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SULPHHYDRYL AND DISULPHIDE STAININGS OF AMYLOID: A COMPARISON WITH HYALINE BODY

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Abstract. Histochemical staining for sulphhydryl (-SH) groups and disulphide (S–S) bonds was undertaken in 10 cases of cutaneous amyloidosis using the new flurorescent thiol reagent. A weak S–S fluorescence (but not –SH) was detected in dermal amyloid, suggesting that cross-linking of –SH groups may occur in amyloidogenesis. Since in normal skin S–S bonds were stained only in the horny layer and negligibly in the dermis, our findings seem to support the view that amyloid is derived at least partially from epidermal cells.

Key words: Amyloid; Hyaline body; Histochemical staining; Sulphhydryl group; Disulphide bond

Although the origin of amyloid is still controversial, the role of epidermis has been implicated in the formation of papillary deposits of amyloid in cutaneous amyloidosis. This concept is derived primarily from the observations of Black and his co-worker (1, 3) that amyloid shares a number of morphological and histochemical similarities with hyaline bodies which originate from degenerated epidermal cells (4, 7, 8, 11, 18).

On the other hand, Ogawa et al. (15) demonstrated that cross-linking of sulphhydryl (-SH) groups takes place at the terminal stage of keratinization, using histochemical techniques with the new fluorescent thiol reagent, named DACM (*N*-(7-dimethylamino-4-methylcoumarinyl) maleimide) (Wako Pure Chemical Industries, Osaka, Japan). DACM elicits specific blue-green fluorescence only when it binds to -SH groups of molecules (exciting wavelength 400 nm; emission wavelength 485 nm).

Using this technique, we have found fluorescence for disulphide (S–S) bonds in hyaline body of lichen planus (6). In this study, DACM staining of amyloid was compared with that of hyaline body to investigate more of the relationship between these two structures.

MATERIALS AND METHODS

Skin specimens

Biopsy specimens were obtained from typical lesions in 6 cases of lichen amyloidosus, 4 cases of macular amyloidosis. A tissue was divided and a half was immediately frozen in acetone–dry ice mixture and stored at −80°C until use. Some of the tissues were embedded in Tissue-Tek II O.C.T. compound (Lab-Tek Products, Division of Miles Laboratories, Illinois, USA) and frozen. The other half was fixed in 10% formalin for histological stainings using hematoxylin and eosin, periodic acid-Schiff, van Gieson, crystal violet, thioflavin T and alkaline Congo red fluorescence. Thioflavin T- and Congo red-stained sections were observed using a Nikon fluorescence microscope with appropriate optical conditions, described elsewhere (17, 19). Diagnosis was confirmed by the combination of these stainings.

DACM stain techniques

Adjacent 3 μm cryostat sections were prepared in pairs. One of them was fixed in 4% formalin and stained with thioflavin T or Congo red fluorescence. The other, unfixed section was stained with DACM for -SH groups or S–S bonds *ad modum* Ogawa et al. (15). Fluorescence for –SH and S–S stainings was examined under the fluorescence microscope using a 200W high pressure mercury lamp, an exciter filter (IF385–425) which excites wavelengths between 385 and 425 nm and a set of barrier filters (470K) which suppress waves below 450 nm. To examine the localization of –SH gorups and S-S bonds, DACM staining was compared with thioflavin T or Congo red fluorescence staining.

RESULTS

Papillary deposits of amyloid were shown in all skin specimens. No apparent difference concerning –SH and S–S stainings was noted among lichen amyloidosus and macular amyloidosis. The following observations are thus the summary of these two types (Table 1).

