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Ultrastructural Study of a Hitherto Unknown Disturbance of the Synthesis of Collagen Precursor Molecules in Human Cutis

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Summary. The ultrastructural characteristics of on hitherto unknown disturbance of collagen precursor molecules in human skin were studied. A severe disturbance of the synthesis of collagen exists at the procollagen stage. The paracrystalline accumulation of a form of procollagen in the tubes of the smooth ER and an increase in its appearance in the extracellular space, distinguish this clinical picture from all other previously described connective tissue disorders, especially from Dermatosparaxis and Ehlers-Danlos syndrome type VII. For this clinical picture we suggest the name "Dermatosparaxis endoplasmatica".

Key words: Procollagen – Endoplasmic Reticulum – Dermatosparaxis endoplasmatica.

Introduction

Hanset in 1971 described a genetic defect of the skin which appeared for the first time in inbred Belgian cattle. He named this anomaly "Dermatosparaxis" (torn skin). The particular feature of this condition is a dramatic fragility of the skin; healing of lacerated wounds is very bad. The dewlap, forelimbs and eyelids are often edematous. The skin is not hyperelastic.

The ultrastructural and biochemical studies of the skin of these cattle revealed

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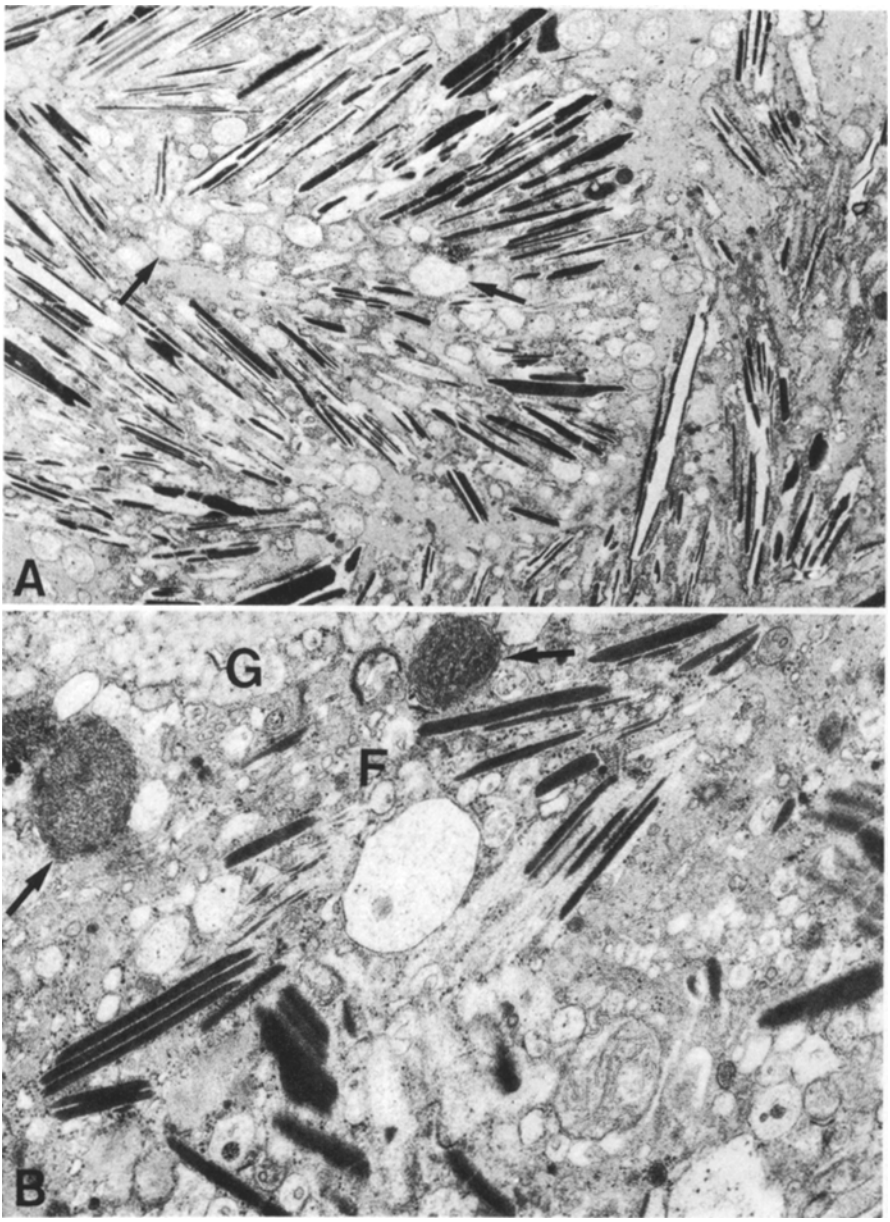
the presence of procollagen instead of collagen forming the extracellular fibrils. This change results from the lack of activity of procollagen peptidase, an enzyme required for the final processing of procollagen into collagen. The groundsubstance shows qualitative and quantitative changes in their content of glycosaminoglycans. Abnormalities of fibrocytes and small vessels have not been observed (Winand and Nusgens, 1971; Piérard and Lapiére, 1976). We have studied ultrastructurally in man a clinical picture with a similar disturbance of collagen precursor molecules but with very characteristic abnormalities of fibrocytes.

