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Sclerema Neonatorum

Light and Electron Microscopic Studies

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Summary. Specimens from four cases of sclerema neonatorum were studied by light and electron microscopy. The connective tissue bands of the cutis and subcutis were thickened and alternated with loosely distributed bands in broad, distended areas of basal substance. Extensive areas filled with cross-banded structures were observed in the dermis. In the interlobular septa of the subcutaneous and perirenal fat splitting of fibers into microfibrils was visible. Changes in the capillaries and small blood vessels were also noted. Neither fat cells filled with rosettes of needle-like crystals, nor any inflammatory infiltrates of foreign body type giant cells were found in subcutaneous tissue.

Key words: Electron microscopy of sclerema neonatorum – Dermal and interlobular connective tissue – Splitting of collagen fibers – Cross banding structures – Fat cells – Blood vessels.

Introduction

Sclerema neonatorum (SN) is an extremely rare alteration of the subcutaneous tissue which affects weak, premature infants or term infants suffering from a severe disease, congenital defects, intracranial hemorrhage or shock (Bielicka and Oppenheim-Fischler 1955; Stinson et al. 1962; Prod'hom et al. 1974; Stanley-Brown and Allendorf 1976). It is characterized by a diffuse, rapidly spreading, hardening of the skin to the whole body except for the palms, soles and genitalia. Respiration, sucking and movements of limbs may be restricted. The skin is cold, waxy-indurated and pale with purplish mottling. The prognosis is poor despite of treatment with antibiotics and steroids, and the mortality is still very high (73–84%) (Levin et al. 1961; Milunsky and Levin 1966). The aetiology

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is unknown. It is possible that SN is a symptom-complex resulting from the interaction of a number of factors (Warwick et al. 1963; Gupta et al. 1968). Kellum et al. (1968) suggested the hypothesis that in SN there is defective lipolysis of subcutaneous triglycerides with decreased mobilization of fatty acids from the adipose tissue – a source of energy for metabolic processes.

There are three contradictory, completely different descriptions of the histological lesions in SN. Some authors (Gray 1926; 1933; Zeek and Madden 1946; Flory 1948; Wright and Symmers 1966; Graham 1967; Lever 1967) considered that the histological picture of SN is the same as that in Adiponecrosis subcutanea neonatorum (ASN), namely degeneration, necrosis and crystallization of the fat tissue together with an inflammatory reaction, and hyperplasia of foreign body type giant cells and fibroblasts. Others (Prokš 1961; Prokš and Fikejz 1961; Stinson et al. 1962; Prokš and Volvoda 1965, 1966; Kellum et al. 1968; Pinkus and Mehregan 1969; Irgang 1971; Jabłońska 1975; Lever and Schaumburg-Lever 1975) failed to observe anything significant in sclerema, except for the presence of needle-shaped crystals and hypertrophy of interlobular septa, and still others (Siwe 1934; Grotts 1951; Potter 1952; Brescia and Tartaglione 1954; Søndergaard and Nielsen 1954; Mc Donald 1955; Stowens 1959; Warwick et al. 1963; Levin and Milunsky 1965; Tedeschi 1965; Gupta et al. 1968) found no changes on histological examination, or observed only thickening of the bands of connective tissue. According to Hughes and Hammond (1948), and Montgomery (1967) in SN there may be variably observable enlargement of collagen fibers and fibrosis in the dermis and subcutaneous tissue, yet there is a lack of any inflammatory change – giant cells and rosettes of crystals.

Ultrastructural features of the SN have not been previously presented in the literature.

The present investigation is an attempt to resolve the histopathological problems in SN and, on the basis of light and electron microscopic studies of the indurated skin, to answer the following questions: 1. Are the epidermis and dermis involved in the pathological process? 2. Could changes in connective tissues be seen? 3. Do the blood vessels change? 4. What kind of changes occur in the fine structure of fat cells in SN?

Material and Methods

Clinical Data

For light and electron microscopic examinations 12 specimens were used, obtained from the indurated skin and subcutaneous tissue (from the trunk and the extremities) and the perirenal fat of four neonates with sclerema, 4 and 24 h post mortem. Owing to the very serious general condition of the infants with SN, biopsies from the skin and subcutis could not be performed.

For comparison control biopsies were taken for light and electron microscopic study from normal skin and subcutaneous tissue obtained during surgical procedures on the buttocks and abdominal region of two 1-day-old neonates (with a birth weight of 2,950 g, 3,250 g) and a 3-week-old infant (with a birth weight of 3,600 g). Specimens were also taken from the unchanged skin and subcutaneous tissue of the abdominal and femoral regions and the perirenal fat tissue of four neonates 2 to 9-day-old at 4 and 24 h after death.

Case 1. A 3-day-old male infant, small for gestational age (birth weight 2,650 g, length 51 cm), was born without complications. On the 2nd day the newborn became anxious, and bloody-brown vomitus appeared. The eyelids were oedematous. Muscular hypertonicity; pale, cold skin and generalized induration of the skin and subcutaneous tissue were found. The indurated skin was immobile and non-pitting. The body temperature was 35.6° C. On the 3rd day, in spite of treatment with hydrocortisone, penicillin, gentamicin and rewarming of the body, the child died.

Autopsy findings included: bilateral, mixed bronchopneumonia (haemorrhagic, exudative and suppurative, with pyogenic abscesses), bilateral mucopurulent bronchitis and bronchiolitis, cerebral and cerebellar hyperemia, haemorrhage into the ventricles of the cerebrum, especially into both sides of the posterior horn, haemorrhagic gastritis and duodenitis, ulcerative oesophagitis, interstitial fibrosis of the pancreas, diffuse fatty infiltration of the liver, hyperaemia of the kidneys, and sclerema.

