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ULTRASTRUCTURE OF DERMAL CONNECTIVE TISSUE IN FIBRODYSPLASIA OSSIFICANS PROGRESSIVA

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Abstract. The ultrastructure of dermal connective tissue in two patients suffering from fibrodysplasia ossificans progressiva (syn. myositis o.p.) was studied. The disorder starts with soft tissue swellings which subsequently ossify. The connective tissue of various organs may be affected. No defects in calcium metabolism have been discovered. It was intended to study the primary event which initiates the ossification. The present ultrastructural study demonstrates an accumulation of proteoglycan microfibrils as well as glycoprotein material in dermal connective tissue. Both substances are prerequisites of calcification resulting from the binding of calcium and phosphorus ions. A subsequent release of proteolytic enzymes in the tissues may set free the bound ions and, thus, initiate mineralization.

Key words: Fibrodysplasia (myositis) ossificans progressiva; Connective tissue; Proteoglycan; Glycoprotein; Ossification

Fibrodysplasia ossificans progressiva (FOP; *syn.* myositis o.p.) is an extremely rare disease of the connective tissues (20, 21). An acute occurrence of soft tissue swellings may develop into ossified lumps. The connective tissues of fasciae, tendons, aponeuroses, ligaments, joint capsules, and skin are usually affected. The disease starts in the first decade of life and usually progresses through the second or third decade, finally resulting in a crippling condition. Reduced joint mobility and secondary muscle atrophy are predominant features.

Previous laboratory studies have shown few significant findings. The tissue alkaline phosphatase level may increase (7), while serum alkaline phosphatase remains normal (12, 21). No defects in calcium metabolism have been discovered. The early histologic changes are not known (21). Previous light microscopical studies on long-standing lesions have demonstrated soft tissue calcification and secondary muscle atrophy (29, 30). The dermal connective tissue becomes generally involved at the time when the subcutaneous tissue swellings develop. Calcification of skin connective tissue was reported long ago by Rosenstirn (25) and, more recently, by Vastine et al. (32).

The etiology of the disease is still unknown, though several hypotheses have been proposed. In 1964, Lutwak (20) suggested abnormalities of collagen to be the primary event. However, at that time no collagen changes had been detected which could explain the calcification. The aim of the present study was, therefore, to reveal the early changes in the dermal connective tissue during the acute phase of FOP, which may be responsible for the initiation of ectopic ossification in FOP. To the best of our knowledge, no previous work has been performed on this subject. Hitherto, histological studies on FOP have dealt with the problem whether the disorder primarily involves connective tissue or striated muscles (7, 19, 29).

MATERIAL AND METHODS

Two patients suffering from fibrodysplasia ossificans progressiva were studied. Both were young women in their early twenties and both had had the disease since birth. A more detailed case report has been published elsewhere (11).

Four skin biopsies were taken from acute-developed lumps in the proximal parts of extremities. The specimens were fixed in a 6% glutaraldehyde solution in Veronal acetate buffer, pH 7.4, with 7.5% sucrose. After osmification the specimens were dehydrated and embedded in Epon 812. Ultrathin sections were cut with LKB and Reichert ultramicrotomes. The sections were stained