Material and Methods

From a patient (male, 44 years) with the clinical diagnosis of an acquired dermatochalasis and urticaria pigmentosa, four skin biopsies from the eyelid and body were examined. The biopsies were fixed immediately after removal in 1% osmic acid in 0.1 M phosphate buffer (pH 7.3). After washing in buffer and dehydration with alcohol they were embedded in Epon. Thin sections were stained with uranylacetate and lead citrate after Reynolds (Ref.). A Zeiss EM 9S2 microscope was used. Ultrahistochemically, thin sections were digested with trypsin und pepsin after Geyer (1973).

Results

Both intra- and extracellular abnormalities of collagen formation are immediately apparent (Figs. 1 and 2). The rough endoplasmic reticulum (ER) is not as well developed as in normal active fibrocytes. In many fibrocytes dilated sections of smooth ER are present. These cisterns often contain a fine granular material which is deposited on the inner side of their membranes (Figs. 1, 2). They ultimately form paracristalline rod-shaped masses, often filling the ER (Figs. 1 and 2). In the affected cells, metabolism is apparently altered with suggestive cristolysis in the mitochondria. The inclusions contain parallel filaments which through end-to-end connections may reach a length of several thousand Å and have a diameter of 60–80 Å (Fig. 2A). They are generally resistant to trypsin but degraded by pepsin (Fig. 2A). Exocytosis of this apparent elementary collagen stage does not lead to the formation of collagen by transformation of tropocollagen, rather, an accumulation of spherical masses of these 60–80 Å filaments is usually observed in the ground substance (Fig. 2B). Next to this, collagen fibers are also found in the matrix and in cross section present themselves as electron microscopically translucent and swollen (approx. 1400–1700 Å; Fig. 2B). These fibers reveal the classical periodicity of 640 Å, and we assume that this collagen arises from the less affected fibrocytes. The proteoglycan composition of the extracellular matrix is likewise out of balance, a fact probably related to the surface erosion of elastic fibers (Fig. 2B). Mast cells are apparently involved in these phenomena, a fact which is evidenced by their presence in increased numbers. Conspiciously, mast cell granules can often be observed in the modified fibrocytes (Fig. 1B).



Figs. 1 and 2. Dermatosparaxis endoplasmatica. Dermis

Fig. 1. **A** Section of a fibrocyte. Paracrystalline packing of collagen precursor molecules in the ER. Crystolysis in the mitochondria (*arrows*). ×10,600; **B** Intracytoplasmic uptake of mast cell granules (*arrows*) from the ground substance G into a likewise affected fibrocyte *F*. ×21,400