Case 2. A 3-week-old girl, had been delivered normally, with a weight of 3,300 g and a length of 53 cm, after a full-term uncomplicated pregnancy. In the first week of life the infant was treated persistent diarrhoea and vomiting. Dyspnoea and symptoms of CNS injury appeared some days before admission to the hospital. On the day of admission there was dehydration, bronchopneumonia and diffuse induration of the skin of the trunk, buttocks, extremities and cheeks. The skin was cold, bluish, unable to form folds and non-pitting. Despite treatment with antibiotics, hydrocortisone and cardiac drugs, intravenous administration of electrolyte and fluid, transfusions of plasma, albumin, and warming of the body, the general condition of the infant deteriorated. There was a persistent metabolic acidosis. The body temperature fell to 35° C. The child died on the 6th day of hospitalization.

Autopsy findings: acute exudative gastroenterocolitis, sepsis, bilateral haemorrhagic pneumonia, haemorrhage into the kidneys, hyperaemia and fatty infiltration of the liver, hyperaemia of the cerebrum and leptomeninges, dilatation of the right side of the heart, persistent ductus arteriosus, and sclerema.

Case 3. A 4-day-old male infant, 5 weeks premature (birth weight 2,500 g, and length 50 cm) was delivered vaginally with breech presentation. The Apgar score was 4 at 1 min. The child did poorly with decreased activity, transient cyanosis, and weak muscular tone. On the 2nd day of life there were spells of apnoea and diffuse hardening of the skin and subcutaneous tissue of the lower limbs, buttocks and trunk was noted. In spite of treatment with oxygen, antibiotics, hydrocortisone, blood transfusions and cardiac drugs the child died.

Autopsy showed: prematurity, extensive bilateral pulmonary haemorrhage and hyaline membrane disease, patent ductus arteriosus, hyperaemia of liver, spleen and kidneys, and sclerema.

Case 4. A female infant, delivered normally with umbilical cord wrapped twice around the neck. The birth weight 2,480 g. The Apgar score was 4 at 1 min and 9 at 30 min. On the first day of life the infant was pale and developed irregular breathing with long periods of apnoea resulting in cyanosis. Diffuse induration of the dorsal portion of the feet and legs was observed. Hydrocortisone, penicillin, cardiac drugs and oxygen were administered. On the next day, respirations were more difficult and irregular. Body temperature was 35.7° C. The induration of skin spread rapidly. The skin of the whole body including the face became cold, board-like, could not be picked up, did not pit on pressure, and was bound down to underlying tissues. The child died on the 3rd day of life.

The autopsy revealed: bilateral pulmonary haemorrhage complicating hyaline membrane disease, cerebral and cerebellar hyperaemia, dilatation of right side of the heart, patent ductus arteriosus, hyperaemia of the liver and kidneys, and sclerema.

Morphological Techniques

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 haematoxylin and eosin, van Gieson, Alcian blue and PAS, Gomori, orcein, von Kossa and the Mallory method, and toluidine blue for metachromatic reaction. The frozen sections were cut

using a Frigistor and examined in the polarizing microscope (Bausch and Lomb) and viewed after staining with Sudan III, or Nile blue sulphate.

For electron microscopy tissues were cut into 1 mm^3 cubes and fixed in 3% glutaraldehyde buffered with 0.1 M cacodylate (pH 7.4) in 4° C and left overnight, and next 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.

For light microscopy thick sections were made from the epon blocks and stained with 1% methylene blue and Azur II, they were used for selection of areas to be cut for electron microscopy.

Ultrathin sections were cut with a glass knife on LKB ultratome MT2-B, and double-stained with uranyl acetated and Reynold's lead citrate, then examined in a Philips EM 300 electron microscope.

Results

Normal Skin and Subcutaneous Tissue Obtained During Surgical Procedures and at 4 and 24 h After Death from Neonates with no Skin Lesions

Light Microscopy. In paraffin embedded preparations the fat lobules were separated by bands of collagen fibers. Very loosely distributed single fibroblasts, mastocytes, macrophages, pericytes and small blood vessels were seen. Sections stained using the Van Gieson, Gomori, Mallory and von Kossa methods and orcein, Alcian-blue+PAS, and toluidine-blue techniques did not show any changes.

In unstained frozen sections of subcutaneous and perirenal fat tissue viewed in the polarizing microscope neither intracellular, nor extracellular crystal elements were found.

Electron Microscopy. The epidermis and the dermis from a biopsy during life showed the same features as have been described in the literature (Breathnach 1971; Rhodin 1974). The fat cells of normal lobules were round or oval in shape. Their size varied from 50–100 μm in diameter. The central lipid droplet was seen as an uniform, moderately electron-dense material with no empty spaces, surrounded by a very thin ring of cytoplasm, which in the region of the nucleus was slightly thicker. Inside the cytoplasm, especially around the area of the nucleus, typical cellular organelles were found. The interlobular septa contained a lattice of connective tissue composed of collagen and reticular fibers, small vessels, fibrocytes, mast cells and reticulum cells. The blood vessels between the fat cells and in the interlobular septa appeared normal. The basal lamina of each capillary was in close apposition with the cell membrane of the fat cell.

In the specimens of normal skin and subcutaneous tissue (obtained 4 h after death from neonates with no skin lesions), the epidermis did not show any changes. However, in small blood vessels of the dermis found some autolytic damage, i.e. the endothelial cells had oedematous and injured mitochondria, dilated cisterne of rough-surfaced endoplasmic reticulum and Golgi zones and in some part damage of the cell membrane. The fat cells in size and shape were similar to those taken during operation. The central lipid droplet was filled with moderately osmiophilic material and a lot of fine, differently shaped,