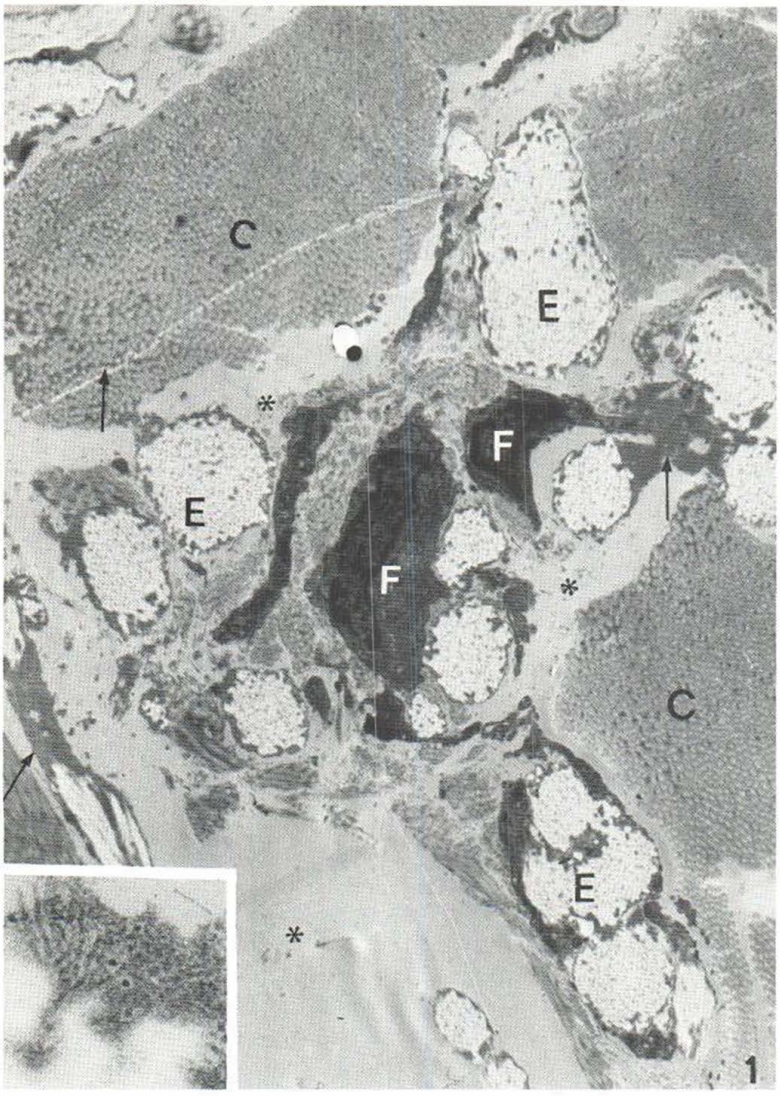


Fig. 1. Fibroblasts (*F*) surrounded by numerous elastic fibres (*E*). Proteoglycan fibrils appear between bundles of collagen fibrils (asterisks). Between the individual collagen fibrils (*C*) and the elastic fibres an amorphous material is clearly seen (arrows). ×6 000. Inset shows amorphous material between elastic fibrils. ×60 000.

with uranyl acetate and lead citrate, ruthenium red and periodic acid silver proteinate after Thiéry (31). The stained sections were studied in a Siemens electron microscope (Elmiskop 1A) at 80 kV with a double condensor system.

RESULTS

The intercellular compartment of the dermis

A large quantity of microfilaments of varying thickness formed a network between the bundles of collagen fibrils (Figs. 1, 2). An abundant quantity also appeared in the surroundings of fibroblasts and mast cells (Figs. 1, 12). The microfilaments were stained selectively with ruthenium red (Fig. 3). The periodic acid silver proteinate staining only partly visualized the microfilaments (Fig. 4).

An amorphous material was revealed between the individual collagen and elastic fibrils (Figs. 1, 5, 6). This material was located close to the fibrils without coating them. A distinct clear halo around each collagen fibril was always present (Fig. 5). The amorphous material was stained with periodic acid silver proteinate (Fig. 6), but not with ruthenium red.

The elastic fibrils and matrix appeared normal (Fig. 1). The collagen fibrils had a normal axial periodicity and diameter (Fig. 5). Calcification of the connective tissue was not observed.

