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SECONDARY LOCALIZED CUTANEOUS AMYLOIDOSIS ASSOCIATED WITH ACTINIC KERATOSIS*

KEN HASHIMOTO, M.D., AND LLOYD E. KING, JR., M.D., PH.D.

ABSTRACT

Histochemically identifiable amyloid associated with actinic keratosis was studied with the electron microscope. Typical amyloid filaments forming islands identical to collagen strands were found in the upper dermis. Beyond the lesion of actinic keratosis, no amyloid was found. In the healing wound of a previously biopsied lesion, amyloid was regenerated only when the epidermis covered the wound. In such epidermis-covered granulation tissue, electron-dense amorphous substance was initially formed in the upper dermis and amyloid filaments subsequently emerged within it. In both original and regenerated lesions, perivascular spaces were free from amyloid, and actinic elastosis was absent. It was concluded that secondary amyloidosis associated with actinic keratosis is produced by local fibroblastic cells under an abnormal influence of the epidermis.

Amyloid deposition associated with various tumors, particularly skin tumors of epithelial origin, has been well documented by light microscopic histochemical methods [1–7]. The specific ultrastructural criteria for amyloid fibrils have only recently been applied to tumor-associated amyloid in calcifying epithelioma of Malherbe or pilomatrixoma [8], and basal cell epithelioma [9]. In otherwise healthy patients, the finding of amyloid only in the immediate vicinity of localized tumor growth [9] suggested that "epithelioma-associated" amyloid may reflect specific, localized tumor-induced alterations.

We now report studies on localized amyloid associated with multiple actinic keratoses. The presence of large amounts of amyloid in the skin of a cooperative, otherwise healthy patient allowed us to test the hypotheses [10] that (i) cutaneous amyloid is produced in response to local influences, and (ii) epithelial influences are required for cutaneous amyloid production. The recent evidence for these hypotheses rests on the demonstration, in basal cell epitheliomas [9], that (a) the amyloid deposition was strictly limited to the areas of the epidermal hyperplasia; and, (b) as shown in the lichenoid and macular amyloidoses [10], amyloid regeneration in the healing biopsy wounds occurred only when the abnormal epidermis regenerated and covered the wounds. In the present study, crystallization of amyloid filaments within and surrounding the dense amorphous substances initially deposited in the biopsy wounds was demonstrated.

MATERIALS AND METHODS

Case History. The patient was a 73-year-old white male admitted to the Veterans Administration Hospital after an initial skin biopsy (from the dorsum of the left hand) revealed amyloid deposits. The patient had had no chronic disease. Specifically, the patient denied "pinch purpura," papulonodular or pruritic skin lesions. Review of systems, family history, and social history were unremarkable. Physical examination, including tongue, heart, lung, abdomen, and lymph nodes, was within normal limits except for multiple actinic and seborrheic keratoses on the face, neck, around the ears, and on the dorsum of the upper extremities.

Hemogram including platelet count was normal. Proctosigmoidoscopy and rectal biopsy were performed and no histologic evidence of amyloid deposits was found. Serum immunoelectrophoresis (IgM, IgG, and IgA) showed no abnormalities. Urinalysis showed no abnormalities with a negative Bence-Jones protein test on two occasions. Twenty-four-hour urine samples and serum tests for creatinine, protein, calcium, and phosphorus were normal. The following tests were normal or negative: Serum electrolytes, glucose, LDH, SGOT, alkaline phosphatase, acid phosphatase, BSP, cholesterol, total bilirubin, amylase, carotene, VDRL, rheumatoid factor, fluorescent antinuclear antibodies, C-reactive protein, uric acid, and protein electrophoresis. Bone marrow examination was interpreted as normal, with stainable iron present, slight lymphocytosis, and no amyloid deposits seen with Congo red stain. EKG was normal, with occasional sinus arrhythmia. The following x-rays were interpreted as normal except for generalized osteoporosis: chest, esophagus, upper G.I., barium enema, intravenous pyelogram, and metastatic survey.