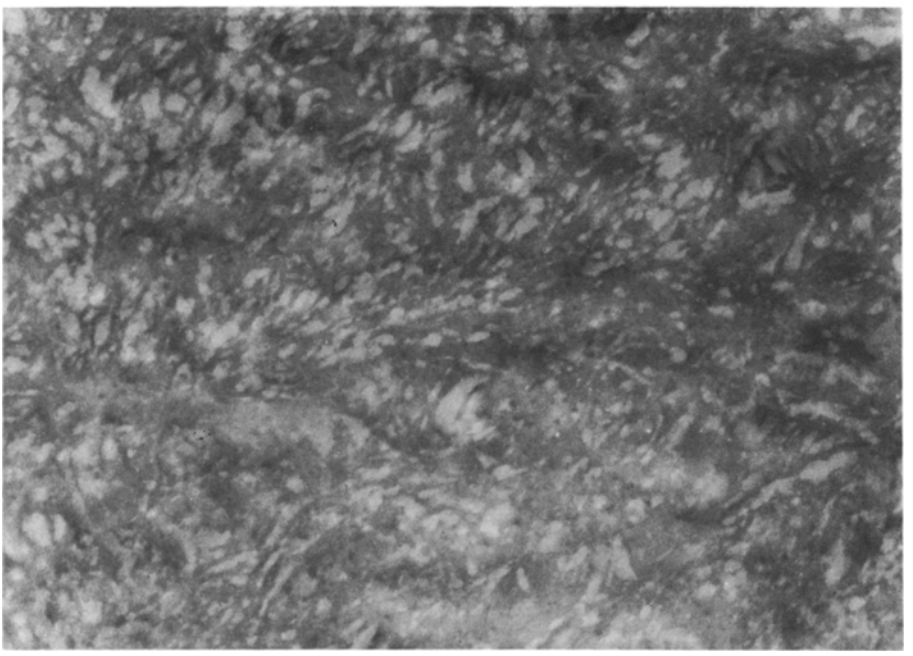

Fig. 1. Normal subcutaneous fat tissue taken 4 h post mortem from 2-day-old newborn without skin lesions. Part of the fat cell. Within the central lipid vacuole numerous, small, irregularly dispersed, electron-translucent clefts are seen. ($\times$ 30,000)

electron-translucent spaces were seen (Fig. 1). Cytoplasmic organelles and membranes of these fat cells were damaged. The same changes can be seen in the fat cells from perirenal areas. Interlobular septa were unchanged.

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Light Microscopy. In the epidermis no changes were found in paraffin sections. In the dermis the number of blood vessels was considerably decreased. The lumen of capillaries was narrow, and contained very few blood cells. Within the dermis no cellular infiltrations were found. In the deeper layers of the dermis thickening, distension, and separation of the bundles of collagen fibers uniformly stained with the van Gieson method were seen. The elastic and reticular fibers, stained with orcein and with the Gomori method, showed no changes. In the basal substance of the connective tissue, neither increase in the quantity of the glycosaminoglycans nor hyalinization were found. The subcutaneous tissue was composed of very poorly developed fat lobules, separated by broad, distended septa of the connective tissue (Fig. 2). Many small fat cells contained a centrally situated nucleus and 2–3 lipid vacuoles. Among the fat cells, single macrophages and fibroblasts were visible. The network of capillary vessels of the fat lobules was very scantily filled with blood cells. The fat cells in the sections stained with Sudan III and Nile blue sulphate showed no crystals in the frozen sections viewed in the polarizing microscope.

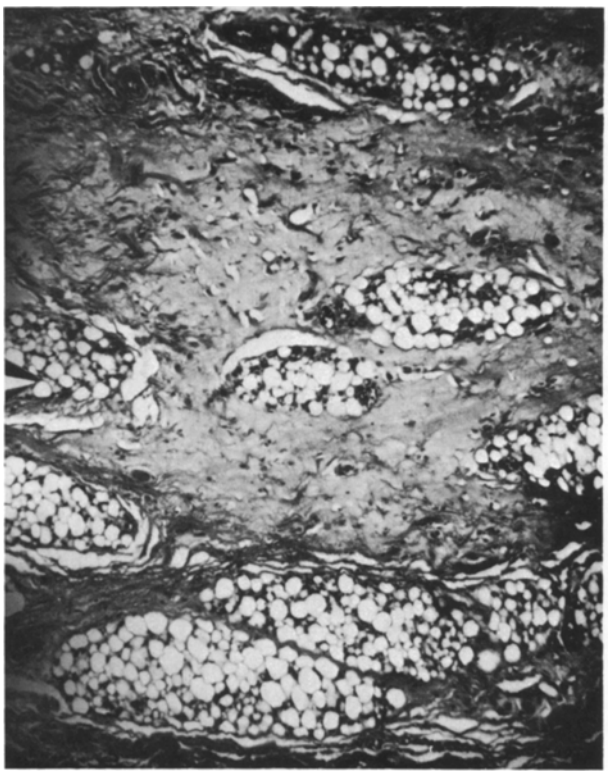

Fig. 2. Sclerema neonatorum. Poorly developed fat lobule of the subcutaneous tissue, separated by very distended septa of connective tissue. (H.+E. ×80)

In the interlobular septa, very thick bundles of collagen fibers were seen, lying among wide, distended spaces. In some places these were delaminating. No cellular infiltrations were found. Vascularization of the connective tissue septa was very poor. In the specimens stained with Alcian-blue+PAS method, no increase in the glycosaminoglycans was observed. The elastic fibers stained with orcein were fragmented in the distended areas. Special staining of the reticular fibers (Gomori method) did not show any changes.

In the perirenal adipose tissue poorly developed fat lobules were separated by very broad distended bands of connective tissue septa with focally thickened bundes of collagen fibers. In the interlobular spaces, apart from regularly dispersed, fairly numerous fibroblasts, no cellular infiltrations were found. The fat cells were small, about 30–50 μm in diameter. They contained a centrally placed nucleus and numerous small lipid vacuoles in the cytoplasm. In the lipid vacuoles, no crystal formations were found, either in the stained specimens or in the unstained, frozen sections viewed in the polarizing microscope. The fat cells were distributed among a dense network of capillaries abundantly filled with erythrocytes. In the interlobular septa no increase in glycosaminogly-

