

PSEUDOXANTHOMA ELASTICUM

An Ultrastructural Study of Scar Tissue

Lis Danielsen and Takasi Kobayasi

*From the Department of Dermatology (with the Connective Tissue Research Laboratories),
University of Copenhagen, Rigshospital, Copenhagen, Denmark*

Abstract. Pericicatricial lesions from one patient with pseudoxanthoma elasticum (PXE) showed typical ultrastructural alterations of PXE, i.e. large calcium deposits inside normal and irregular elastic fibres as well as similar deposits without relation to elastic tissue, and large areas of a thready material and twisted collagen fibrils with increased diameters. In addition, some of the collagen fibrils were found to be split into thin filaments with the periodicity of collagen and into thin filaments and masses of threads without banding. Fibroblasts showing evidence of abnormal synthesis were observed in close relation to the abnormal collagen fibrils. The centre of the scar was normal except for a few filamentous split collagen fibrils and threads. Lesions from 8 other PXE patients showed alterations of the fibroblasts like those observed in the scar lesions, only less pronounced, while normal-appearing skin from 4 of these patients contained fibroblasts similar to those observed in the skin of 5 control subjects. A defect in the synthesis of collagen resulting in the formation of large areas of a thready material and of twisted collagen fibrils is suggested.

Pseudoxanthoma elasticum (PXE) is a genetically determined disease involving elastic and collagen fibres in skin, eyes and vascular tissues. The skin shows small yellow papules in the flexural folds. Previous ultrastructural studies of the skin lesions have shown calcification inside and around elastic fibres, twisting, and increased diameter of collagen fibrils, and the presence of thready masses of unknown origin (3). A report is presented on the ultrastructure of scar tissue in PXE with special reference to the fibroblasts and the thready masses.

MATERIAL

The material consists of biopsy specimens from typical PXE lesions located around a broad appendectomy scar and from skin in the centre of the scar. The appendectomy

was performed 46 years previously, when the patient was 11 years old and a peritonitis developed. The patient was not aware of the development of PXE lesions around the scar. In addition, lesions were present in the flexural folds, periumbilical area and in the oral mucosa.

For comparison, biopsy specimens from lesions of 8 other PXE patients previously reported (3) and from normal-appearing skin of 4 PXE patients and 5 control subjects were studied. All biopsies were taken from light-protected skin areas. The control subjects were age-matched with the PXE patients. Three scars of non-PXE patients were also studied.

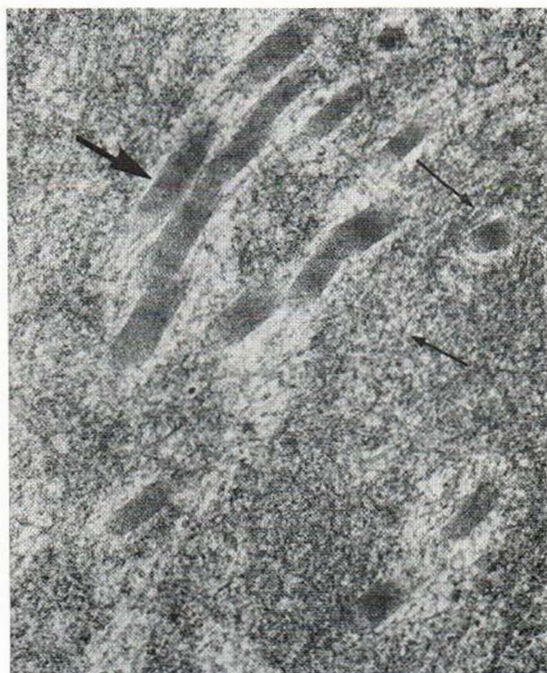


Fig. 1. Pericicatricial lesion. Masses of threads (thin arrows) containing a few obliquely cut collagen fibrils (thick arrow). $\times 60\ 000$.



Fig. 2. Pericatricial lesion. Part of a round area containing long thin filaments (*thin arrow*), granular material, and empty spaces. The filaments bound irregular villi,

the pattern of which is surrounded by collagen fibrils (*thick arrow*). $\times 50\,000$.