Biopsy materials. Specimens were taken with a 6-mm punch from four typical actinic keratoses on the dorsum of his left hand, clinically normal skin adjacent (0.5–1 cm) to these lesions, normal skin of the right upper eyelid, and normal skin of the abdomen. In three lesions on the dorsum of the left hand, amyloid was found, using histochemical and ultrastructural methods. Two of the amyloid-positive lesions were rebiopsied (4-mm punch) after one month, and one after seven months. All lesions were photographed before biopsy, and care was taken to obtain the tissue from the center of the healing wounds and to exclude the nonbiopsied areas at the periphery. The speed of wound healing varied from one lesion to another; thus, one biopsied lesion was already covered with epidermis at the time of the second biopsy after one month, whereas the other was not. All three amyloid-

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* From the Memphis Veterans Administration Hospital and Division of Dermatology, Department of Medicine, The University of Tennessee College of Medicine, Memphis, Tennessee 38103.

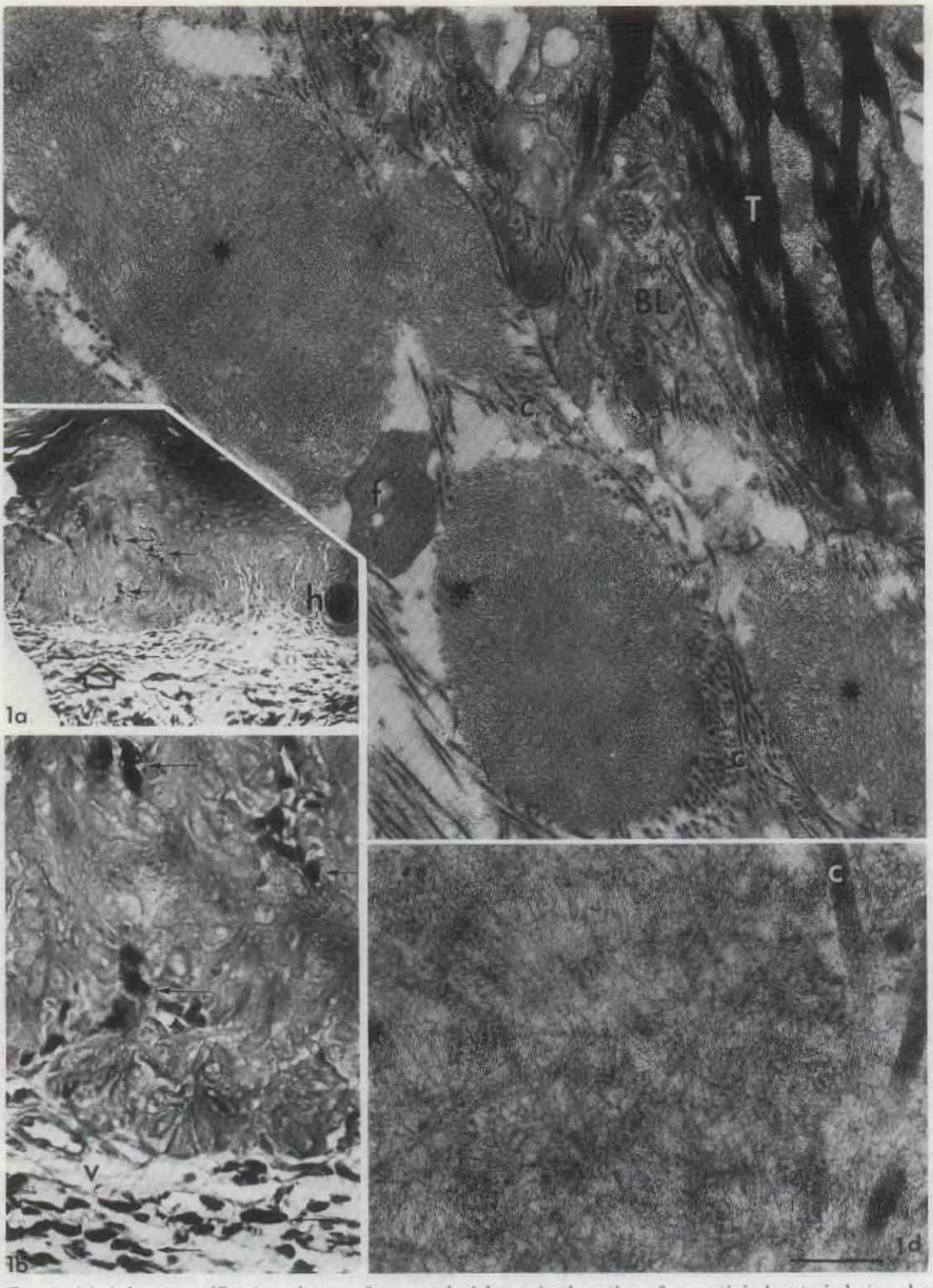

FIG. 1. (*a*) A low magnification picture of a crystal violet-stained section of an actinic keratosis lesion shows hyperkeratosis (k), horn pearl (h), and metachromatic amyloid deposits (thin arrows), either trapped between hyperplastic rete ridges or intermingled with collagen in the upper dermis. Hollow arrow indicates the area to be magnified in Fig. 1b. Actinic keratosis, left hand. × 100.

(*b*) Higher magnification of the area marked by a hollow arrow in Fig. 1a. Thin arrows point to metachromatic amyloid deposits. Notice that perivascular spaces (v) are free from amyloid deposition. Actinic keratosis, left hand. × 250.

(c) In actinic keratosis, amyloid (*) was deposited in an island-like pattern. Each amyloid island is separated by normal collagen bundles (c). BL: basal lamina; f: processes of dermal fibroblasts; T: tonofibrils of an epidermal basal cell. First biopsy from left hand lesion. × 13,000.

(d) Enlargement of an amyloid island (*) with normal collagen (c) at its periphery. First biopsy from the lesion of the left hand. The bar indicates 0.5 μ. × 75,000.