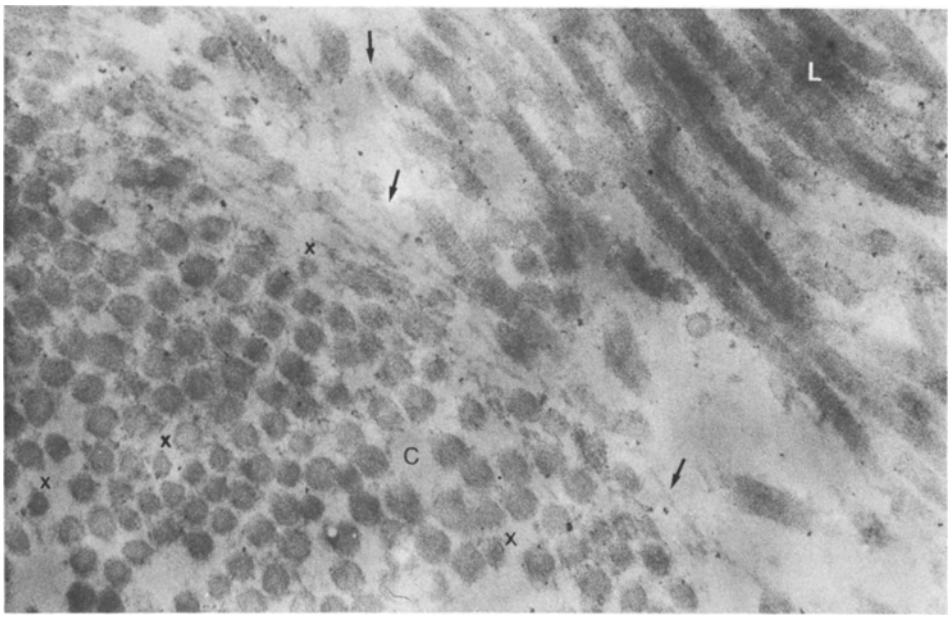

Fig. 3. Sclerema neonatorum. Collagen fibers of the dermis are seen in longitudinal (*L*) and cross (*C*) section with obliterated outline. Notice the microfibrils (*arrows*) and the small diameter of some collagen fibers (*x*). (× 126,000)

cans was observed. The reticular fibers were unchanged and elastic fibers showed ruptures only in the focally distended parts.

Electron Microscopy. In electron microscopic examinations, the epidermis appeared unchanged. Single cellular elements (fibroblasts, mast cells, histiocytes, lymphocytes), were found in the dermis. In the deeper layers of the dermis broad bands of compact bundles of collagen fibers were found, running in various directions. They lay in very extensive, amorphous electron-lucent areas, containing foci of small granulations and amorphous substance of moderate electron-density. In the vicinity of collagen fibers, numerous microfibrils were observed. Cross-sections of collagen fiber bundles showed a non- uniform diameter of the fiber and obliterated outline (Fig. 3). In other parts, large spaces filled with fine granular substance, were seen having a partly striated distribution and moderately electron-density with regular cross banding at intervals of about 650 Å (Fig. 4).

Capillaries of the reticular layer showed great damage. In the endothelial cells, distension of the mitochondria and damage to the cellular membranes, with the presence of desmosome-like formations or maculae adhaerentes structures were observed. The basement membrane formed a broad homogeneous layer (Fig. 5).

In the subcutaneous tissue the cellular membranes of most of the fat cells, both small (20 30 μm in diameter) and large (80–100 μm) were damaged, with contours obliterated in some places. In their cytoplasm, dilated mitochondria,

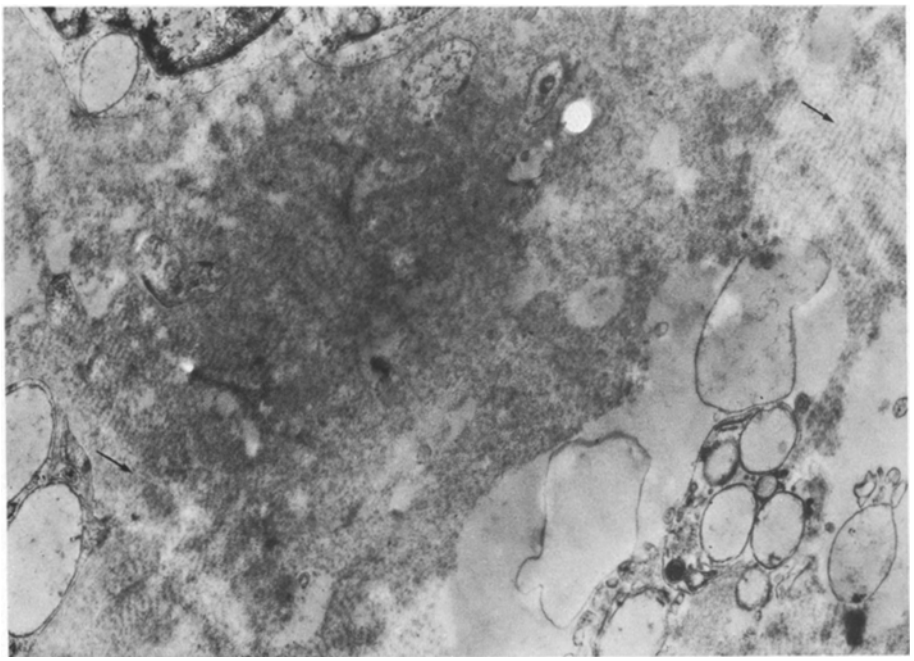

Fig. 4. Sclerema neonatorum. Area of the dermis filled with fine granular, moderately electron dense, striated substance – "cross-banded structures" (*arrows*). Cell debris with large vacuoles and post mortem injured mitochondria. (× 30,000)

