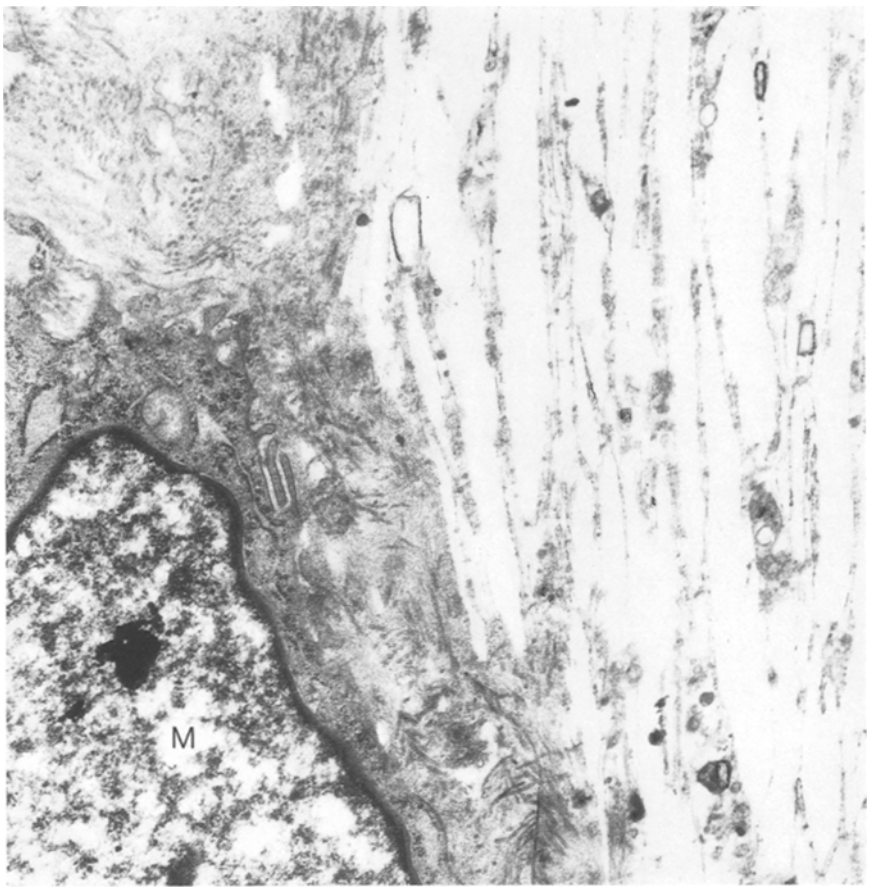

Fig. 5. Large accumulations of needle-like clefts. Completely obliterated the border line of fat cell. Macrophage (*M*) (×18,360)

of rosettes, in a radiating pattern, or in wheel-like arrangements were present. Similar clefts and lipid droplets of various sizes were observed in the foreign-body giant cells and in the macrophages (Fig. 2).

II. Electron Microscopy

A. The Fat Cells. Three stages were established in to the ultrastructural changes in the fat cells.

1. The least altered cells were non-uniform in size, the majority of large cells having a diameter about 140–150 μ, and were uniformly filled with a moderately electron-dense material. Other fat cells showed irregular circular forms and often kidney-shaped excavations. A considerable numer of cells had a ruptured cell membrane. The spaces between cells were wide with loosely distributed collagen fibres (Fig. 3).

2. In other fat cells, the central lipid droplet was composed of a moderately electron-dense amorphous material and an electron-lucent substance, which toge-

Fig. 6. Macrophage (*M*) with ingested lipid droplet and small cristal clefts (*F*). Large numbers of cytoplasmic protrusions of macrophages which have braided themselves together (arrows). Collagen fibres (*C*). (× 11,700)

