Ultrastructural Findings in the Skin Lesions of Patients with Anetoderma

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Venencie PY, Winkelmann RK, Moore BA. Ultrastructural findings in the skin lesions of patients with anetoderma. Acta Derm Venereol (Stockh) 1984; 64: 112–120.

Eight biopsy specimens from the skin lesions of five patients with anetoderma were studied for their ultrastructural findings. In all of them, normal elastic fibers were absent and a few very thin, irregular elastic fibers with a more or less complete loss of the amorphous substance and a relative conservation of the microfibrils were observed. The collagen fibers were normal. Inflammation composed of macrophages and lymphocytes, with some plasma cells, was a prominent finding. It is suggested that anetoderma and acquired cutis laxa are part of the same spectrum of elastolytic disease. *Key words: Anetoderma; Elastolytic disease; Elastic fibers; Cutis laxa.* (Received May 30, 1983.)

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Anetoderma is a clinicohistopathologic entity that was first described by Jadassohn (1) in 1892. Our review of 16 patients with respect to clinical findings and their associations (Venencie PY, Winkelmann RK, Moore BA: Arch Dermatol 1983, in press and to histopathology (Venencie PY, Winkelmann RK: Submitted for publication) showed lesions that persisted for many years. Clinically, the disease is characterized by circumscribed areas of loss of substance and elastic tissue with a herniation phenomenon, spontaneously or under palpation. The histologic changes of anetoderma consist of a more or less complete focal loss of normal elastic fibers, together with a perivascular inflammatory infiltrate consisting mainly of lymphocytes but also of plasma cells and histiocytes with some granuloma formation. The histopathologic changes together with the associated disorders suggest that anetoderma could be part of a large spectrum of elastolytic diseases which includes acquired cutis laxa. Ultrastructural studies represent an important additional step, having confirmed the presence of inflammation and the loss of normal elastic tissue in the anetoderma lesions.

MATERIAL AND METHODS

Five of the 16 patients were studied for ultrastructural changes in their skin lesions of anetoderma.

Patient 1 was a 5-year-old boy with a 3-year history of increasingly numerous noninflammatory lesions of anetoderma—some saclike, others depressed below the level of the skin of the face, neck, trunk, and extremities—when he had a biopsy of a lesion of the back. Five years later, he had a second biopsy from a lesion on the left upper chest. At that time he had been taking phenytoin for 2 years at 100 mg daily, without any improvement; an elevated level of antinuclear antibody (ANA) (1:64, speckled) and an elevated concentration of ceruloplasmin (80 mg/dl, normal range 22.9 to 43.1 mg/dl) were thought to be related to the phenytoin. After withdrawal of this drug and during the follow-up, the ANA was negative, and anti-native DNA, blood copper, and alpha$_1$-antitrypsin levels were within normal limits. The chest and bone X-ray films were normal.

Patient 2 was a 35-year-old man with Addison's disease and a 10-year history of increasingly numerous inflammatory saclike lesions of anetoderma affecting mainly the trunk when he had biopsies of two lesions of the low back region. During the follow-up, an isolated elevated anti-native

Fig. 1 (patient 3). Aneto-
derma lesions, including
"saclike" lesions.

DNA at 2.1 μg/ml (normal range 0 to 0.85 μg/ml) was found without any other findings that met the American Rheumatism Association criteria for systemic lupus erythematosus. Blood and urine copper, ceruloplasmin, and alpha$_1$-antitrypsin levels were within normal limits. The chest, esophago-gastroduodenal, and bone radiographs were normal.

Patient 3 was a 21-year-old woman (Fig. 1) with an 11-year history of increasingly numerous noninflammatory lesions of anetoderma—some saclike, others depressed under the normal level of the skin, affecting the face, neck, trunk, and extremities—when she had a biopsy from a lesion of the left arm. Two years later, she had a biopsy from a lesion of the right arm. ANA and anti-native DNA were negative, and urine copper, ceruloplasmin, and alpha$_1$-antitrypsin were within normal limits. Chest, esophagogastroduodenal, and bone X-ray films were normal.

Patient 4 was a 58-year-old man with noninflammatory saclike lesions of anetoderma—they had appeared when he was 10 years old on the neck, trunk, and extremities—together with a congenital bilateral hip dislocation when he had a biopsy from a lesion of the low back. ANA and antinative DNA were negative. Blood and urine copper, ceruloplasmin, and alpha$_1$-antitrypsin were within normal limits. Chest and esophagogastroduodenal radiographs were negative.