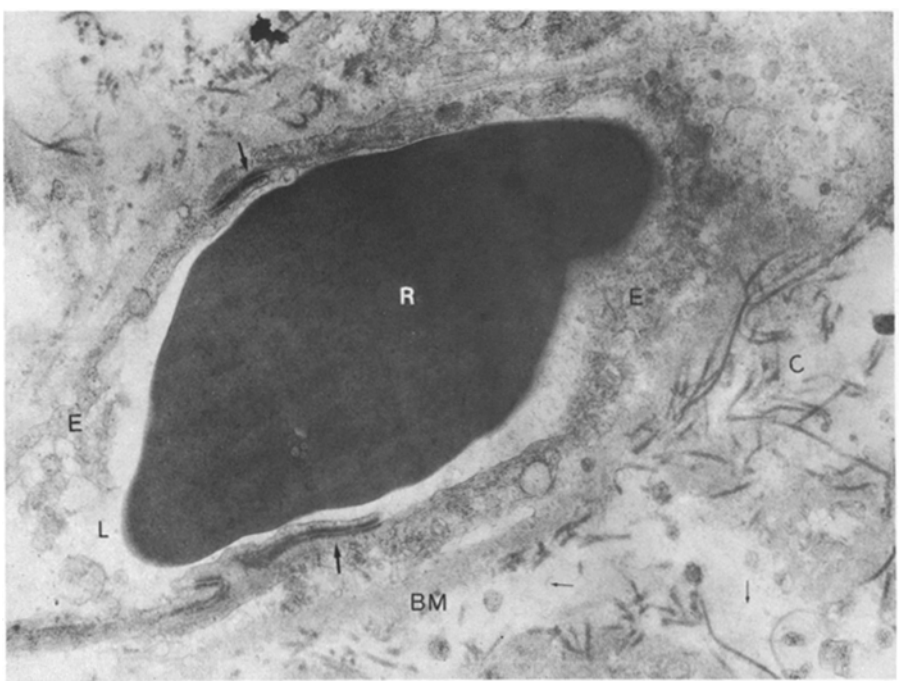

Fig. 5. Sclerema neonatorum. Capillary of the dermis. Endothelial cells (*E*) show dilatated mitochondria and damaged cell membranes. Notice desmosome-like structures (*thick arrows*). Wide and homogeneous basement membrane (*BM*). Collagen fibers (*C*). Microfibrils (*thin arrows*). Erythrocyte (*R*). Lumen (*L*). (× 30,000)

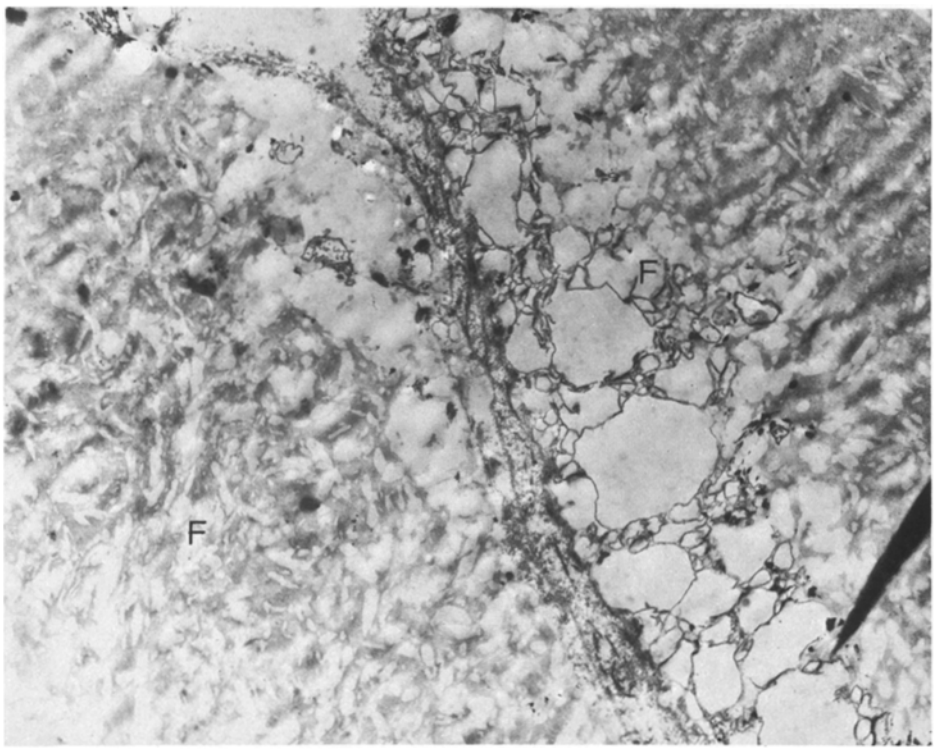

Fig. 6. Fragments of two fat cells (*F*) with damage of cell membranes and cytoplasmic organelles. Central lipid droplets possess numerous, small, irregularly dispersed, variously shape, electron-lucent spaces. On the periphery larger spaces forming a spider's web-like pattern. (× 16,000)

numerous vacuoles and a nucleus with dispersed and peripherally compact chromatin were observed. The central lipid droplet was filled with moderately electron-dense material, containing small, electron-lucent spaces of various shape. On the periphery of the lipid vacuole there were larger spaces which formed a spider's web pattern (Fig. 6).

In the interlobular spaces, the collagen fibers were arranged in thick, compact bundles or were distributed singly. They were oriented in different directions and separated by wide spaces of electron-lucent ground substance. In 30,000 × magnifications, microfibrils were seen adhering to the collagen fibers or were separately grouped (Fig. 7). Greater magnifications (about 100,000×) showed longitudinal splitting of the collagen fibers into smaller fibrils of microfibril type. These phenomena could also be interpreted as a result of defective polymerization of tropocollagen during its maturation or by the lack of cross covalent bonds between small collagen fibers (Fig. 8). Cross-sections of collagen fibers demonstrated the existence of single fibers having a considerably decreased diameter (about 500–800 Å) and concentration of microfibrils around the fibers.