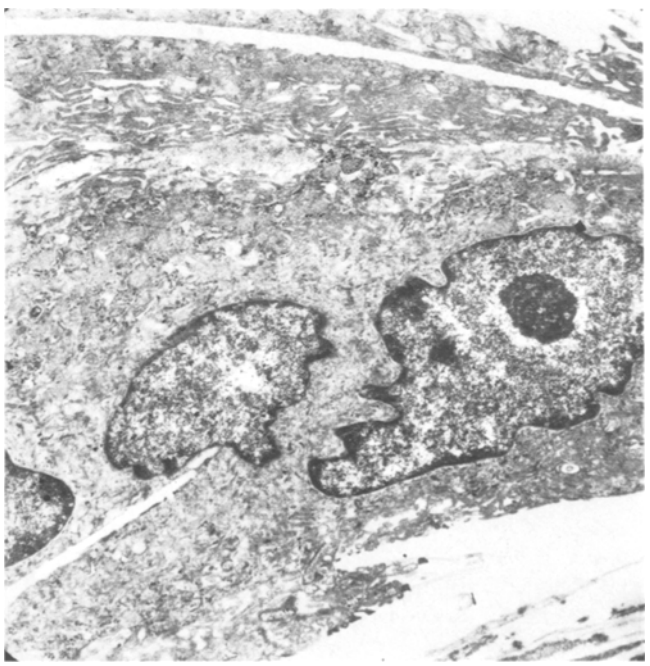

Fig. 7. Part of the foreign-body giant cell with needle-like spaces inside the cell. Note large number of pseudopodia on the periphery of the cell. (× 10,530)

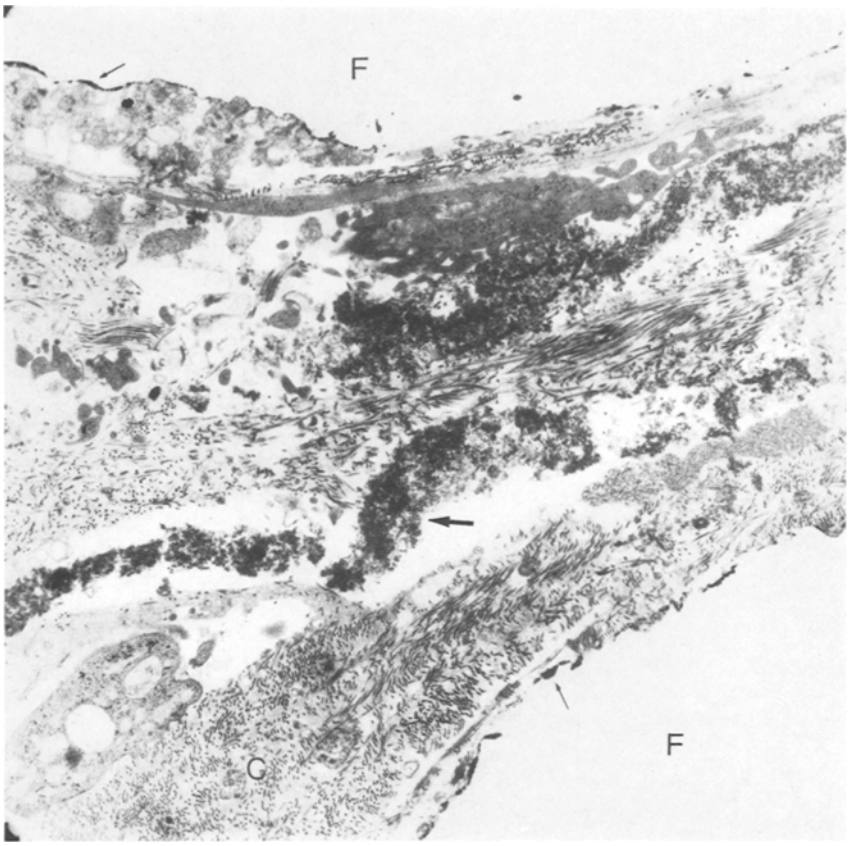

Fig. 8. Space between two changed fat cells (*F*) with areas of highly electron-dense granules (thick arrow) located around needle-like spaces together with collagen fibres (*C*). Note the same concretions on the margin of the central lipid droplets (thin arrow). (× 8,910)

ther formed a kind of net. In the cytoplasm of these cells pathological changes were observed which involved the obliteration of structures inside the cytoplasm, an uneven, often interrupted border line between the central lipid droplet and cytoplasm, as well as a lack of cellular membranes. In numerous segments bordering the transformed cytoplasm and the margin of the lipid droplet, highly electron-dense granules were observed (Fig. 4). These granules were similar in distribution and structure to calcium salts stained with the von Kossa method.