The fluorescence pattern of –SH and S–S stainings in the epidermis was identical with that in nor-

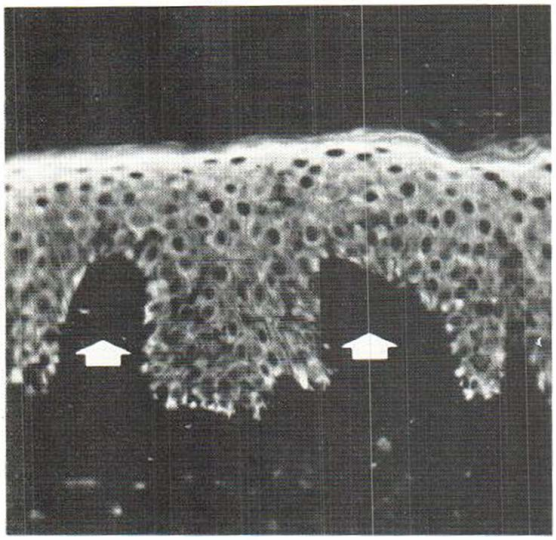


Fig. 1. –SH staining in lichen amyloidosus (Case 1). Fluorescence is seen in the cytoplasm of living layers of epidermis. No fluorescence is detected in papillary deposits of amyloid (arrows) (×800).

mal skin (15): fluorescence for -SH groups was seen mainly in the cytoplasm of living layers (Fig. 1), whereas that for S–S bonds was observed intercellularly in the horny layer (Figs. 2a & 3a). Occasionally, eosinophilic staining ovoid bodies were found in the epidermis (Cases 2 & 5). Since these structures had histochemically no properties of amyloid, they were considered to be degenerated epidermal cells or hyaline bodies, as noted by others (1, 3, 9). By DACM staining, they showed a homogeneously-packed mass of intense S–S fluorescence (Fig. 4). Intra-epidermal amyloid deposits were found only in case 8, in which neither –SH nor S–S fluorescence was detected.

In dermal amyloid, fluorescence for –SH groups was not detected (Fig. 1). S–S staining was observed in 7 cases, in some parts of papillary and upper dermal deposits of amyloid (Figs. 2a, b & 3a, b). It looked like a clump of granular fluorescence. The intensity of the fluorescence was rather weak, compared with that in the hyaline body which fluoresced as brilliantly as the horny layer. In the other 3 cases, S–S staining was not detected. Fluorescence of other dermal components for DACM was negligible. Under the optical conditions used, amyloid itself showed a weak pale-blue autofluorescence, but was clearly distinguished from the blue-green fluorescence for DACM.

Table 1. *DACM staining of dermal deposits of amyloid*

–, negative; +, weak, and ++, moderate fluorescence as compared with strong (+++) fluorescence in the horny layer

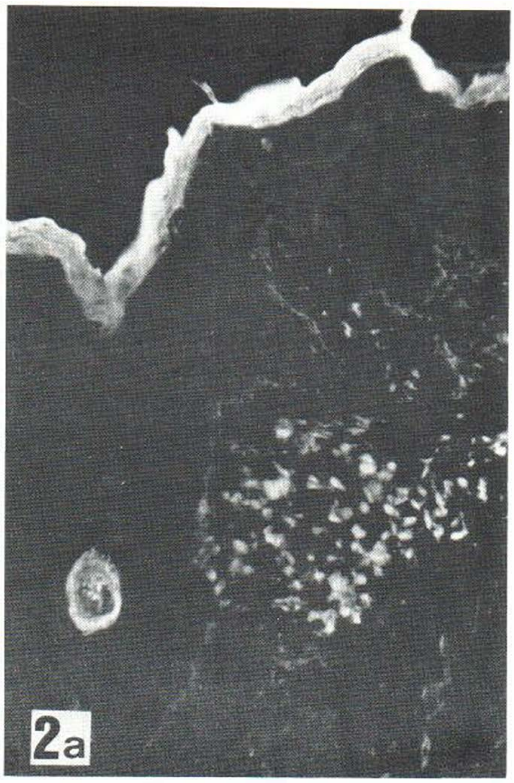
Case	–SH staining	S–S staining
Lichen amyloidosus		
1	–	–
2	–	+
3	–	+
4	–	–
5	–	+
6	–	+, partially ++
Macular amyloidosis		
7	–	+
8	–	+, partially ++
9	–	+
10	–	–