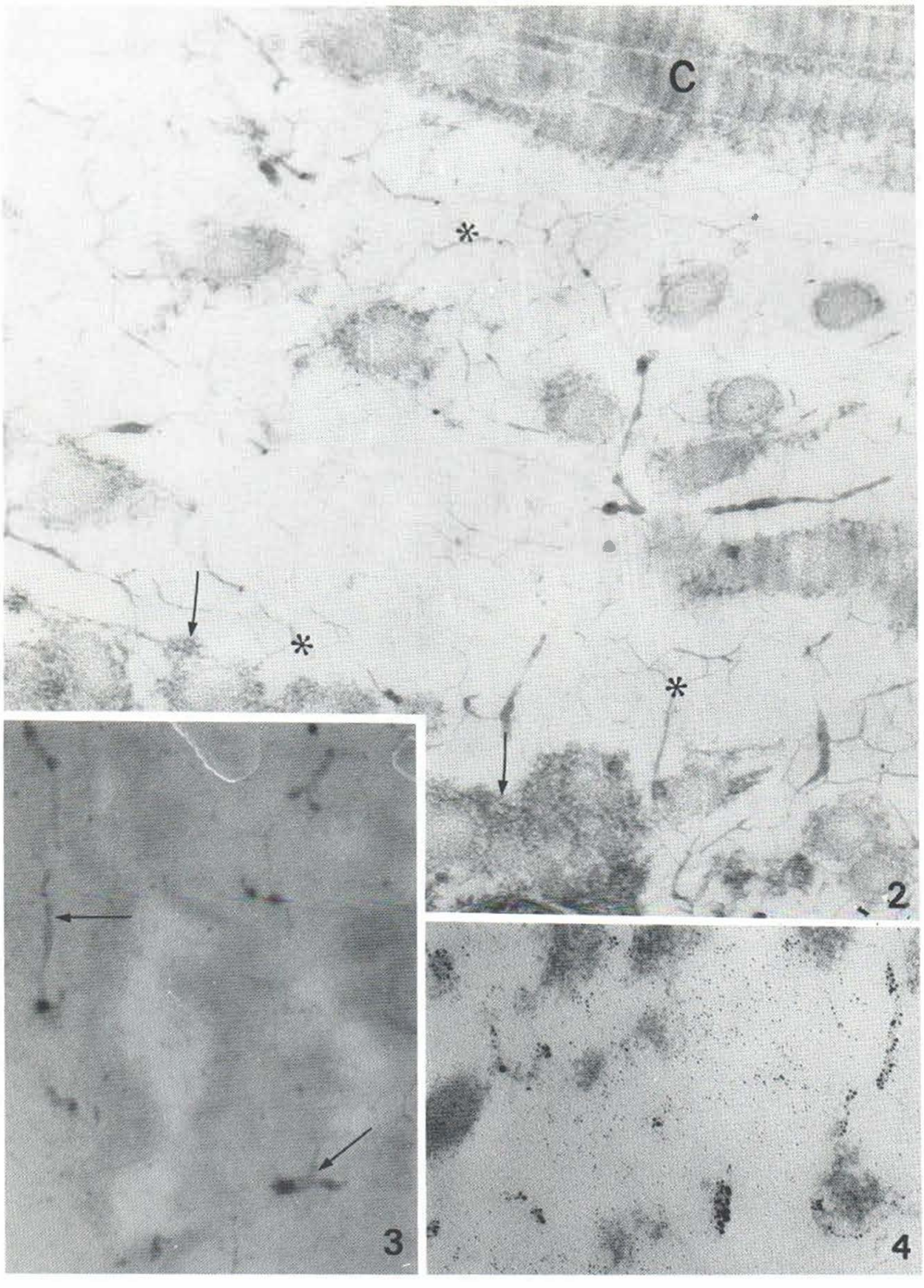
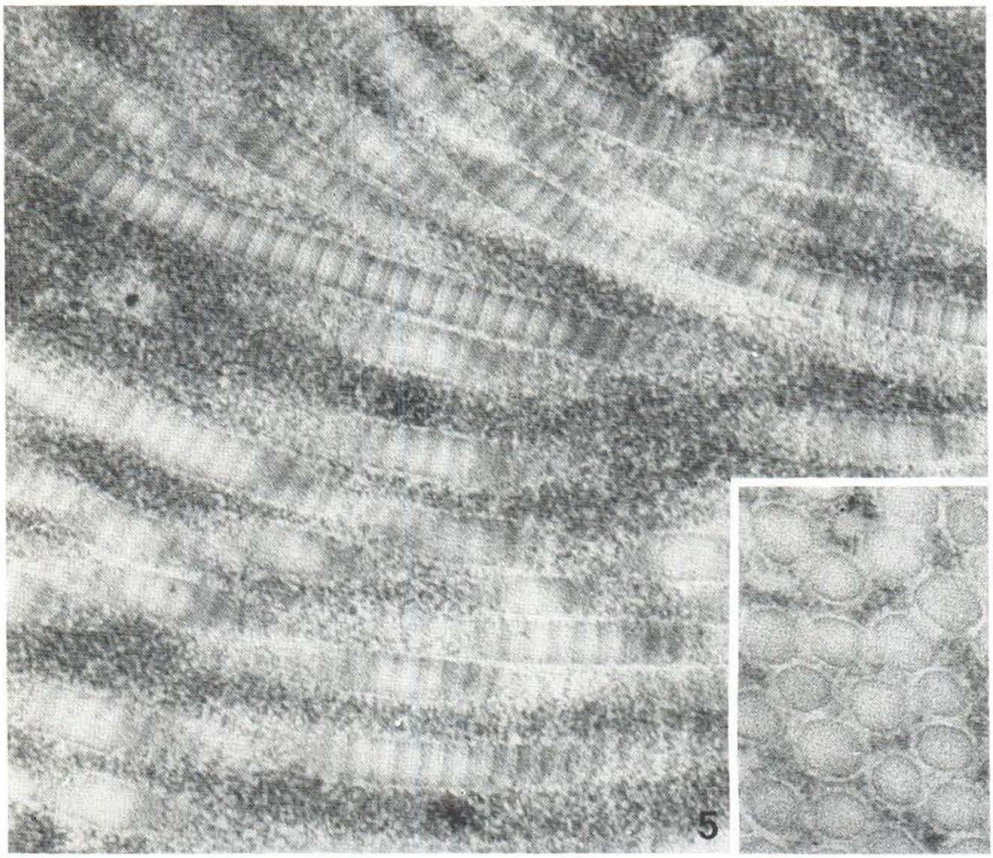