METHODS

The specimens were fixed in a 4% glutaraldehyde solution, buffered at pH 7.4 with phosphate salts for 1 hour at 4°C. For after-fixation, 1% osmic acid solution in 0.1 M phosphate buffer (pH 7.4) was used. After stepwise dehydration in increasing concentrations of ethanol the specimens were embedded in epoxy resin, and ultrathin sections were cut by a Reichert OM 2 ultramicrotome. The sections were stained by a combined technique using uranyl acetate and lead citrate and studied by a Siemens electron microscope (Elmiskop 1A) operated at 80 kV with double condensers.

RESULTS

In specimens from the lesions around the scar tissue, calcium was observed both inside elastic fibres and without any relation to elastic tissue. Many twisted thick collagen fibrils and large thready masses (Fig. 1) were observed around the calcified areas. Large round areas of granular material and empty spaces separated by irregular

villous structures made up of long thin filaments were seen (Fig. 2). In the surroundings the collagen fibrils showed a high degree of abnormality. Many were split into thin filaments appearing partly with, partly without the typical periodicity of collagen fibrils (Fig. 3). These filaments often formed little round or oval bulgings and were often in close proximity to the long thin filaments of the central area described above (Figs. 3 and 4). The long filaments showed no periodicity. Banded filaments were seen to be in continuity with masses of fine threads without periodicity (Fig. 5). This thready material was similar to that observed in large areas around the calcified material described above (Fig. 1). Several bizarre fibroblasts showing changes in the endoplasmic reticulum were observed (Figs. 6 and 7). The endoplasmic reticulum was often seen to be dilated and surrounded by a row of ribosomes or by a few or no ribosomes. In some areas the centre of the dilated reticulum contained a dense gran-



Fig. 3. Pericardial lesion. Collagen fibrils appear split into thin filaments showing the typical banding of collagen (thin arrow). Some filaments form small round (R) and

oval (O) structures. Long thin filaments with dubious banding (thick arrow) are seen in close relation to the abnormal collagen. $\times 60\,000$.

ular material. However, it frequently appeared almost empty with few dispersed grains or thin filaments. Similar little cysts without ribosomes were also seen between the laminae of the nuclear envelope (Fig. 7). In large areas no cell membrane could be seen (Fig. 7), and some cytoplasmic areas of these cells showed only cystic reticulum irregularly bounded by a thin membrane without ribosomes. There was a close relationship between these fibroblasts and the long thin non-banded filaments. Some of the filaments were directly contiguous with the dilated reticulum of the cells (Fig. 6). Pinocytosis was often observed. Large areas of calcified material were noticed in relation to both the long thin filaments (Fig. 6) and the thready material. Around the areas containing long thin filaments, normal-appearing and irregular elastic fibres were observed. Fibres of both types were seen to contain calcified material (Fig. 8). The degree of degeneration of the

elastic fibres varied according to the age of the patient (4).

The specimens from the centre of the scar contained normal elastic fibres and collagen fibrils and a few filamentous split collagen fibrils and threads. The fibroblasts were similar to those of the skin of 5 control subjects.

Besides the previously described alterations (3), the specimens from PXE lesions of 8 other patients showed changes in the fibroblasts. The cells showed a more prominent cystic endoplasmic reticulum than those of the skin of 5 control subjects. In one of the patients the reticulum was extremely dilated. As a rule, the ribosomes of all the patients were numerous, though dilated cystic reticulum with few or no ribosomes was also observed. The cysts contained thin filaments or dispersed grains. Cystic nuclear envelope with ribosomes was observed in 7, and without ribosomes in 4 of the patients. Often no cell mem-