Patient 5 was a 14-year-old girl with a 5-year history of increasingly numerous noninflammatory anetoderma lesions on the neck, trunk, and extremities when she had a biopsy of a lesion of the neck. At that time the ANA was slightly elevated (1 : 32, mixed), but it was negative during the follow-up; the anti-native DNA and chest radiographs were normal.

In none of the patients studied was there any evidence of syphilis serologically or tuberculosis radiologically. The biopsies were done with a 5-mm punch under 1 % lidocaine anesthesia. The age of the lesions biopsied was impossible to assess absolutely, but all were well developed clinically and showed light-microscopic absence of normal elastic fibers. Half of the biopsy specimen was placed in formalin for histopathologic studies with hematoxylin and eosin, Alcian blue-periodic acid-Schiff, and aldehyde-fuchsin-Giemsa. Half of the biopsy specimen was used for ultrastructural studies, and 1-mm cubes were immersed in cold 4 % glutaraldehyde buffered with 0.08 M cacodylate and maintained at pH 7.2. After rinsing with cacodylate buffer for 3 hours, the tissue was postfixed with 1 % osmic acid. After dehydration and embedding in Epon, thin sections were stained with lead and uranyl acetate. The early biopsy material (up to 1978) was observed with an RCA EMU 3 electron microscope and subsequent tissue was observed with a Philips 200 electron microscope.

RESULTS

Histopathologic findings. Normal elastic fibers were absent in the superficial and mid dermis in patients 1 (one of two biopsies) 2, 4, and 5. In patients 1 (one of two biopsies) and 3, normal elastic fibers were absent in the mid dermis but were present in the superficial dermis. Abnormal very fine and irregular elastic fibers were found in all the biopsies (Fig. 2). Some of the fine fibers were individual and some were twisted together.
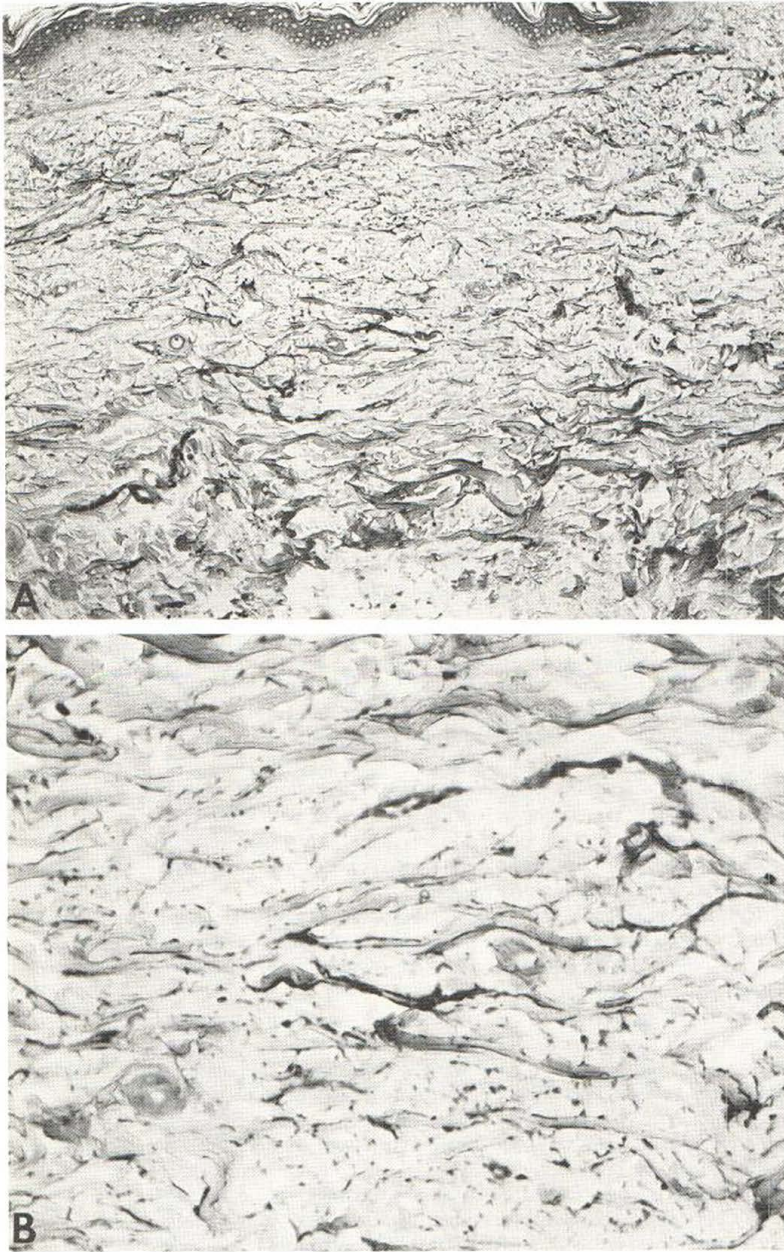
Fig. 2 (patient 1). Biopsy specimen from a lesion of the chest. (*a*) Absence of normal elastic fibers in the superficial and mid dermis. (Aldehyde-fuchsin-Giemsa; ×100.) (*b*) Presence of fine irregular elastic fibers. (Aldehyde-fuchsin Giemsa; ×250.)