Fig. 2. The proteoglycan microfibrils (asterisks) and the amorphous material (arrows) are shown at higher magnification. The section is stained with uranyl acetate and lead citrate. ×60 000.

Fig. 3. Proteoglycan microfibrils stained with ruthenium red (arrows). ×60 000.

Fig. 4. Silver grains on proteoglycan microfibrils indicate reaction with periodic acid silver proteinate (Thiéry). ×60 000.



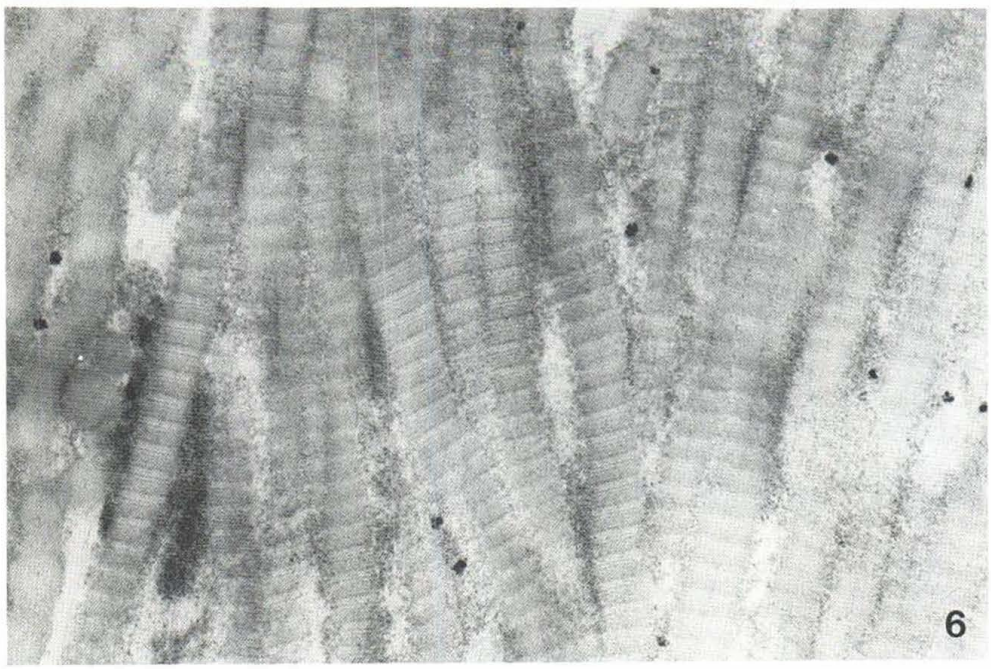


Fig. 5. Amorphous material between individual collagen fibrils. Inset shows the lucent halos separating each collagen fibril from the surrounding amorphous material. ×60 000.

Fig. 6. The amorphous material reacts with periodic acid silver proteinate. ×60 000.