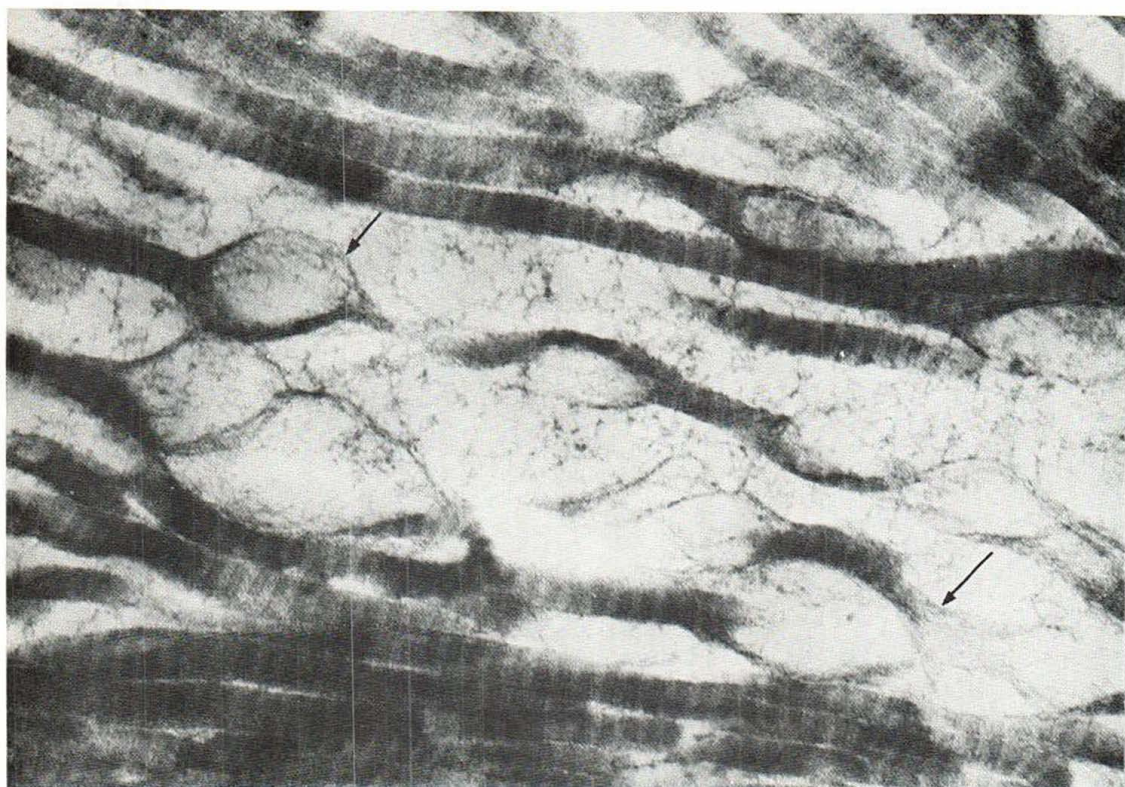


Fig. 4. Pericatricial lesion. Collagen fibrils are split into thin unbanding threads forming oval figures (arrows). $\times 60\,000$.

brane was observed. In one of the patients the cytoplasm appeared partly destroyed. Pinocytosis was often observed. Calcification was frequently noticed in areas with thready material.

The specimens from normal-appearing skin of 4 PXE patients showed fibroblasts, as were observed in the skin of 5 control subjects. In one of the specimens from a control subject, long thin parallel-arranged filaments showing the periodicity of collagen were observed near a fibroblast. Collagen fibrils were seen parallel to and in close proximity to the filaments. Three scars of non-PXE patients showed no filamentous splitting of collagen.

DISCUSSION

The finding of continuity between thin filaments with banding typical of collagen fibrils, and thready masses without periodicity, suggests severe alteration of collagen in PXE. The thin

parallel-arranged filaments with a periodicity like that of collagen observed in normal skin probably represent normal precursors of collagen fibrils (8). When comparing these filaments from normal skin with the above-mentioned from PXE pericatricial lesions, the idea of a defect in the chemical composition of the thready material inhibiting its further development into collagen fibrils seems reasonable. The occurrence of twisted collagen fibrils in PXE (3) may also be related to an abnormal composition of collagen. Biochemical studies of PXE lesions have shown increased lysine and hydroxylysine contents in the newly synthesized collagen (1). Both amino acids are known to be involved in the cross-linking of collagen (6, 10). In all patients studied, the appearance of a well-developed cystic endoplasmic reticulum, with disappearance of ribosomes in the fibroblasts of the lesions, may evidence an abnormal protein synthesis. The additional findings of cystic nuclear envelope with or without ribosomes and of absent