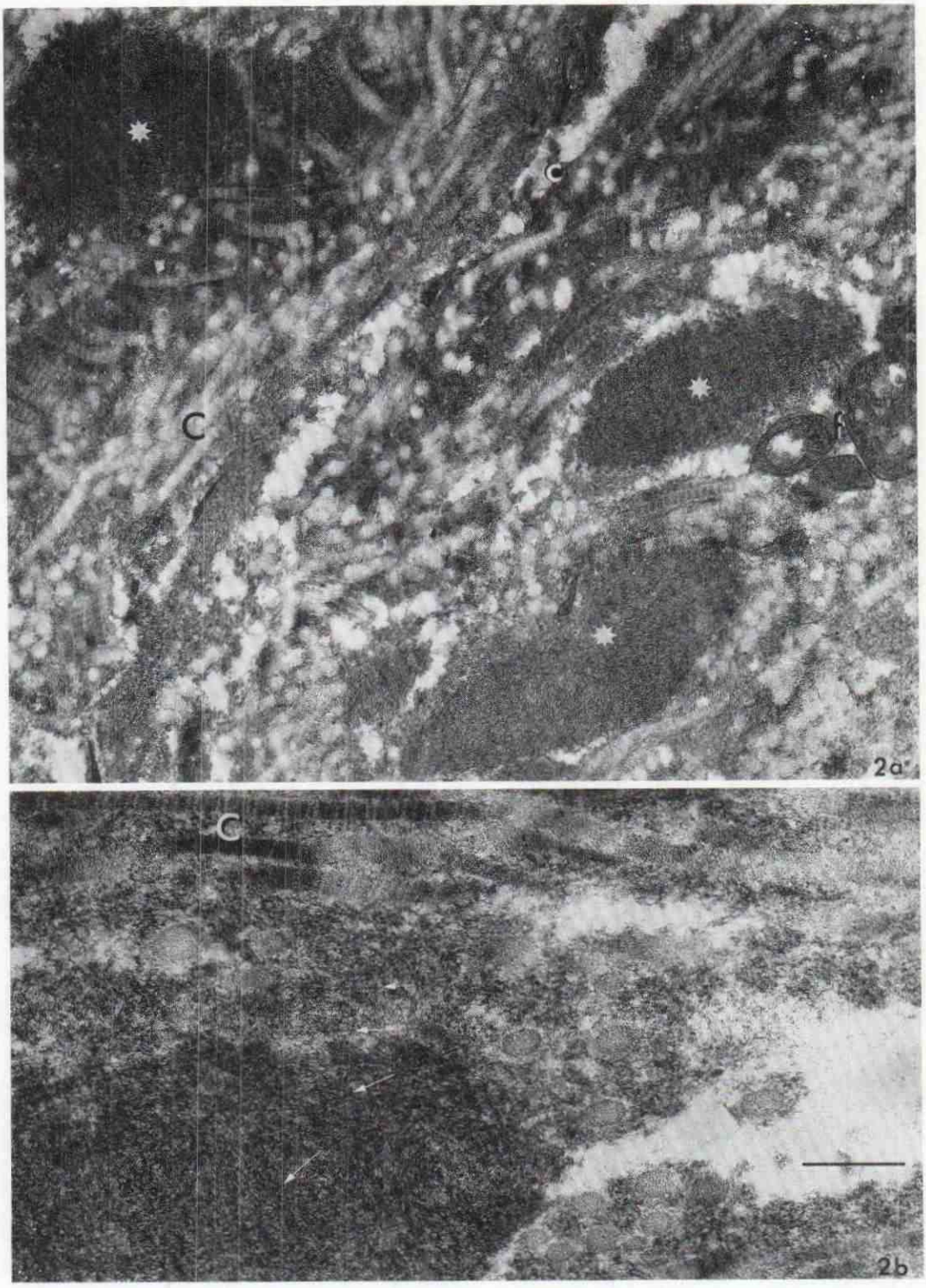

FIG. 2. (*a*) Actinic keratosis rebiopsied one month after the initial biopsy shows small aggregations of dense substance (*) accumulating between irregularly arranged collagen fibrils (C). f: parts of processes of fibroblast. × 25,000.

(*b*) An enlargement of a similar area. Although there are a few fine filaments (arrows), the major portion of this accumulation is composed of dense, amorphous substance. C: collagen. A bar indicates 0.5 μ. × 75,000.

positive lesions were treated with 5% 5-fluorouracil cream (Efudex), with subsequent disappearance of the hyperkeratosis. These three treated lesions, when rebiopsied after six months, still contained amyloid.

As control and comparative studies, typical amyloid lesions found in lichenoid and macular amyloidoses were used. The case histories, methodology, and results of these control studies have been previously reported [10–12].

Specimen preparations for light and electron microscopy. All specimens were taken under local anesthesia with 4- or 6-mm punches and immediately divided into two parts. One part was either fixed in 10% formalin in preparation for paraffin sections or frozen-sectioned at 2–4 μ. The other part was processed routinely [9, 10, 13] for electron microscopic studies.

Paraffin and frozen sections were stained with alkaline Congo red [14] and crystal violet. Hematoxylin and eosin-stained sections were used to define the extent of the actinic keratosis in relation to the areas of amyloid deposition.

RESULTS

Light microscopy. Three out of four actinic keratoses were amyloid-positive. The fourth lesion was biopsied too superficially and omitted from the subsequent rebiopsy studies. All specimens taken from the vicinity of these three hand lesions, and normal skin from the eyelid and rectal mucosa, were amyloid-negative. Amyloid deposits were localized in the dermal papillae and upper dermis (Fig. 1a). When the hyperplastic epidermis elongated deeply into the dermis, deposition of amyloid also accompanied such elongation (Fig. 1b). Amyloid deposits were often found between such elongated epithelial cords and, therefore, appeared as if they were within the hyperplastic epidermal (tumor) cells (Fig. 1b). These amyloid deposits were not diffuse, but were limited to island-like areas (Fig. 1b). Perivascular spaces were usually free from amyloid deposition (Fig. 1b).