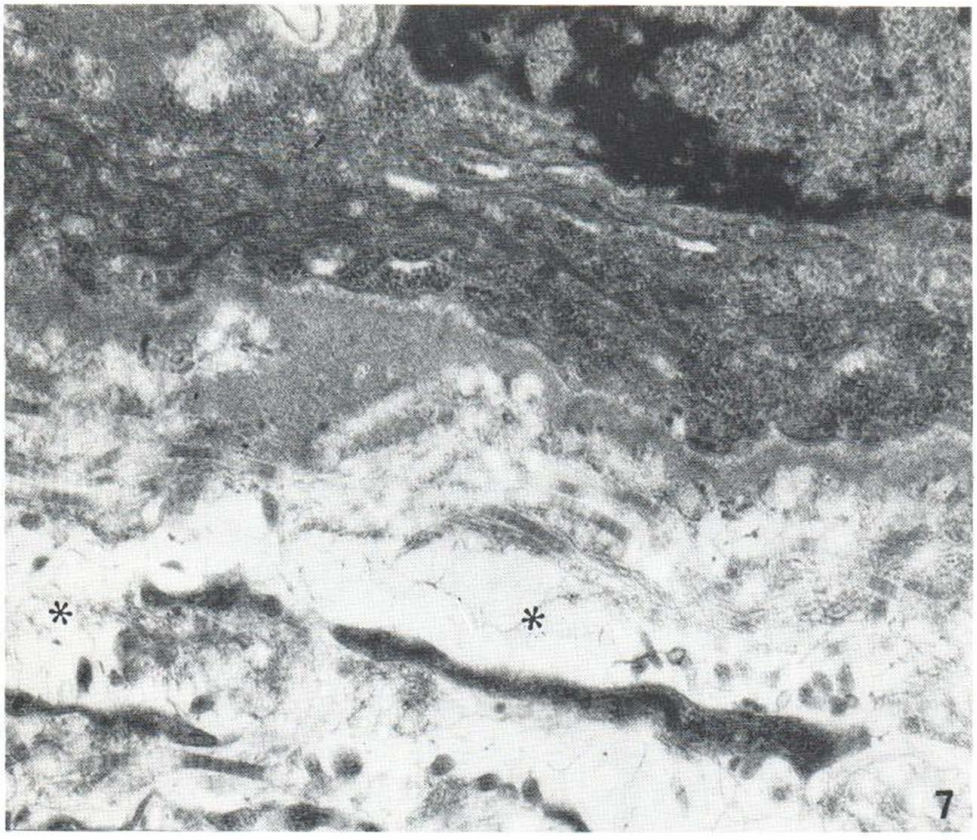


Fig. 7. The basal lamina of the dermo-epidermal junction is thickened. The epidermal cells appear normal. Beneath the junction zone, large amounts of proteoglycan microfibrils are seen (asterisks). ×15000.

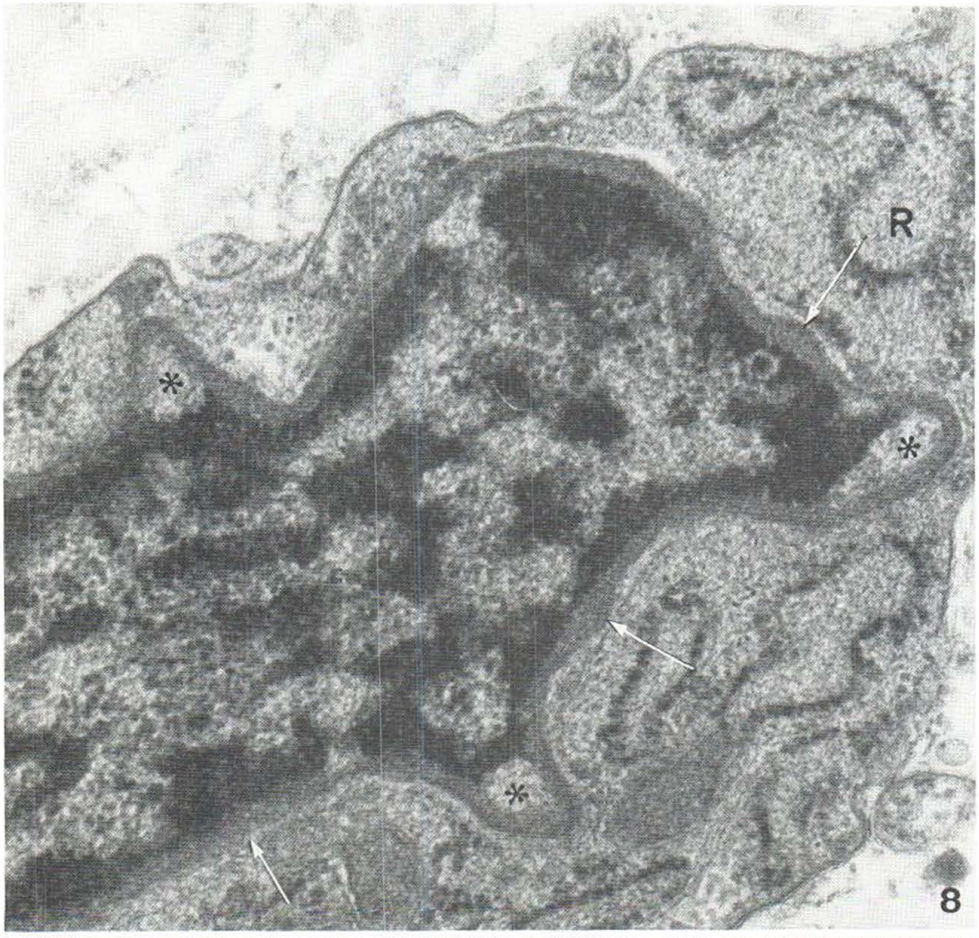
The dermo-epidermal junction was thickened as visualized by accumulation of Thiéry positive amorphous material (Fig. 7). The anchoring fibrils showed a distinct banding and were of varying width.