An inflammatory infiltrate was found in all the biopsy specimens, even those from clinically noninflammatory lesions, and consisted mainly of lymphocytes but included also some plasmacytes in patients 2 and 5. Mild, focal thickening of the basement membrane zone was found in biopsies from patients 1, 2, and 5.

Ultrastructural findings. In the eight biopsy specimens from five patients, the changes were identical. In none of them were elastic fibers identified in the papillary dermis. In the reticular dermis, a few, generally very thin, fibers were seen (Fig. 3). Some of these thin
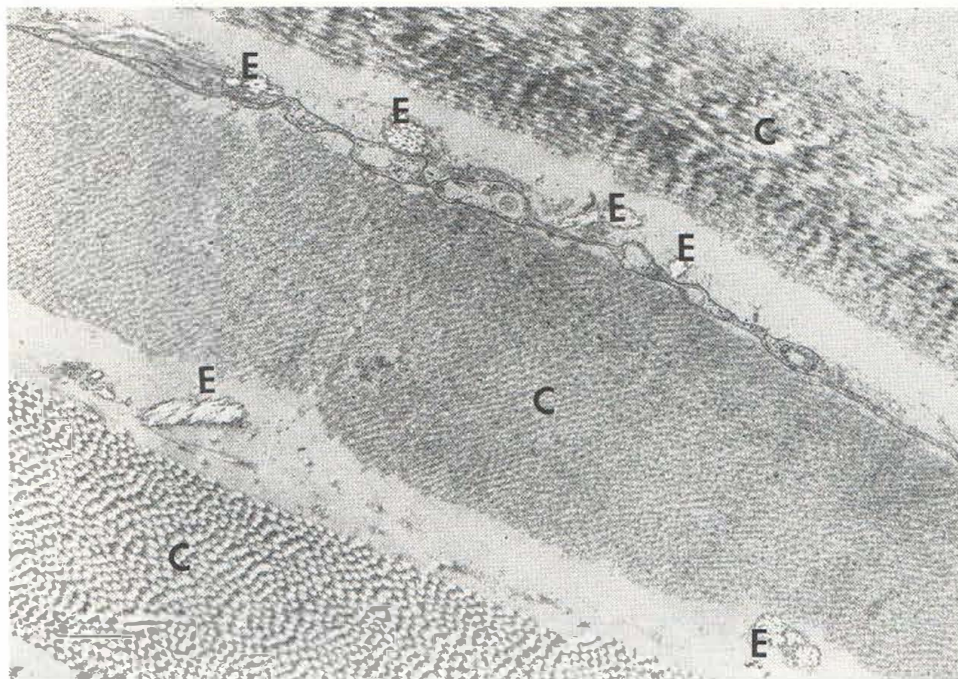
Fig. 3 (patient 1). Biopsy specimen from a lesion of the chest. Normal-sized elastic fibers are absent. Only very thin elastic fibers are found in the reticular dermis. *E*, very fine elastic fiber; *C*, collagen. (×12 500.) Scale line on this and subsequent illustrations represents 1.0 μm.
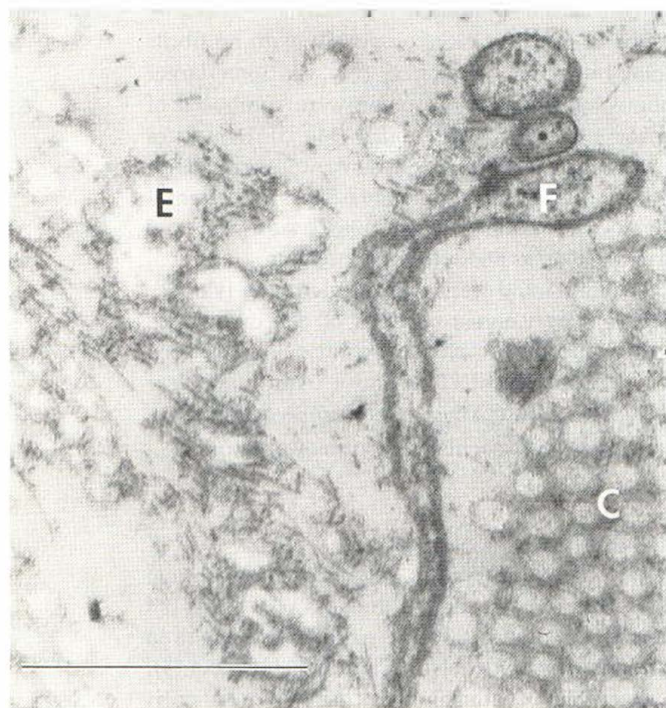