3. In the most altered fat cells, in the marginal part of the lipid droplet large aggregations of band-shaped and reticular material was observed, containing electron-lucent spaces in the form of clubs, cylinders, spindles, and needles, which were surrounded by a non-uniform thin membrane. These aggregations were usually arranged in parallel and they invaded progressively larger areas and caused a progressive reduction of the central lipid droplet. The peripheral ring of cytoplasm exhibited disappearance of its cellular components; the nucleus was absent. These cells, or their fragments, were surrounded by macrophages or foreign-body giant cells (Fig. 5).

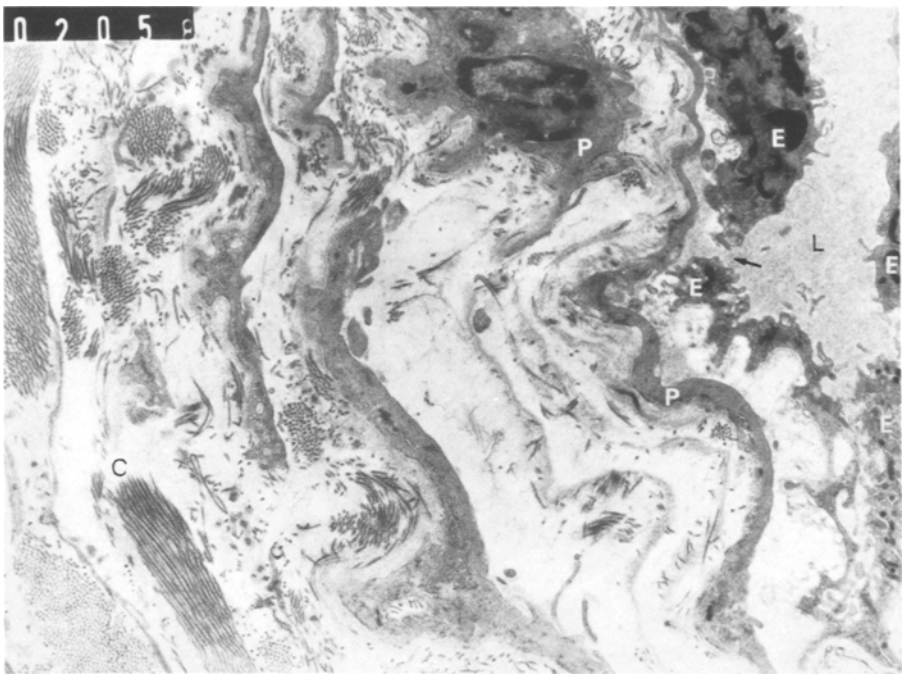

Fig. 9. A vessel from the deeper part of the dermis, partially closed by oedema of the walls. Endothelial cells (*E*) possess enlarged nucleus with folded margins and large number microvilli protruding to the lumen. Note the large fenestration (arrow). The basement membrane shows obliteration of the normal structure. Lumen (*L*). Protrusions of pericyte (*P*). Collagen fibres (*C*). (×13,500)

B. Granulomatous Infiltration. The cells of the infiltrate extended from the interlobular septa to the centre of the altered fat lobules, and included the collagen fibres. Among the cells, the most numerous were macrophages, foreign-body giant cells, fibroblasts, and lymphocytes, but occasional single neutrophilic granulocytes, eosinophiles, and mast cells were seen.