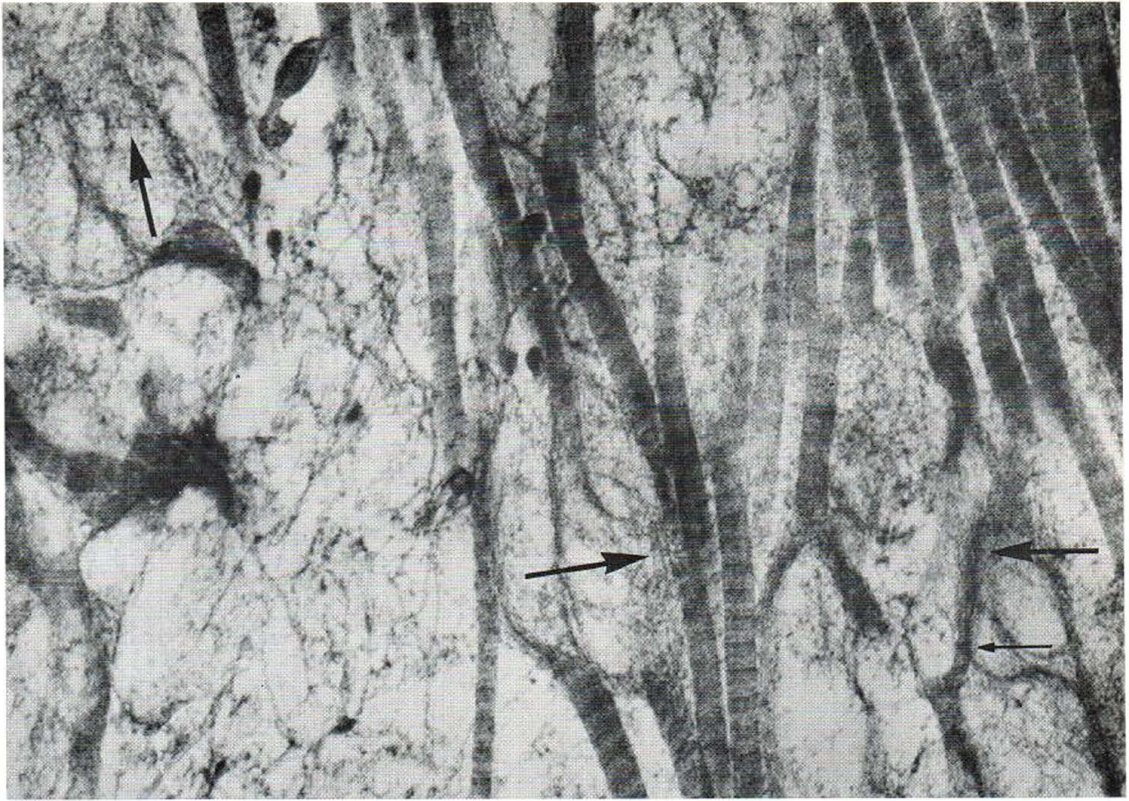


Fig. 5. Pericardial lesion. Collagen fibrils split into banded filaments (*thin arrow*) and unbanded masses of threads (*thick arrows*). $\times 60\,000$.

cell membrane support this idea. The origin of the long thin filaments (Fig. 2) of the pericardial lesions is unknown. The filaments may represent abnormally developed limiting membrane of the cytoplasmic reticulum or abnormal precursors of collagen. Dilated cystic reticulum of the fibroblasts has previously been reported in 3 PXE patients (11). A similar combination of large twisted collagen fibrils, a filamentous material and a dilation of endoplasmic reticulum in the fibroblasts has been observed in one case of hyalinosis (7). The authors postulated that the major portion of the hyalin was produced by abnormal fibroblasts. A thready material around abnormal collagen fibrils with whisk-like ends and twistings in 3 patients with shagreen patches was suggested to represent precursors of abnormal collagen (9). The observation of calcified material in relation to the thready material suggests a calcification of malformed collagen. However,

the possibility cannot be ruled out that pre-existing elastic fibres were covered by the calcified material. Besides the usual ultrastructural changes of PXE, calcification of collagen fibrils was observed in a gastric artery of a PXE patient (5).