Interestingly, the amyloid-positive lesions did not contain elastotic (basophilic) material characteristic of actinically damaged senile skin. In contrast, amyloid-negative control specimens taken from the adjacent normal skin showed a marked increase of elastotic (basophilic) degeneration of the papillary and upper reticular dermis.

In the rebiopsied specimens, amyloid was not detected unless the biopsy sites had reepithelialized. In newly epithelialized lesions, small amyloid deposits were detected mainly along the epidermodermal junction and in the upper dermis. Elastotic material was not found in the healing wounds.

Electron microscopy of the initial biopsies. Amyloid depositions were seen either as variously sized islands in the papillary and upper reticular dermis or often invaginated or entrapped between proliferated epithelial cords (Fig. 1c). The basal lamina of the basal cell was often obscured by the infiltrating amyloid. Normal collagen fibrils were mainly present along the periphery of each amyloid island and seldom found in the island's center (Fig. 1c). The size and shape of these amyloid islands were very similar to those of dermal collagen strands. Fibroblasts with well-developed, rough, endoplasmic reticulum were often seen encircling individual amyloid islands and, when the main bodies of such fibroblasts were not cut, cytoplasmic processes were seen surrounding many amyloid islands. The pattern of actinic keratosis amyloid was, therefore, identical to the typical island-forming pattern of amyloid deposition seen in primary localized cutaneous amyloidosis such as the lichenoid or macular varieties [10–12]. At high magnification, individual amyloid islands were seen to be composed of fine filaments, 60–100 Å in width, which were straight, nonanastomosing and nonbranching (Fig. 1d). In some islands these filaments were embedded in a variable amount of dense, amorphous substances. The dimensions and ultrastructural characteristics of the actinic keratosis-associated amyloid was identical to the typical amyloid filament found in either lichenoid or macular amyloidosis [10–12]. The blood vessel walls were free of amyloid infiltration. Although amyloid islands could be found close to the vascular wall, a layer of normal collagen usually distinctly separated the islands from the basal lamina.

Control specimens taken from the area adjacent to each lesion and from the right hand, eyelid, and abdomen were all negative for amyloid. The rectal biopsy was not examined with the electron microscope. Control specimens taken from sun-exposed areas contained a large amount of elastotic substance.

Electron microscopy of the rebiopsies. Regeneration of amyloid was observed only in epithelialized lesions. The initial stage of amyloid formation in the upper dermis appeared to be numerous small aggregations of amorphous or fine, filamentous material (Figs. 2a, 2b). These aggregates were found between or admixed with irregularly arranged collagen fibrils (Figs. 2a, 2b). In larger (or more mature) aggregations, typical amyloid filaments were observed in the peripheral portion, while the center of the island was occupied with dense amorphous material (Fig. 3). This pattern of dense amorphous core and peripheral ring of amyloid filaments was noted in various stages of amyloid aggregation. In many instances, active fibroblast-like cells with dilated rough-surfaced endoplasmic reticulum were intimately associated with newly developing amyloid deposits (Fig. 3). Some of these cells also contained dense bodies and the possibility that they were phagocytes could not be excluded. No plasma cells were encountered, nor was true perivascular amyloid deposition seen (Fig. 3).

The epidermodermal junction area showed a similar deposition of dense substances often admixed with newly synthesized collagen. When the aggregates filled the dermal papillae, the basal lamina below basal cells was often obscured. Interestingly, the basal lamina covering the basal surface of melanocytes was intact. The fibroblast-like