Macrophages were irregular in shape with numerous pseudopodia. In the cytoplasm, variously sized phagocytized lipid droplets and needle-shaped electron-lucent spaces were found surrounded by a membrane, with various lysosomal structures around them. Macrophages were present in the immediate vicinity of necrotic fat cells. Large numers of cytoplasmic protrusions surrounded the electron-lucent spaces, and lipid droplets and other fat cell elements braided themselves together, forming a syncytium—a giant cell (Fig. 6).

The giant cells possessed a large number of mitochondria, a vast Golgi apparatus, vesicles, fine filaments, and lysosomal structures. In the peripheral portions abundant rough-surfaced endoplasmic reticulum and numerous pseudopodia were present. In the cytoplasm phagocytized lipid droplets were seen and, dispersed or aggregated in patches and in various numbers, electron-lucent spaces which were needle-like, oval, or spindle-shaped. These spaces, lying intracellularly as well as extracellularly, showed a delicate membrane (Fig. 7).

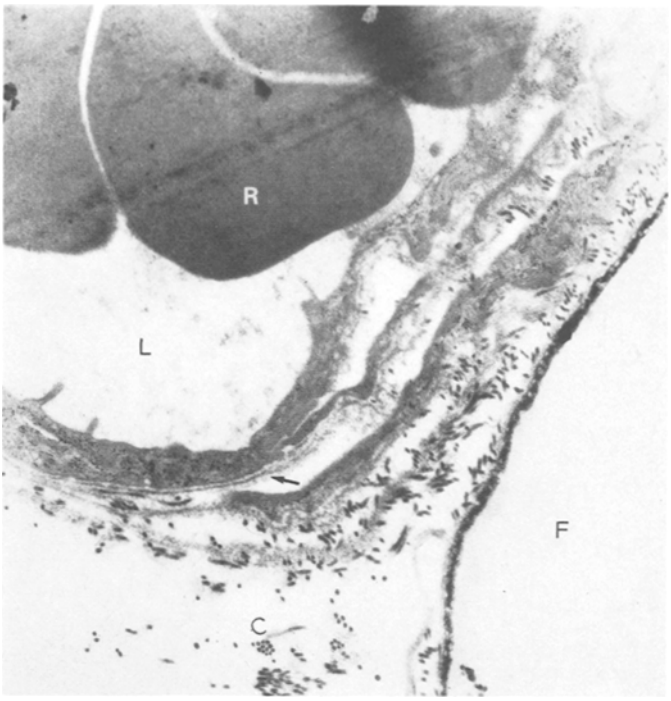

Fig. 10. Multiple dissection and homogenization of the basal membrane of a capillary vessel (arrow). Changed endothelial cells with microvilli. Lumen (*L*). Erythrocyte (*R*). Fat cell (*F*). Collagen fibres (*C*). (×18,360)

Fibroblasts, and abundant bundles of collagen fibres, were seen in the vicinity of most altered fat cells. These fibroblasts showed the typical characteristics of an actively synthetizing cell; they possessed wide cisterns of rough-surfaced endoplasmic reticulum and an large Golgi apparatus.

Large concretions of highly electron-dense crystals of calcium were seen extracellularly, either in the form of aggregates or dispersed around needle-shaped fissures. Calcium often occurred together with collagen fibres (Fig. 8).

C. Blood Vessels. In capillaries between the fat cells, alterations were seen in all components of the wall.

The endothelial cells exhibited irregular swelling of the nucleus with folded margins and irregularly dispersed chromatin. In the cytoplasm, a few mitochondria, distendend endoplasmic reticulum, a small Golgi apparatus, and fairly abundant glycogen were apparent. A large number of endothelial cells possessed numerous microvilli of various sizes that projected into the lumen of the vessel (Fig. 9). In other segments the endothelial cells had a homogenous cytoplasm, consisting of many free ribosomes, whereas other cellular organelles were less apparent. Finally, in the most altered capillaries, the endothelial cells showed a greatly attenuated cell membrane.