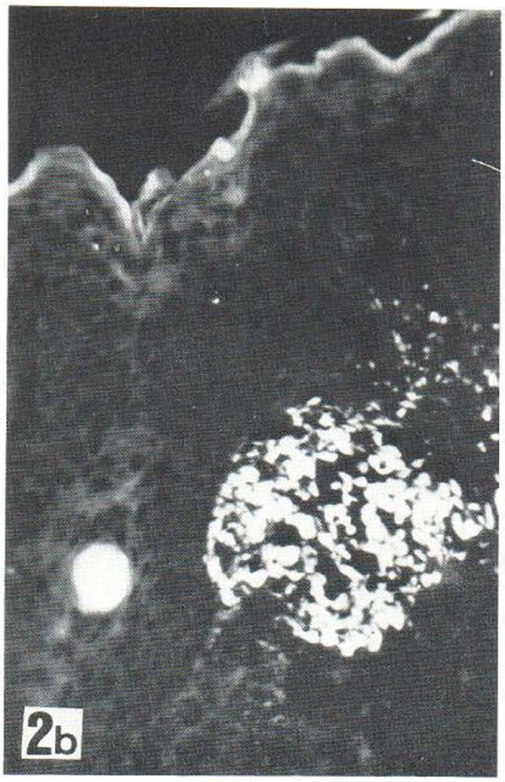
DISCUSSION

The histochemical observation by Black and Wilson-Jones (1, 3) that cutaneous amyloid formation is related to the epidermal changes has been confirmed by electron microscopic studies (2, 12, 13). Fibrils of both amyloid and hyaline bodies were shown to be produced by epidermal cells via filamentous degeneration (12, 13). Furthermore, amyloid was stained with anti-keratin antibody by indirect immunofluorescence, suggesting the immunological resemblance between amyloid and keratin proteins (14). Using DACM stain technique, we found fluorescence for S–S bonds but not for –SH groups in dermal deposits of amyloid and intra-epidermal hyaline bodies. It has been shown that cross-linking of -SH groups occurs in filamentous cells (bodies) such as hyaline body in lichen

Fig. 2. Comparison between S–S staining (*a*) and thioflavin T fluorescence (*b*) in macular amyloidosis (Case 8). Granular fluorescence for S–S bonds is observed as a clump in thioflavin T-positive amyloid deposits. Hair keratin shows S–S staining in (*a*) and nonspecific fluorescence for thioflavin T in (*b*) (×820).

Fig. 3. Comparison between S–S staining (*a*) and Congo red fluorescence (*b*) in lichen amyloidosis (Case 6). Aggregates of granular fluorescence for S–S bonds are localized in Congo red fluorescence-positive amyloid deposits. In (*a*) only negligible S–S fluorescence is seen in the dermis. The wavy one is autofluorescence of collagen bundles (×830).


2a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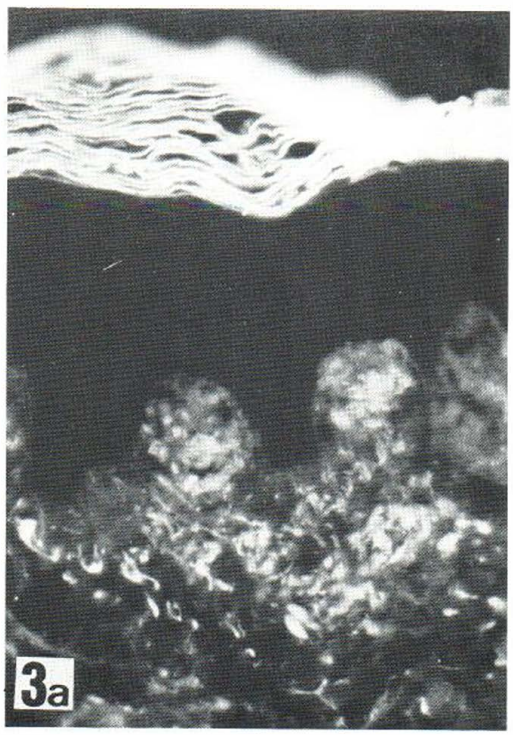
3a