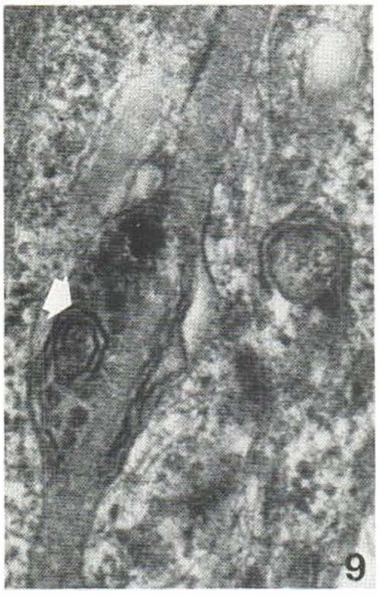
The dermal cells

The number of fibroblasts was not increased. The cells were generally polygonal and not spindle-shaped (Fig. 1). The shape of the nucleus was often irregular, and there was a dense zone of chromatin granules along the periphery. Between the nuclear membrane and the chromatin granules, a thick fibrous lamina appeared, which surrounded the nucleus except at the sites of nuclear pores (Fig. 8). Foldings of the nuclear membrane and fibrous lamina occurred frequently (Fig. 8). In the cytoplasm a well-developed granular endoplasmic reticulum appeared. The endoplasmic reticulum was usually dilated and contained a material which stained with periodic acid silver proteinate. The Golgi apparatus was abundant and contained many vesicles and flat saccules. In the surroundings of the fibroblasts, bundles of elastic fibres occurred (Fig. 1).

The macrophages were seen less frequently. These cells contained prominent lysosomes and some endoplasmic reticulum. Some macrophages were seen to have engulfed collagen fibrils (Figs. 9, 10). Lysosomal degradation of such engulfed fibrils appeared in some areas (Fig. 9). A condensation of the cytoplasm was usually seen around the ingested collagen fibrils (Fig. 10). Inside the channel with ingested collagen fibrils, round membrane-bounded bodies occurred. Residual bodies inside these channels were also present (Fig. 9).

Mast cells were frequently found in both patients, although their numbers were not significantly path-





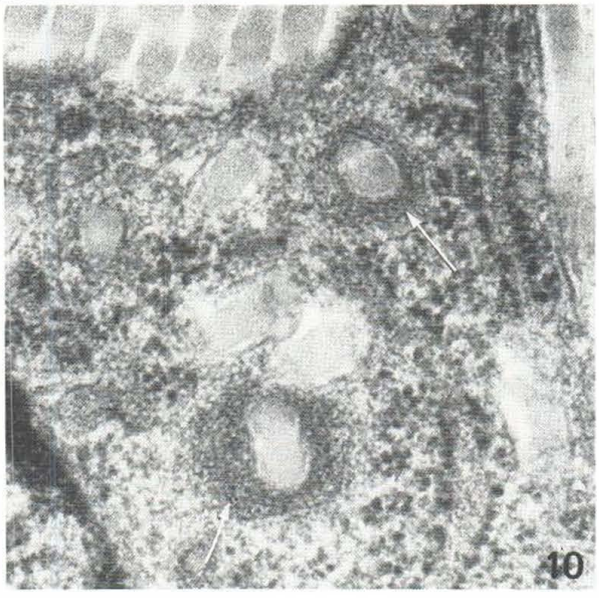


Fig. 8. A fibroblast with dilated granular endoplasmic reticulum (*R*). The nucleus appears with a thick fibrous lamina (arrows) and chromatin-free membrane foldings (asterisks). ×60 000.

Fig. 9. Detail of a macrophage with an engulfed collagen fibril and a residual body (arrow). ×60 000.

Fig. 10. A macrophage with engulfed collagen fibrils. Note the dense cytoplasm surrounding some of the fibrils (arrows). ×60 000.