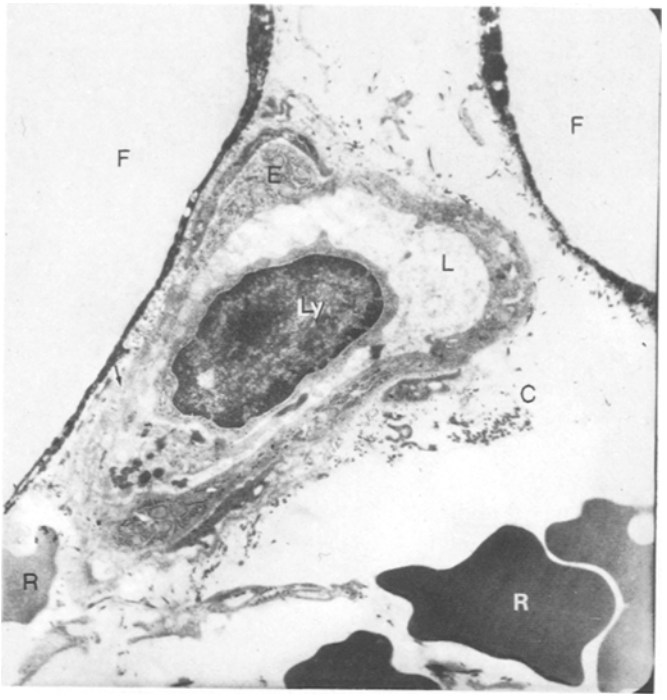

Fig. 11. The most changed capillary vessel. Obliterated contours of endothelial cells (*E*). Rupture of the basal membrane (arrow) with extravasation. Erythrocyte (*R*). Lymphocyte (*Ly*). Lumen (*L*). Fat cell (*F*). Collagen fibres (*C*). (×8,910)

Many capillaries exhibited a multiple layered basal lamina (Fig. 10). In various segments apart from changes in the endothelial cells, rupture of the basilar membrane of the capillaries and extravasation of the erythrocytes were seen (Fig. 11).

The external layer of the capillaries (adventitia) contained individual fibroblasts, leucocytes, lymphocytes, macrophages, mast cells, and loosely distributed bundles of collagen fibres.

III. The Differences Between the Cases with ASN

The fat cells in lobules lying superficially in case 2 and 4 did not exhibit any change, but in the deeper areas there were varying changes. In case 1 and 3 alterations in fat cells were present in superficial and deeper lobules.

Morphological changes in fat cells and giant cell type granulation tissue reaction was similar in all cases, but in those in which the pathological process persisted for 2 and 4 weeks (Case 1 and 2) there were considerably more altered fat cells with great numbers of needle-shaped crystals, and fewer foreign-body giant cells. Conversely, in the specimens from lesions lasting 6 weeks (Case 3

and 4) the giant cells predominated, while pathologically changed fat cells were less abundant.

In the specimens excised from skin and subcutaneous fat without clinical lesions after 5 months, some of the fat lobules contained single necrotic fat cells and foreign-body giant cells. Calcium deposits were only seen in case 2 in the specimen taken after 4 weeks disease.

Discussion

By electromicroscopy, 3 morphological pictures could be established reflecting changes in fat cells. The pathological process probably begins in the central part of the lipid droplet, which in the least altered cells was enlarged but still uniform in structure. In the more altered fat cells a state of preliminary crystallization was observed, in the form of a delicate network in the central part of the lipid droplet. In other fat cells, the crystallization of fat also involved the periphery of the central lipid droplet with the formation of spindle- or needle-shaped crystals. These, in the electron microscope appeared as empty spaces – "ghost" crystals – surrounded by a band-like, reticular substance. The most necrotic altered fat cells were present as structures without a cell membrane, nucleus, or cytoplasm, but containing numerous needle-shaped fissures, sometimes with accompanying residual fat. They were surrounded by macrophages and foreign-body giant cells. Similar needle-shaped fissures surrounded by a membrane and fat droplets could be seen in the cytoplasm of giant cells and macrophages.