Fig. 4 (patient 1). Biopsy specimen from a lesion of the chest. Elastic fiber is seen with central amorphous substance of low density and elastin microfibrils at the periphery. *E*, very fine elastic fiber; *C*, collagen; *F*, fibroblast. (×45 600.)
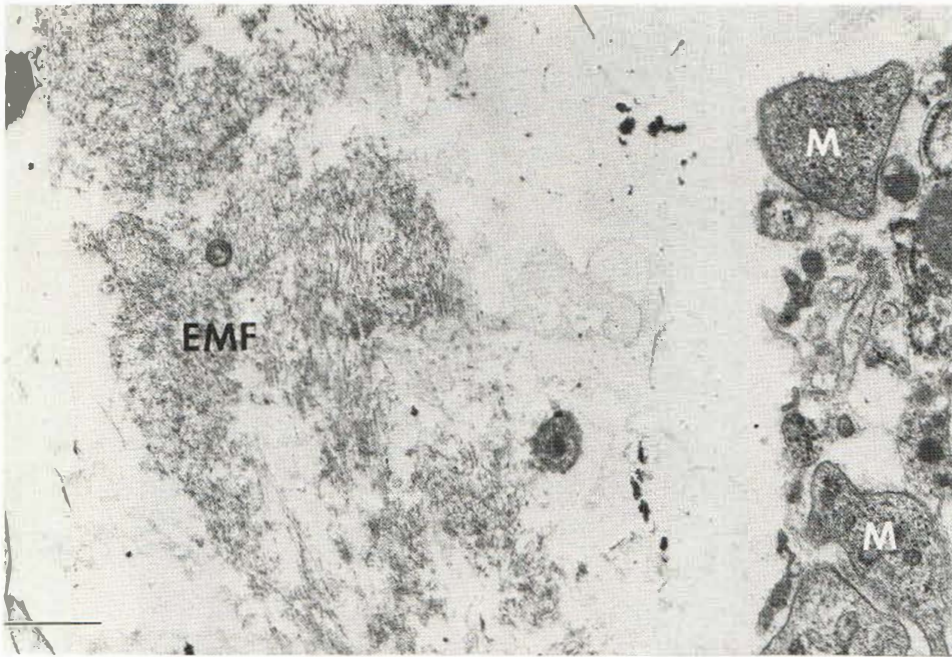
Fig. 5 (patient 2). Biopsy specimen from a lesion of the back. An irregular elastic fiber is seen composed of masses of elastin microfibrils, with lack of amorphous substance. To the right are membrane-bound portions of cell cytoplasm of macrophages. *EMF*, elastic microfibrils; *M*, macrophage. (×16 700.)

fibers showed a central amorphous substance of very low electron density with no fibrils within it but with elastic microfibrils fanning out at their periphery (Fig. 4). Some larger fiber masses with areas in which amorphous substance was lacking were composed of numerous bundles of parallel microfibrils, some of which were tubular (Fig. 5). In most of the small fibers, central dense bundles were absent or, when present, were irregularly distributed. Occasional vacuolar changes were observed within some fibers in patient 4 (Fig. 6). The collagen fibers were normal in all cases. Numerous fibroblasts were seen, and many contained vacuoles in addition to well-developed endoplasmic reticulum.