Within the interlobular septa of the subcutaneous fat tissue, among the collagen fibers, single, elongated or oval fibroblasts were seen. The nucleus

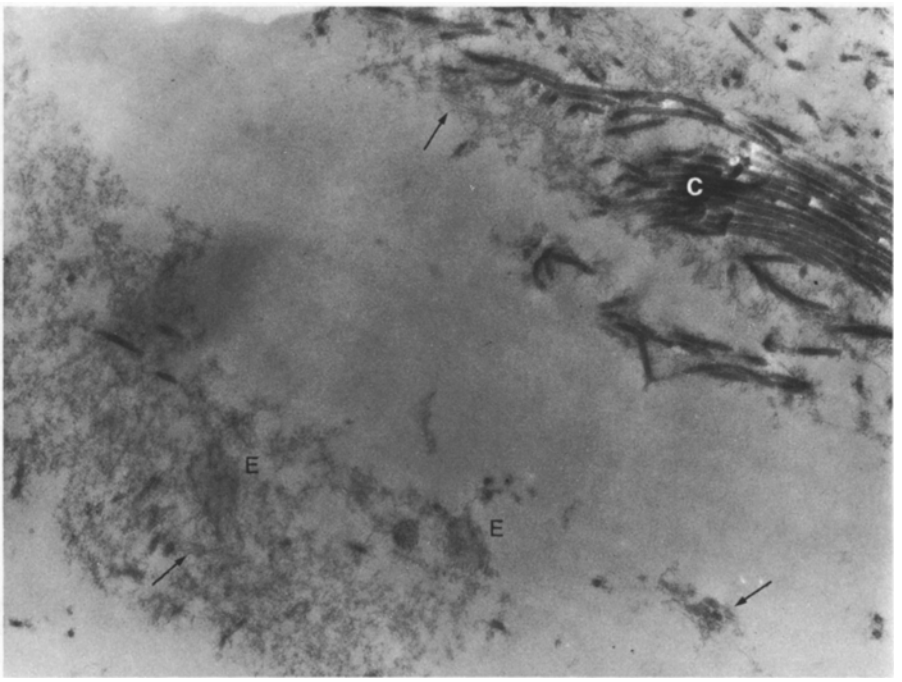

Fig. 7. Accumulation of microfibrils (*arrows*) in the close vicinity of collagen fibers (*C*) and elastic fibers (*E*) of the interlobular septa in SN. Notice wide spaces of the amorphous ground substance. (× 30,000)

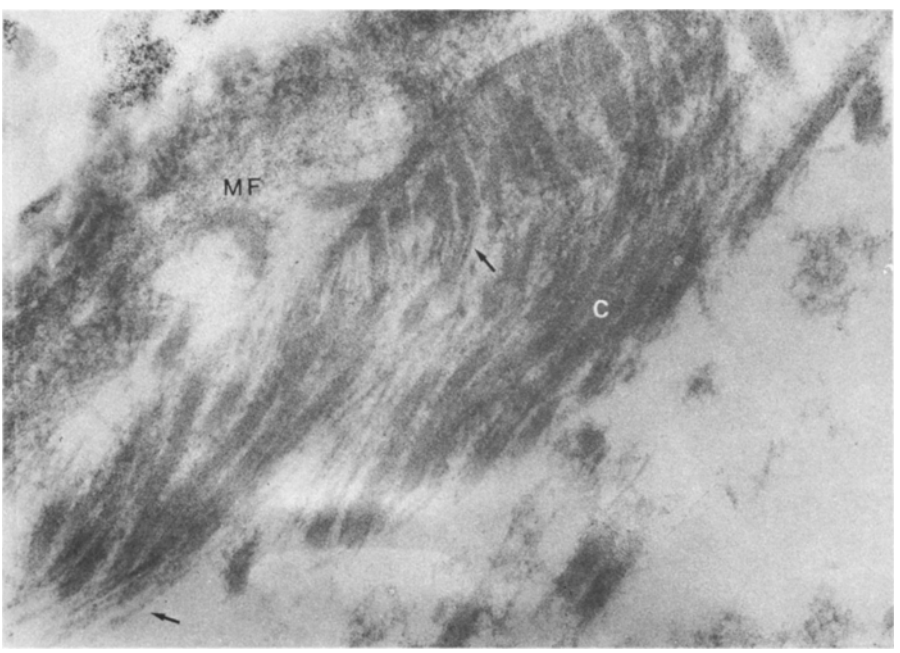

Fig. 8. Collagen fibers (*C*) and aggregations of microfibrils (*Mf*) of the interlobular septa in SN. Splitting of collagen fibers into microfibrils (*arrows*) or defective polymerization of tropocollagen into larger collagenous fibers are seen. (× 126,000)

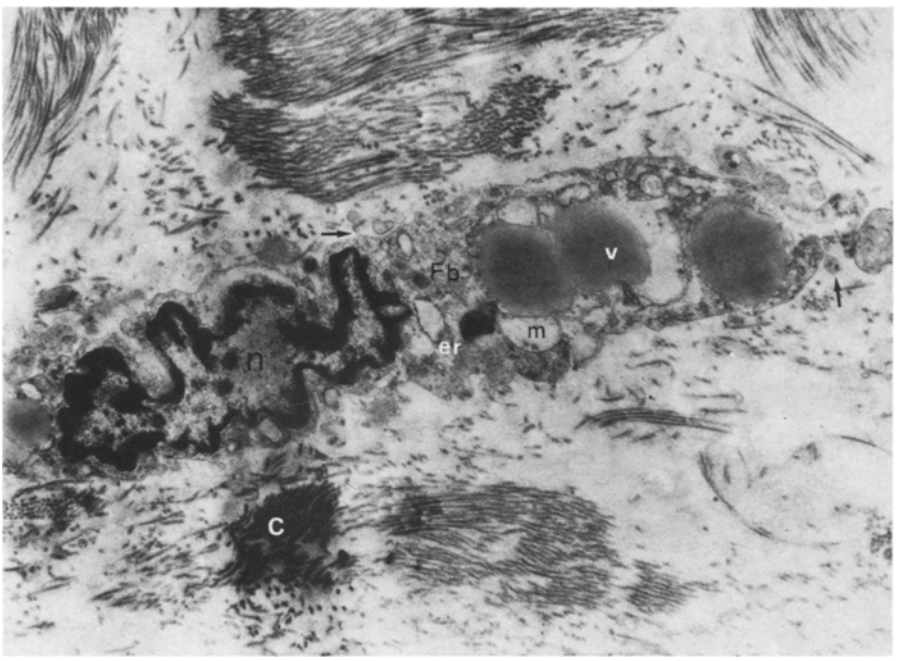

Fig. 9. Sclerema neonatorum. The fibroblast (*Fb*); nucleus (*n*) with folded margins; elongated cytoplasm packed with mitochondria (*m*), vacuoles (*v*) and rough-surfaced endoplasmic reticulum (*er*). The peripheral cytoplasm seems to be segregated into the extracellular spaces. Note peripheral budding of the fibroblast (*arrows*). Collagen fibers (*C*). (× 16,800)