Chemical studies on the structure of the crystals in ASN have suggested that they are cholesterol and its esters (Carol and von der Zande, 1926), neutral fat (Noojin et al., 1949) or fatty acids (Channon and Harrison, 1926; Prokš, 1964; Horsfield and Yardley, 1965; Lindlar and Misgeld, 1967). Horsfield and Yardley (1965), using X-ray diffraction technique, concluded that the crystals consisted of triglycerides with a large quantity of palmitic acid. From electron microscopic findings these suggestions cannot be ruled out. The chemical composition of the subcutaneous tissue in ASN is well-known. It is agreed that, in comparison with adults, the fatty tissue of the newborn and infants exhibit a low content of fatty acids (oleic acid) causing a shift in the coagulation point of these fats, facilitating crystallization. Enzymes may also play an important role in the process of crystallization. These enzymes are responsible for the desaturation of palmitic and stearic acids, which, according to Sweeney et al. (1963) and Horsfield and Yardley (1965) are less developed in infants with ASN than in healthy infants.

The crystallization of lipid in fat cells causes the immediate formation of an infiltrate with proliferation of macrophages; later foreign-body giant cells appear. This process may be connected to a second phenomenon in ASN, i.e. phagocytosis of crystals and lipid droplets by macrophages and giant cells. Here, the rare phenomenon of so-called "hyperphagocytosis" occurs, which takes place when the particle is too large for one single cell. Several macrophages then gather around it and form and areola. Many authors (Davis, 1964; Gusek,

1964; Gilman and Wright, 1966; Sutton and Weiss, 1966; Sutton, 1967; Carter and Roberts, 1971; Mariano and Spector, 1974) pointed out that one of the mechanisms in the formation of giant cells is fusion of macrophages. In our studies the cytoplasmic protrusions of the macrophages adhered very closely to the needle-shaped fissures and lipid droplets and formed compact, syncytial connections, which could be identified as giant cells.

It is known that macrophages play an important role in fat metabolism in chronic inflammatory states (Carr, 1973); because of their rich supply of various enzymes they have the ability to hydrolyse and esterify different fats (Day, 1964). The fat released from a damaged fat cell undergoes hydrolysis to glycerol and fatty acids, the latter produce a reaction with the characteristics foreign-body giant cell granuloma (Cairns, 1968). Macrophages also release a substance, probably lysosomal in origin, which causes proliferation of fibroblasts and synthesis of collagen (Spector, 1973). We observed large bundles of collagen fibres in the most advanced alterations in the proximity of macrophages and giant cells.

The accumulation of calcium concretions in fatty tissue in ASN, sometimes with accompanying hypercalcaemia has previously been observed with the light microscope (Martin and Steven, 1957; Wesener, 1957; Michael et al., 1962; Duhn et al., 1968). In the present studies we also found foci of calcium deposition in the central part of altered fat lobules. In the electron microscope, we have shown calcium crystals located near collagen fibres and fissures of fat crystals and have detected an accumulation of calcium concretions in the altered cells. These occur on the border of the lipid droplet and the rim of the cytoplasm. It is assumed that free fatty acids and calcium ions form calcium soaps insoluble in water (Prokš, 1964; Kendrick et al., 1966).

Changes in blood vessels in ASN have been observed by some authors. Hering and Undeutsch (1956) described extravasations in the proximity of larger vessels. Vignolo and Piccardo (1971) observed hypertrophy of the muscle layer and of certain areas of the endothelium. Zeek and Madden (1946) found contracted vessels in the necrotic parts of subcutaneous fat and on this basis proposed a theory of vasomotor origin of the changes in ASN. We observed changes in blood vessels both the light and electron microscope. The lesions involved enlargement of the veins in the dermis and in the intralobular septa of the subcutaneous fat where the blood vessels exhibited thickening of the walls and narrowing of the lumen.

In the electron microscope, capillaries and smaller blood vessels lying inside the fat lobules showed pathological changes of the endothelium, leading to total destruction of the vessel wall and of the basal lamina. In a large number of capillaries, the endothelial cells possessed numerous microvilli, which may restrict the lumen and thus influence metabolic functions. Above all, they may affect the circulation of blood in the capillaries.

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