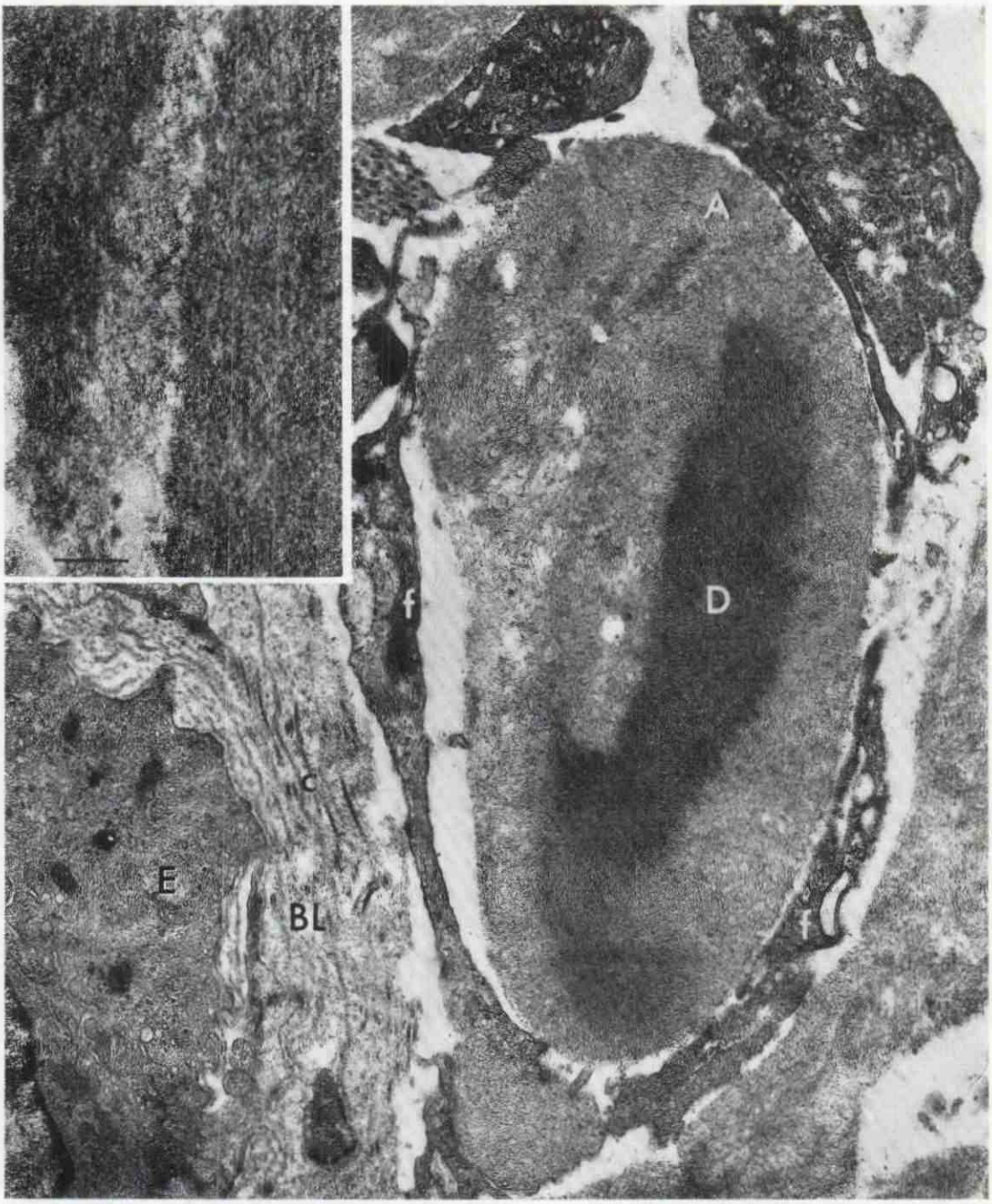

FIG. 3. More mature island than those shown in Fig. 2 has central dense core (D), mainly composed of dense, amorphous substance (insert), and peripheral accumulation of amyloid filaments (A). The entire island is surrounded by processes of fibroblasts (f). Vascular endothelial cells (E) are separated from this island by multi-layers of basal lamina (BL) and some collagen fibrils (C). The bar indicates 0.5 μ. Lesion rebiopsied one month after the initial biopsy. × 12,500. Insert: × 75,000.

cells were frequently apposed or in contact with the basal cells and in these areas the basal lamina was often absent.