of these cells was characterized by an irregular outline with folded margins and showed margination of chromatin. The cytoplasm contained dilated mitochondria and rough endoplasmic reticulum. The endoplasmic reticulum was characterized by a series of interconnected saccate or tubular structures. A dilated cisterne of rough endoplasmic reticulum contained thin cytofilaments. In most of the fibroblasts the rough endoplasmic reticulum and Golgi apparatus were poorly visible. The peripheral cytoplasm showed budding and segregation into the extracellular spaces in numerous segments. Most of the fibroblasts contained several vacuoles in the cytoplasm filled with osmiophilic substance (Fig. 9.).

There were also changes in the capillaries of the fat lobules and small blood vessels of the interlobular spaces. The walls of numerous vessels were contracted or collapsed. These showed severe damage. In many endothelial cells the nucleus had compacted chromatin; in large areas there were no cell membranes and extrusion of cell debris such as vacuoles and large mitochondria into the extracellular spaces was observed. In the cytoplasm of other endothelial cells dilated cisternae of rough endoplasmic reticulum were found. There was an extended Golgi apparatus, distended mitochondria with homogenization of the cristae, and large vacuoles filled with moderately electron-dense substance; however, no pinocytotic vesicles were visible. Intercellular connections took the form of maculae adhaerentes or desmosome-like structures.

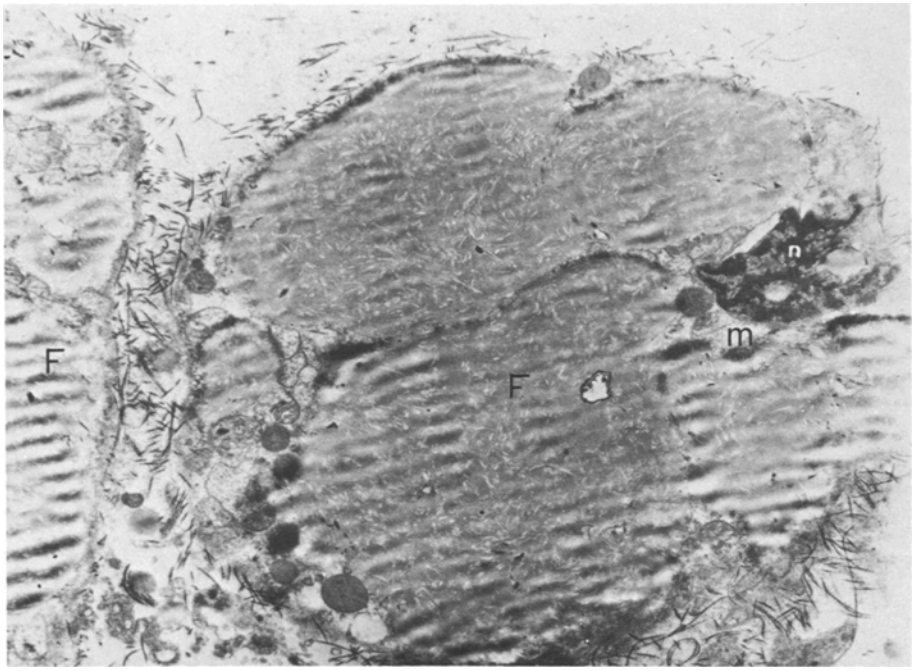

Fig. 10. Perirenal fat tissue in sclerema neonatorum with multilocular fat cells (*F*). Numerous, small spaces within the central lipid droplet. Damaged cell membrane and mitochondria (*m*). Small vacuoles filled with highly electron-dense substance. Nucleus (*n*) with compact chromatin. (×11,700)

The surrounding basement membrane of capillaries was distended in many areas, homogeneously thickened, and multiplied. Only the endothelial cells of the few capillaries situated in the connective tissue spaces were surrounded by an interrupted layer, composed of cytoplasmic protrusions of the pericytes, and also by single collagen fibers. The lumen of some blood vessels contained single erythrocytes. No extravasation was observed.

The fat cells of perirenal adipose tissue contained several lipid vacuoles and usually a centrally positioned nucleus, which showed in some parts excessively compact or dispersed chromatin. In the cytoplasm dilated mitochondria as well as small vacuoles filled with osmiophilic substance were seen. In many cells the cellular membranes were obliterated. The lipid vacuoles in all cells – filled with medium electron-dense material – exhibited many very small clefts of various shape and electron-lucent density. The longitudinal axes of these clefts ran in various directions and they formed neither bundles nor aggregations (Fig. 10).

The wide interlobular septa were composed of thick bundles of collagen fibers alternating with single ones loosely dispersed in the ground substance, with very numerous microfibrils in between them. In cross-sections, just as in the subcutaneous tissue, a few collagen fibers about 500–800 Å in diameter

were seen. Apart from single fibroblasts exhibiting features of active fibrillogenesis, no cellular infiltration was found.

The endothelia of capillaries entwining the fat cells and endothelia of small blood vessels and lying in the connective tissue spaces of the perirenal fat tissue contained nuclei with excessively compact chromatin, often shifted to the periphary. In the cytoplasm of these cells, dilated mitochondria as well as very numerous vacuoles were found of different size and filled with an osmiophilic substance. The rough endoplasmic reticulum showed obliterated contours. The basement membrane of the vessels was uniformly dilated in some segments.