Macrophages were seen in patients 1, 2, 3, and 5 and were intermingled with lymphocytes (Fig. 7). The tissue was often permeated by isolated portions of cell cytoplasm, presumably parts of disrupted macrophages. Some well-developed plasma cells were seen in patients 1, 2, and 5. Mastocytes were found in patient 2, in whose tissue they were degranulating, and also in patient 5. Focal thickening of the basement membrane zone was clearly seen only in patient 1 (Fig. 8). The nerves and vascular structures were normal.

DISCUSSION

Our ultrastructural findings confirm light microscopic reports of the absence of normal elastic fibers in lesions of anetoderma. The small number and the thinness of most of the fibers were previously reported by Hashimoto & Kanzaki (2) in a patient with acquired cutis laxa. The same findings have been described in patients with familial cutis laxa (3), but Goltz et al. (4) reported elastic fibers larger than usual in another case of familial cutis
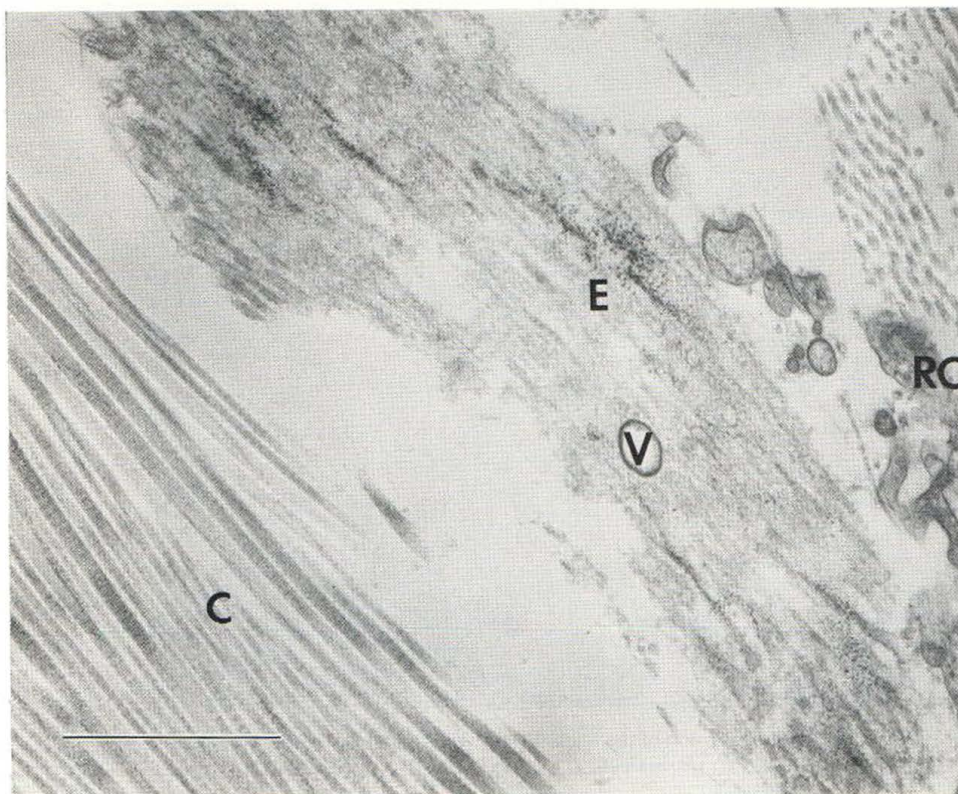
Fig. 6 (patient 4). Biopsy specimen from a lesion of the back. An elastic fiber is seen with irregular borders and focal condensation of amorphous material. Vacuoles (*V*) below and with the elastic fiber may be portions of cell cytoplasm. *E*, elastic fiber; *C*, collagen; *RC*, reticulum cell. (×30 700.)

laxa. Theoretically, the abnormal fibers could represent newly synthesized fibers or partially remaining fibers after elastolysis.