DISCUSSION

In the present study, histochemically identifiable amyloid was found in the vicinity of hyperplastic epidermoid lesions. This "histochemical amyloid" fulfilled all ultrastructural criteria proposed for the identification of cutaneous [10–12] as well as systemic [15] amyloid. Histochemically detectable amyloid has been reported in various skin tumors of epithelial origin such as basal cell epithelioma [1–4, 9], seborrheic keratosis [2], Bow-

en's disease [16], dermal cylindroma [17, 18], and calcifying epithelioma of Malherbe (pilomatrixoma) [8–10]. In three cases of basal cell epitheliomas, this "histochemical" amyloid was shown to contain typical amyloid filaments [9]. In these tumors the amyloid deposits were limited to the immediate vicinity of the parenchyma [9]. In no case was there any associated systemic disease. So far, no convincing documentation has been found that amyloid deposition is associated with purely mesodermally derived tumors. "Amyloid" has been reported in mycosis fungoides [19, 20], but the substance was regarded as collagen rather than true amyloid [16]. In the present study, the essential role played by the neoplastic epithelial components and the close spatial relationship between fibroblasts and epidermal cells in the regenerating amyloid lesion was shown. These findings suggest that interaction between fibroblasts and abnormal epithelial components is essential to produce tumor-associated amyloid. Previously, it was demonstrated in the primary lichenoid and macular amyloidoses of the skin that epidermal regeneration was required for the reproduction of amyloid in the biopsied lesions [10]. Our previous studies on primary cutaneous amyloidosis, as well as the present study, emphasize the lack of either plasma cells or perivascular amyloid deposition. On the other hand, an analogy between the mechanisms of dermal amyloid and collagen production was suggested by (1) the morphologic similarity and association of amyloid islands to collagen strands, and (2) the intimate spatial association of fibroblast-like cells with amyloid islands. If this is true, then it may be postulated that a stroma-modifying agent or agents of epithelial tumor cells influence collagen biosynthesis by dermal fibroblasts.

The true nature of amyloid has been controversial and recently more so since Glenner and his group [21] revived an old concept that amyloid was related to immunoglobulins. They presented data that the amino acid sequence of an amyloid fibril protein derived from systemic [21] as well as localized [22] amyloidoses was very similar to that of the variable portion of several species of Bence-Jones protein. In epidemiologic studies of age-related amyloid accumulation, amyloid deposition in the myeloma patients was shown not to be significantly different from the control [23]. Franklin et al [24] showed that purified amyloid fibrils from five cases with secondary amyloidosis have a homogeneous protein whose partial amino acid sequence is unlike that of any known immunoglobulin. Other studies also strongly support the non-immunoglobulin nature of some amyloid fibrils [25–27].

The preliminary reports [28, 29] that, in secondary amyloidoses and in experimental amyloidoses, cellular and humoral immunity may be affected, are interesting in considering the pathogenesis of tumor-associated amyloid. As we have previously emphasized [9–11], in primary and secondary cutaneous amyloidoses of the skin the afflicted individuals are otherwise entirely healthy. It has been our belief [30] that the purely cutaneous form of amyloid is a product of a local abnormality and most likely produced by fibroblasts under the influence of epidermis (lichenoid and macular amyloidoses) or epithelial tumors. The findings obtained in the present investigation support this view. The concept that amyloid, in all forms of systemic and localized amyloidosis, is a local product of cells belonging to the reticuloendothelial system was summarized by Cohen in 1967 [31].