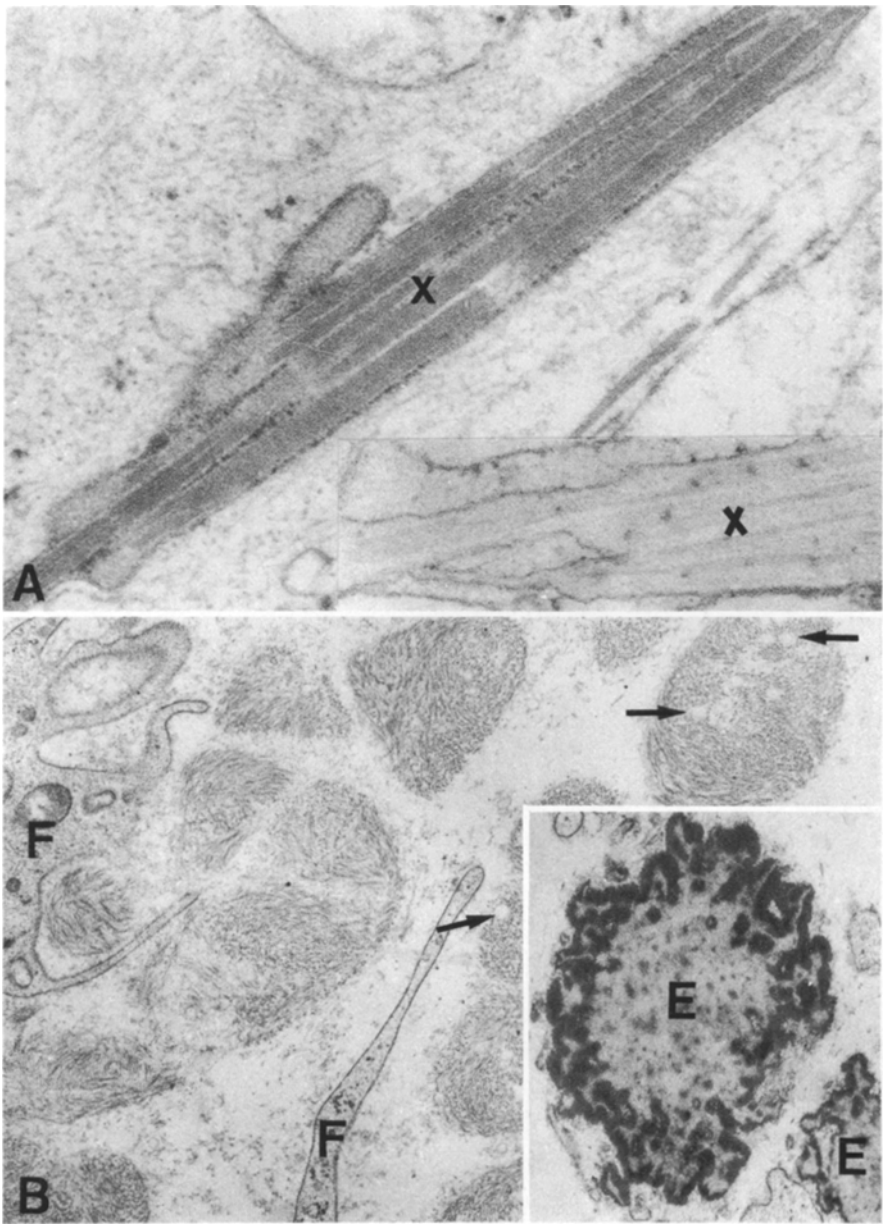


Fig. 2. A Higher enlargement of the paracrystalline inclusions *X* in the smooth ER. Parallel arrangement of collagen precursor fibrils. ×6400; Inset: The same after pepsin digestion *X*. ×60,000; **B** Groundsubstance. Ball-like condensation of collagen precursors including cross sections of swollen pale collagen fibers (*arrows*). Between the clumps pale edematous groundsubstance. *F* sections of fibrocytes. ×21,400; Inset: Crossections of elastic fibers *E*. Marked erosions of the fiber surfaces. ×20,400

Discussion

Fibrocytes synthesize the precursors of the fibrous components of the extracellular space (i.e. collagen, elastin, reticulin) and the proteoglycans of the groundsubstance (reviewed by Balasz, 1970; Kimmig und Kreysel, 1973).

The fibrocytes in the corium of our patient show characteristic ultrastructural disturbances in their ability to synthesize these materials. Under normal conditions the fiber precursors and the proteoglycans would be synthesized in the rough ER and then passed into the smooth ER. The enzymatic coupling of these products occurs in the smooth ER with delivery to the extracellular space through the Golgi apparatus (Kimmig and Kreysel, 1973; Schulze and Staudinger, 1975; Slavkin and Greulich, 1975).

Our electron microscopic findings suggest that in the fibrocytes from the abnormal skin of this patient collagen synthesis is blocked at a very early stage, probably the procollagen stage. The parallel arrangement of the fibers, their length (Weinstock et al., 1975) as well as their susceptibility to pepsin digestion but resistance to trypsin (Wassermann, 1956) support this interpretation. Conversion from procollagen to tropocollagen and then further extracellular polymerization to collagen is markedly reduced and leads to a congestion of the smooth ER with an initial membrane deposition and subsequent paracrystalline packing of the ER tubules with procollagen like molecules. This points to a lack of activity of procollagen peptidase. In association with this, cell metabolism is markedly impaired, as shown by the typical mitochondrial changes (i.e. cristolysis).

The intracellular accumulation of procollagen and its increased appearance in the extracellular space distinguish this clinical picture from all other connective tissue disorders previously described in humans (reviewed by McKusick, 1972). In particular it differs from dermatochalasis but also from syndromes with disturbances in collagen development such as the Marfan syndrome and the Ehlers-Danlos syndrome.

In our case the key problem is the inability to convert procollagen to collagen; probably due to the lack of activity of procollagen peptidase, with a subsequent storage of a form of procollagen in the tubes of the smooth ER. Until now deficiency of this enzyme – but without the endoplasmic phenomena described above – has been reported to exist in man in Ehlers-Danlos syndrome type VII (Lichtenstein et al., 1973). In cattle the clinical picture with the genetic absence of procollagen peptidase is referred to as "Dermatosparaxis". Because of the additional endoplasmic storage phenomena present in our case, we suggest the name "Dermatosparaxis endoplasmatica", instead of a further type of Ehlers-Danlos syndrome; realizing that we can, at present, not say with certainty whether a particular pattern of inheritance is present or if exogenous influences are mainly responsible.

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