Discussion

Histological and ultramicroscopic studies of SN were based on autopsy material, as it was impossible to obtain biopsy material of skin or subcutaneous tissue because of the grave condition of the infants. Hence, many ultrastructural changes detected in the cells may be ascribed to cytolysis taking place as a result of the action of post-mortem enzymes. Nevertheless, both light and electron microscopic studies served to establish some morphological changes in the subcutaneous and perirenal fat tissue and for comparing them with those observed in ASN (Pasyk 1978).

Thus, in SN, in the electron microscope destruction of the cell membrane, damage to cytoplasmic organelles, and changes in the nucleus could be seen in numerous fat cells. These ultrastructural features have not been taken into consideration. However, very numerous, small irregularly dispersed fissures could be observed in the central lipid droplet of the fat cells, as well as in subcutaneous and perirenal fat tissue. Their size, shape, and lack of surrounding membrane differed from the needle-shaped clefts in ASN. Similar small fissures were found in the central lipid droplet seen in preparations taken 4 h post mortem in cases of SN (Figs. 6, 10) and in autopsy material taken 4 h post mortem from infants without skin lesions (Fig. 1). These fissures are probably the result of post-mortem physico-chemical phenomena occurring in the fat tissue and cannot be taken as a basis for diagnosing SN. Similarly, nondiagnostic are the small crystals befined by Prokš (1961) as type A, observed in the polarizing microscope in autopsy material from cases of SN (Prokš 1961; Prokš and Volvoda 1965; Kellum et al. 1968). They were also observed in the fat cells of unchanged subcutaneous fat tissue of neonates and infants who died from various diseases (Prokš 1961; Horsfield and Yardley 1965).

The formation of these small crystals depends not only on the chemical composition of the fat but also on the temperature, length of fixation of the tissue, and on the fluids used for fixing (Prokš 1961). In studies on animals it appeared that there is a connection between the temperature and the degree of saturation of the fats in the tissue (Barańska and Włodawer 1966). The fat in brown fat tissue crystallizes much more easily at room temperature than does subcutaneous fat tissue. This is connected with the higher degree of saturation in brown fat tissue and higher coagulation point (Barrnett 1973). The

crystallization of fat may also be facilitated by an increased acid level in the subcutaneous fat tissue, as was seen in animals 24 hours post mortem (Sink 1966).

In the interlobular septa of the subcutaneous and perirenal fat tissue in SN, thick, compact bundles of collagen fibers occurred alternately with bands of loosely distributed ones in broad, distended areas of electron-lucent basal substance. Numerous microfibrils adhered to the collagen fibers or formed separate concentrations. The splitting of the fibers into smaller, microfibril types, visible at greater magnifications, might be the result of the action of collagenase, occurring in some bacteria, on newly synthesized collagen, which is especially sensitive to the activity of this enzyme (Stachów 1978); it might also result from the distension of the connective tissue stroma in the presence of a great disturbance of electrolytes. A similar phenomenon, called "fraying" of the collagen fibers, was produced experimentally by placing collagen fibers into a aqueous solution of N-mono-chloro-glycin (Kronman et al. 1977). The splitting of the collagen fibers and concentration of microfibrils around them observed in SN might also be the result of a faulty polymerization of tropocollagen during its maturation and the lack of strong covalent cross-banding between the fibers.

Worthy of note are extensive areas filled with fine-granular striated substance, having bands at regular intervals of 650 Å, detected in the dermis in all cases of SN. Similar cross-banded structures have been described in various dermatological and nondermatological conditions and even in normal tissue (Banfield et al. 1973; Edwards 1975; Bhawan and Edelstein 1977). The origin, mode of development, and significance of these structures are unexplained. They were believed to be a peculiar type of collagen – fibrous long-spaced collagen (Schubert and Adams 1974; Charles et al. 1977; Navas Palacios 1978) – which was also found during experimental reconstruction of collagen fibers from collagen solutions in vitro (Highberger et al. 1950). Hashimoto and Ohyama (1974) suggested that these cross-banded structures may contain acid mucopolysaccharides. However, Sun and White (1975) supposed that they share a common glycoprotein structure with the basement membrane, while Bhawan and Edelstein (1976) considered that they might contain actin and myosin. Hashimoto and Ohyama (1974) postulated that these structures might arise by the degradation of collagen through the action of collagenase and non-specific proteases excreted by malignant tumors. According to Charles et al. (1977) it is possible that their development begins with the separation of collagen fibers into constituent filaments of successively smaller calibre. The subsequent interaction of these collagenous filaments with acid mucopolysaccharides. perhaps at specific binding sites in register, could result in such cross-banded structures. As such, they could be interpreted as an abnormal form of collagen reassembly or repair. It is also possible that both the cross-bands and the finest longitudinal filaments are composed of noncollagenous ground substance.

It is to be expected that tissue material from SN specially prepared by the ruthenium red method and by phosphotungstic acid staining will provide further information as to cross-banded structures and splitting of collagen fibers.

Fibroblasts changes including folding, with numerous pouch-like cavities in the nuclear outline, chromatin shifted to the periphery and distended mitochondria almost entirely lacking cristae, are proof of damage. However, the distended cisternae of the rough endoplasmic reticulum, cytofilaments and budding fibroblast cytoplasm suggest the active formation of such cells in SN.

In the electron microscope changes were also seen in the small blood vessels of the dermis and subcutaneous tissue in preparations taken 4 and 24 h after death. The endothelium showed distension of the mitochondria, damage to the cell membrane, and the presence of structures resembling desmosomes or maculae adhaerentes. The collapsed lumen of the vessels, changes in the endothelial cells, a lack of activity of pinocytotic endothelia, and multiplication and homogenization of the basal laminae may be due to post mortem autolysis, or may result from distension and hypoxia.

Comparison of the histological and ultrastructural changes in SN with results obtained in ASN, show that in SN there is absence of intra- and extracellular needle-like clefts, no foreign-body giant cell granulomatous infiltration, and also no calcium deposits in the fat lobules. These observations are proof that SN is a separate pathological state, coexisting with serious general illness.

The ideal procedure would be to have biopsy material. This material would establish how many ultrastructural features are observed in the blood vessels and collagen fibers in SN, also whether these features are due to only autolytic changes, or are connected with this entity.

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