REFERENCES

1. Sumegi I, Goreczky L, Roth I: Recent studies on the amyloid in malignant tumors. Acta Morphol Acad Sci Hung 4:463–474, 1954
2. Malak JA, Smith EW: Secondary localized cutaneous amyloidosis. Arch Dermatol 86:465–477, 1962
3. Ganter P, Beurlet J: Le stroma amyloide des cancers cutanes baso-cellulaires. Ann Anat Pathol (Paris) 10:233–248, 1965
4. Sumegi I, Rajka G: Amyloid-like substance surrounding mammary cancer and basal cell carcinoma. Acta Pathol Microbiol Scand [A] 80:185–192, 1972
5. Peterson, WC Jr, Hult, AM: Calcifying epithelioma of Malherbe. Arch Dermatol 90:404–410, 1964
6. Peterson, WC Jr: Thioflavine-T reactivity in calcifying epithelioma of Malherbe. Arch Dermatol 97:340–341, 1968
7. Meenaghan MA, Appel BN, Greene GW Jr: Amyloid-containing odontogenic tumors of man. Oral Surg 34:908–919, 1972
8. Hashimoto K, Lever WF: Appendage tumors of the skin: review of histochemistry and ultrastructure. Derm Digest, June, 1968, pp 58–75
9. Hashimoto K, Brownstein M: Localized amyloidosis in basal cell epitheliomas. Acta Derm Venereol (Stockh) (in press)
10. Hashimoto K, Brownstein M: Amyloidogenesis in healing wound. Electron microscopic studies of biopsied wounds in macular amyloidosis. Am J Pathol 68:371–380, 1972
11. Hashimoto K, Onn LLY: Lichen amyloidosus. Electron microscopic study of a typical case and a review. Arch Dermatol 104:648–667, 1971
12. Brownstein M, Hashimoto K: Macular amyloidosis. Arch Dermatol 106:50–57, 1972
13. Reynolds ES: The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J Cell Biol 17:208–212, 1963
14. Cooper JH: An evaluation of current methods for the diagnostic histochemistry of amyloid. J Clin Pathol 22:410–413, 1969
15. Shirahama T, Cohen AS: High resolution electron microscopic analysis of the amyloid fibril. J Cell Biol 33:679–708, 1967
16. Brownstein M, Helwig EB: The cutaneous amyloidoses. I. Localized forms. Arch Dermatol 102:8–19, 1970
17. Freudenthal W: Amyloid in der Haut. Arch Derm Syph 162:40, 1930
18. Heyl T: Amyloid staining with thioflavine T in dermatopathology. Trans St John Hosp Dermatol Soc 52:84–87, 1966
19. Holzmann H, Schott HJ: Amyloid-Nachweis in der Haut bei Mycosis fungoides. Klin Wochenschr 43:1061–1062, 1965
20. Schott JH, Holzmann H: Polarisationsoptischer Nachweis von Amyloid-Ablagerung bei Mycosis fungoides. Arch Klin Exp Dermatol 222:632–641, 1965

21. Glenner GG, Terry W, Harada M, Isersky C, Page D: Amyloid fibril proteins: proof of homology with immunoglobulin light chains by sequence analysis. Science 172:1150–1151, 1971
22. Page DL, Isersky C, Harada M, Glenner GG: Immunoglobulin origin of localized nodular pulmonary amyloidosis. Res Exp Med 159:75–86, 1972
23. Limas C, Wright JR, Matsuzakim M, Calkins E: Amyloidosis and multiple myeloma. Am J Med 54:166–173, 1973
24. Franklin EC, Pras M, Levin M, Frangione B: The partial amino acid sequence of the major low molecular weight component of two human amyloid fibrils. FEBS Letters 22:121, 1972
25. Pearse AG, Ewen SW, Polak JM: The genesis of apudamyloid in endocrine polypeptide tumors: histochemical distinction from immunamyloid. Virchows Arch [Zellpathol] 10:93–107, 1972
26. Husby G, Sletten K, Michaelsen TE, Natvis JB: Alternative, non-immunoglobulin origin of amyloid fibrils. Nature [New Biol] 238:187, 1972
27. Pras M, Reshef T: The acid-soluble fraction of amyloid-a fibril forming protein. Biochim Biophys Acta 271:192–203, 1972
28. Cathcart ES, Scheinberg MA, Benson MD, Cohen AS: B-cell and T-cell dysfunction in human amyloid disease. Clin Res 21:549, 1973
29. Scheinberg MA, Cathcart ES: Cellular events in experimental amyloidosis. Clin Res 21:587, 1973
30. Hashimoto K, Gross BG, Lever WF: Lichen amyloidosus: histochemical and electron microscopic studies. J Invest Dermatol 45:204–219, 1965
31. Cohen AS: Amyloidosis. N Engl J Med 277:522–530, 1967