It also seems reasonable to suggest that a malformation of elastic fibres precedes their calcification. However, a possible abnormal composition of calcifying fibres was not ultrastructurally evident. Biochemically, newly synthesized elastin from PXE lesions has been found to have an elevated glutamine content (2). The occurrence of degeneration products of elastic tissue in relation to the most extensive calcified areas of elastic tissue most probably represents a secondary reaction to the calcification (5).

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Fig. 6. Pericicatrical lesion. Fibroblast (F) showing dilated endoplasmic reticulum (thin arrow) occupying almost all the cytoplasmic area. No cell membrane is seen, and

the reticulum continues into long thin filaments (thick arrow) bounding granular material. Calcified material (C) partly covers such areas. $\times 12\,000$.

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L. Danielsen, M.D.
Department of Dermatology
Rigshospitalet
Blegdamsvej 9
DK-2100 Copenhagen
Denmark

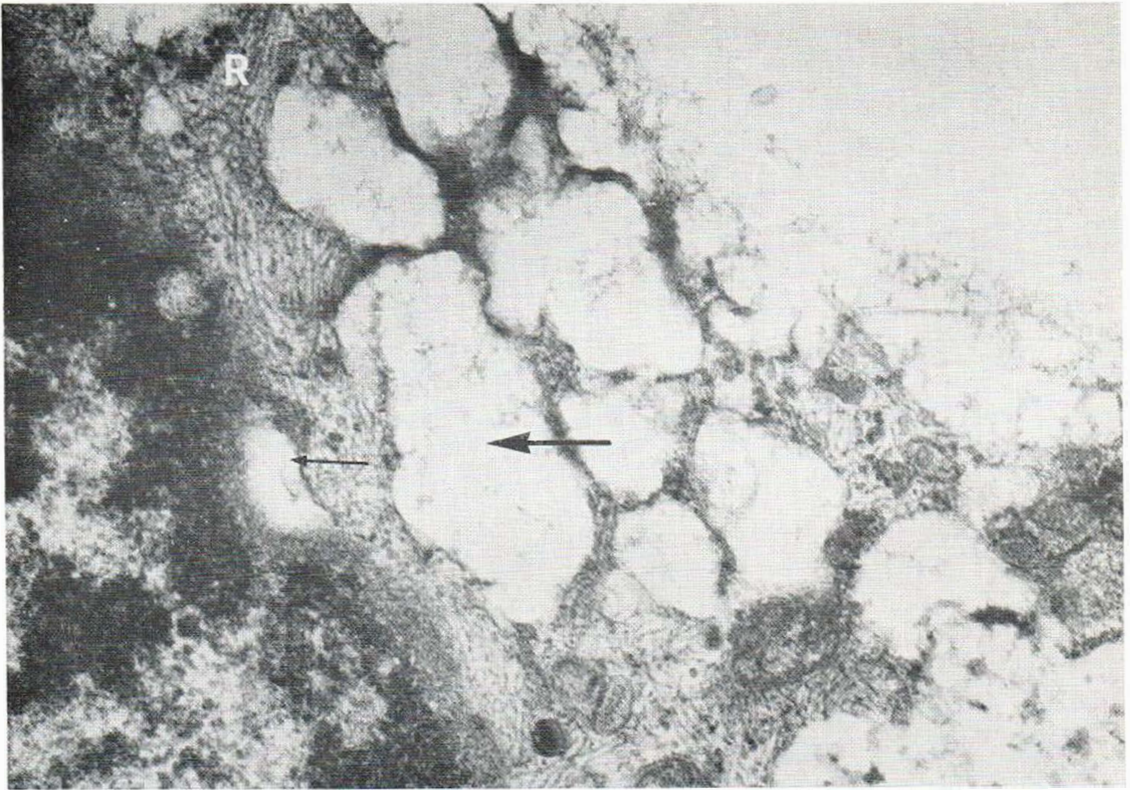


Fig. 7. Pericardial lesion. Fibroblast with cystic endoplasmic reticulum (*thick arrow*) showing few ribosomes (*R*) and a cyst without ribosomes between the laminae

of the nuclear envelope (*thin arrow*). No cellular membrane is seen. $\times 60\,000$.



Fig. 8. Pericatricial lesion. Normal elastic fibre with calcified material (*thick arrow*) surrounded by granular material and long thin filaments (*thin arrow*). $\times 60\,000$.