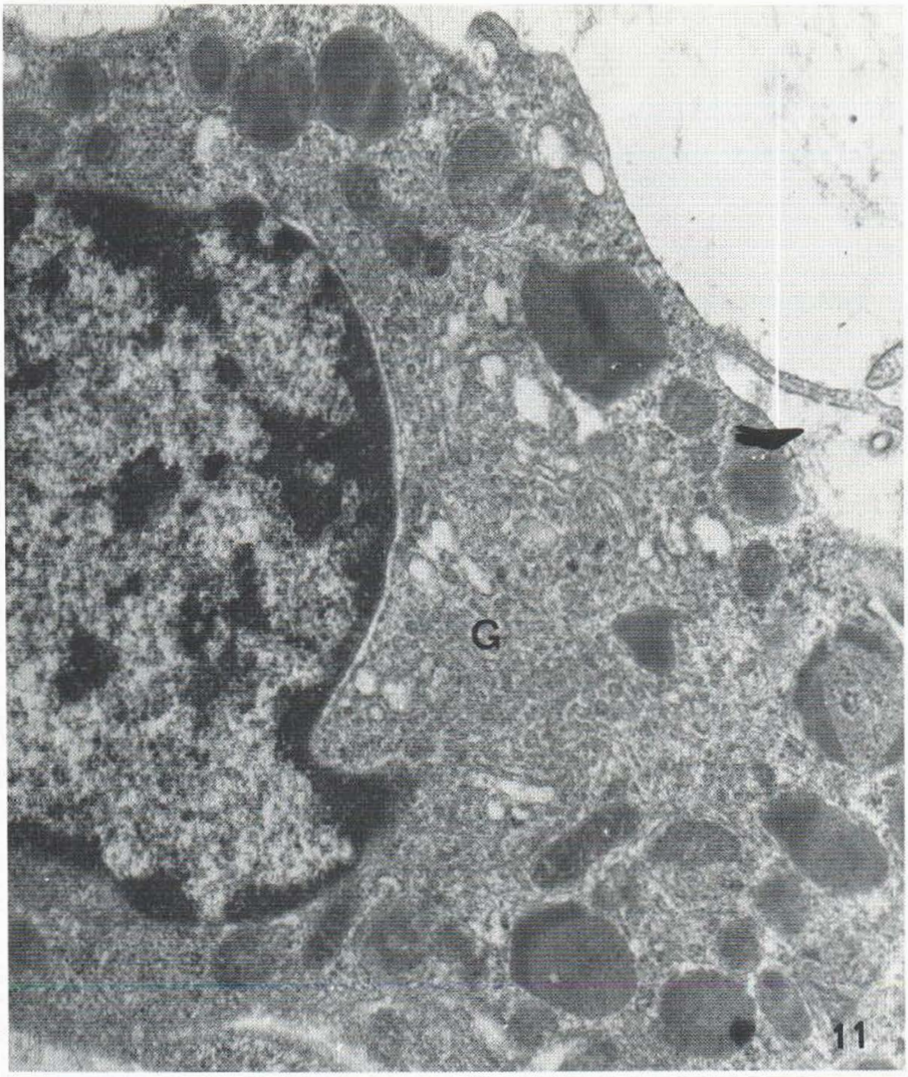


Fig. 11. Mast cell showing a prominent Golgi complex (*G*) and abnormal granules without scrolls. ×30 000.

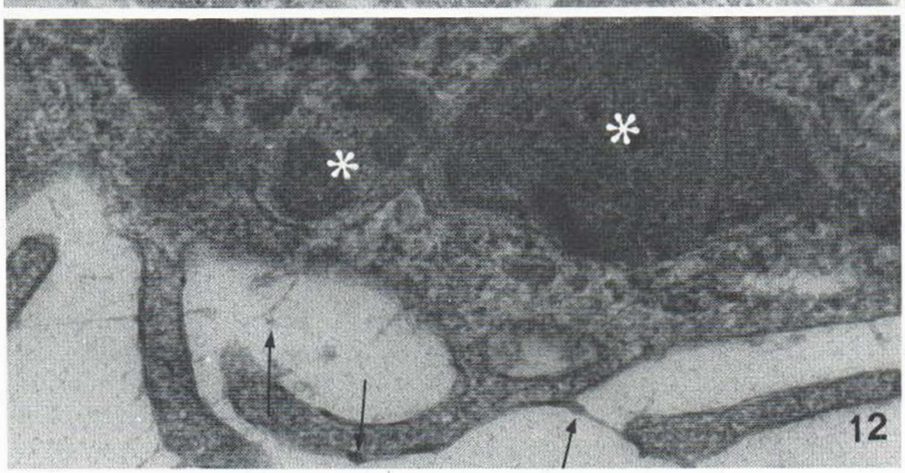


Fig. 12. Mast cell showing proteoglycan fibrils (arrows) on its cell membrane. Note the atypical granules of varying density (asterisks). ×160 000.