Thin fibers with low electron density of their amorphous substance, tubular microfibrils, and microfibril bundles (Fig. 4 and 5) could represent the result of a new synthesis, for they recall the elastic fibers of the ligamentum nuchae of fetal calves reported by Greenlee et al. (5) and Ross & Bornstein (6). The lack of close relation between fine fibers or microfibrils and the fibroblasts could reflect the older age of the well-developed lesions in our series. By this hypothesis, such findings seen in old lesions would mean that the "resynthesis" is abnormal or incomplete. Such an abnormal or incomplete synthesis could be related, as in some patients with inherited cutis laxa (7), to a low activity of lysyl oxidase, a copper-dependent enzyme that plays an important role (8) in the synthesis of elastin. This enzymatic activity has never been studied in patients with acquired cutis laxa or anetoderma; however, in the patients studied in this series, the copper metabolism was normal.

On the other hand, the bundles of parallel microfibrils (Fig. 5), which recall the "oxytalan" fibers described in the papillary dermis by Cotta-Pereira et al. (9) and Tsuji (10), could also represent the remainder of the elastic fiber after its partial destruction by elastases. The fact that the destruction process seems to affect mainly the amorphous substance favours the hypothesis of elastolysis, for it has been demonstrated in vitro that
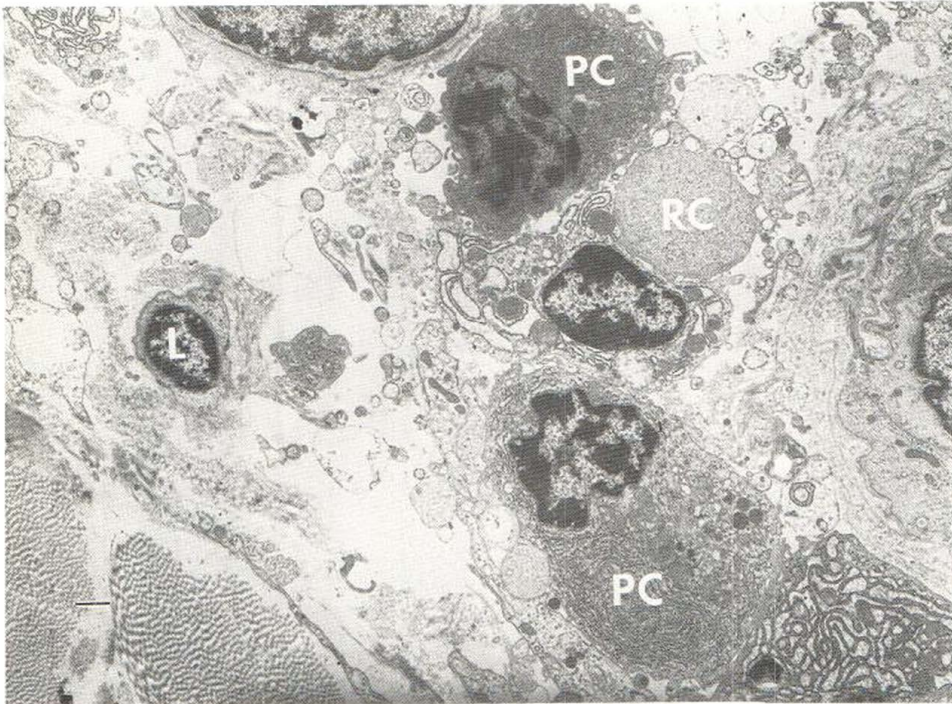
Fig. 7 (patient 5). Biopsy specimen of a lesion of the back. Plasma cells and macrophages are seen in perivascular location in anetoderma. *PC*, plasma cell; *L*, lymphocyte; *RC*, reticulum cell. (×4800.)
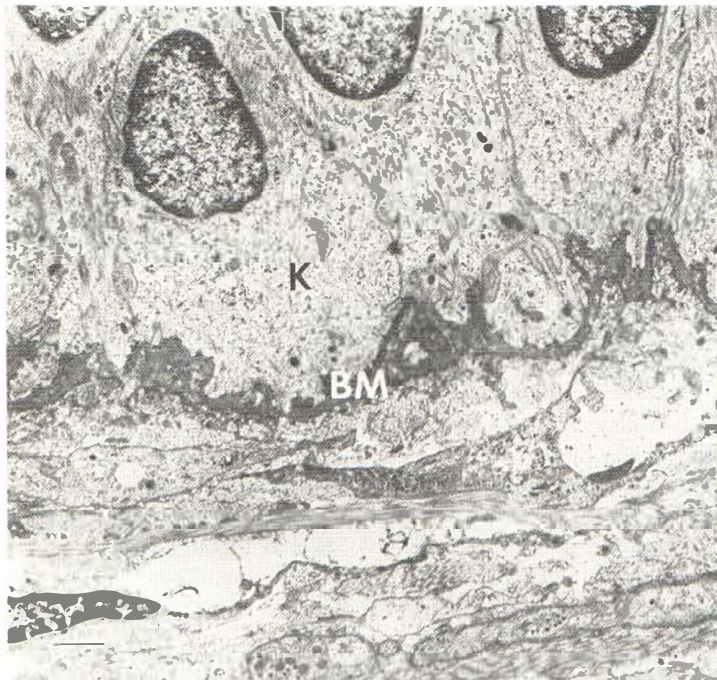