ologic. The cells generally had a large Golgi complex with enlarged lucent vesicles and flat saccules (Fig. 11). The granular endoplasmic reticulum and the mitochondria were less prominent. A few mast cells contained typical mature granules with scrolls, while most of the cells were filled with abnormal granules (Figs. 11, 12). These granules consisted of a homogeneous material which was subdivided into areas of varying density (Figs. 11, 12). Degranulated, honeycomb-like mast cells were seen occasionally. The cytoplasmic villi were often short and scanty. Proteoglycan microfibrils, appearing in the surroundings of the mast cells, adhered to the cell membrane (Fig. 12).

DISCUSSION

Ruthenium red positive microfibrils known to represent proteoglycans have been demonstrated previously in human skin (14, 16). Proteoglycans are known to be of importance for the water content of connective tissues (2), and the abundant amounts of proteoglycans in affected skin of our two patients reflect edematous soft-tissue swellings (21). The findings are similar to the ultrastructural changes found in the progression zone of localized scleroderma (17), in scleromyxedema (5) and localized myxedema (18).

PAS-positive substances in the intercellular compartment of connective tissue (except glycogen and some lipids) are glycoproteins (24). Thiéry (31) modified the periodic acid-Schiff reaction, replacing the Schiff reagent with silver proteinate. The electron-dense silver precipitate renders the modification profitable for electron microscopy. The periodic acid silver proteinate positive amorphous material occurring in the junction zone and between the collagen fibrils is presumed to represent glycoproteins. The material may be synthesized by mesenchymal cells. Both fibroblasts and mast cells appeared active and could be responsible for the production. The amorphous material was never seen in the vicinity of macrophages engulfing collagen fibrils. It is therefore unlikely to be a degradation product. Hascall & Sajdera (8) suggested that connective-tissue glycoproteins link proteoglycan subunits together to form the large chains.

The role of proteoglycans and glycoproteins in the calcification process is still under debate. Both stimulating and inhibitory roles have been suggested (3). Certain purified proteoglycan fractions from cartilage inhibit calcification (27). This inhibition is abolished by proteolytic enzymes (12). Proteoglycans and glycoproteins are both capable of binding calcium and phosphate (3, 4, 6). Ultrastructural studies of calcifying cartilage have shown matrix vesicles in the connective tissue in which the initial hydroxy-apatite crystals appear (1). These vesicles are liberated from chondrocytes and may have a high content of proteolytic enzymes. Howell et al. (12) suggested that proteolytic enzymes may set free the bound calcium and phosphate ions, resulting in a high local supersaturation, and, thus, initiating the calcification. Mast cells may also initiate tissue calcification. The importance of mast cells in experimentally induced calcification in rats was stressed by Selye (28). Human mast cells in cultivated skin of patients with urticaria pigmentosa produce membrane-bounded matrix vesicles as well as proteoglycan microfibrils. They also seem to induce calcification on collagen and elastic fibres (10). An abnormal accumulation of proteoglycans and glycoproteins may be primary to the binding of calcium and phosphate ions.

The engulfment of collagen fibrils by macrophages was a common finding in the present study. The macrophages are known to play an important role in collagen resorption during the involution of rat postpartum uterus (23). Intracellular collagen degradation appeared in vacuoles containing residual bodies and acid phosphatase activity. Engulfment of collagen fibrils is also seen in dermal macrophages of patients with scleroderma (15, 26) and in cultured human skin (9). The significance of collagen resorption in fibrodysplasia ossificans progressiva is unknown.

Mast cells holding abnormal granules have been seen to be surrounded by increased quantities of proteoglycan microfilaments in skin lesions of patients with urticaria pigmentosa (13) and localized myxedema (18). The phenomenon suggests mast cell production of proteoglycans.

The fibroblasts in our patients appeared active, with extensive endoplasmic reticulum and large Golgi apparatus. The nuclear membrane folding is often seen in metabolically active cells (22). The endoplasmic reticulum of the fibroblasts contained a silver proteinate positive material with the same morphology as the extracellular glycoprotein. This suggests that active fibroblasts are responsible for the increased glycoprotein production in fibrodysplasia ossificans progressiva.

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