Fig. 8 (patient 1). Biopsy specimen from a lesion of the back. Focal thickening of the basal membrane zone below a normal epidermis is seen in a lesion of clinically noninflammatory anetoderma. *BM*, basement membrane; *K*, keratinocyte. (×8100.)

elastases preferentially destroy the amorphous substance (6, 11), whereas other processes, such as autoclaving (12), affect mainly the microfibrils. An imbalance between elastase and antielastase activities could lead to such a phenomenon (13). The alpha$_1$-antitrypsin level was normal in the four patients in whom it was studied in this series. The elastase blood activities reported previously (14, 15) in patients with anetoderma are conflicting, and such blood determinations could be a poor reflection of the situation in the mid dermis. The presence of lymphocytic and macrophage inflammation was constant in this series (Fig. 7). The macrophages were synthetic, and many vacuoles, dense bodies, and lysosomal structures were seen. The portions of cell cytoplasm imply cell disruption and the possibility of enzyme release. Thus, elastolysis could be related to elastases from macrophages, for elastase activity has been demonstrated in activated macrophages (16–18). It is not possible to determine whether the macrophages play a primary rather than a secondary role as suggested by the presence of fragments of elastic fibers within them under the light microscope. Similarly, the presence under the light microscope (Venencie PY, Winkelmann RK: Unpublished data) of polynuclear neutrophils within the mid dermis in a very early biopsy of a new lesion in one patient could be of pathogenetic significance, for elastases have been demonstrated in these cells (19, 20). Further biopsies of new lesions are necessary to confirm this hypothesis. It is not known whether the vacuolar changes seen in some elastic fibers in patient 4 (Fig. 6) represent a special kind of degeneration. An artifact cannot be ruled out. Collagen fibers were normal in our cases, in another case of anetoderma (21), and in acquired cutis laxa (2), whereas in one case of anetoderma, Korting et al. (22) reported collagen fibers that were thinner than usual.

Plasma cells are not uncommon in the lesions of anetoderma, for they were found in 6 of 15 patients reviewed for their histopathology (Venencie PY, Winkelmann RK: Unpublished data). We believe that the plasma cells observed on light and electron microscopy represent a unique facet of the histologic and immunologic responses of anetoderma.

The relationship between the mast cells and the anetoderma lesions was suggested previously by the occurrence of anetoderma-type lesions in the sites of previous mastocytosis lesions (23–25). However, mast cells were in normal numbers in the 38 biopsy specimens of 15 patients reviewed for their histopathology. Only one patient showed mast cell disruption.

The focal thickening of the dermoepidermal junction in patient 1 (Fig. 8) is not explained, particularly in a patient with no evidence of lupus erythematosus (LE), whereas anetoderma lesions in our series occurred in one patient with systemic LE without the cutaneous lesions of LE and in patients presenting with lesions of discoid LE elsewhere.

Thus, further biopsies for electron microscopic study must be obtained from very early skin lesions for a better understanding of the initial event. The present findings suggest that anetoderma results from an elastolytic phenomenon and, together with the associations reported in patients with anetoderma, suggest that anetoderma and acquired cutis laxa are parts of the same spectrum. However, in this regard, biopsy specimens from organs other than the skin must be studied in patients with anetoderma for a better assessment of the extension of the disease.

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