3b

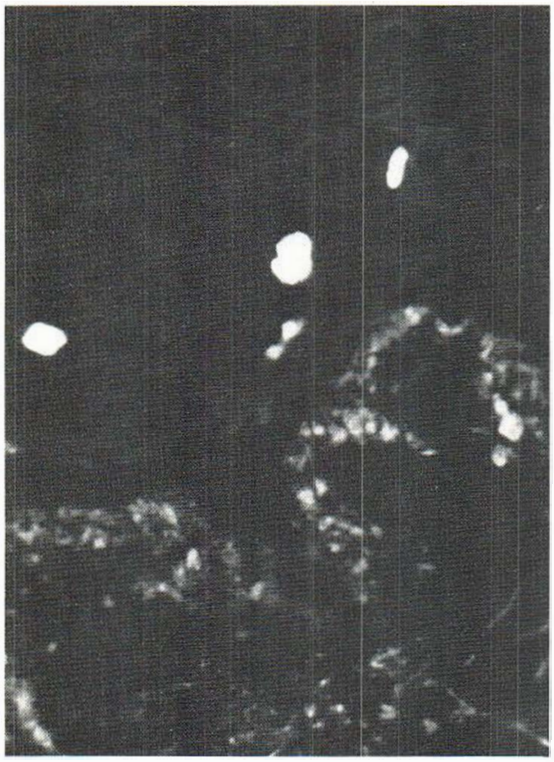

Fig. 4. Epidermal S–S staining in lichen amyloidosus (Case 2), demonstrating intense and ovoid fluorescence in hyaline bodies, which show no properties for amyloid. Small amounts of amyloid deposits in the dermis fluoresce weakly for S–S staining (×820).

planus (6), sunburn cell (5) and individual cell keratinization (16). The staining pattern of amyloid for DACM is basically similar to that of these filamentous cells (bodies), suggesting that S–S cross-linking may be associated with amyloidogenesis too. Since in normal skin S–S bonds are stainable with DACM only at the terminal stage of keratinization and negligibly in the dermis (15), our findings seem to support the view that cutaneous amyloid consists at least partly of epidermal components.

S–S fluorescence in amyloid was much less intense than that in hyaline body and in a few cases even non-existent. According to Hashimoto & Kumakiri (12), amyloid formation consists of four stages: (1) keratinocytes undergo filamentous degeneration (apoptosis), (2) filmentous degeneration produces both thick and wavy filaments of hyaline body (60–80 Å in diameter) and thinner and straighter filaments of amyloid precursors (60–70 Å), (3) degenerated cells lose basal lamina coverage and drop into the dermis, and finally (4) with the addition of unknown dermal components these filaments are converted to the end-product, "amyloid". Looked at in this way, the difference in intensity of S–S fluorescence between amyloid and hyaline body can be explained as follows: cross-linking of –SH groups and disappearance of detectable –SH residues seem to take place over the cytoplasm through stages (1) and (2). Formation of amyloid and hyaline body may share the same processes. However, in contrast to hyaline body—which is a pure product of keratinocytes and hence shows intensive S–S cross-linking—modulation of filamentous degeneration by other dermal factors in amyloidogenesis (stages (3) and (4)) may result in degradation of S–S linkages and may subsequently weaken S–S fluorescence considerably and, in more advanced stages, even quench it completely.

In contrast to the observation by Westermark (20), we found intra-epidermal amyloid only in one case so far examined. As no S–S fluorescence was detected here, this might represent transepidermal elimination of dermal amyloid, as suggested by Freudenthal (10). However, more data will be required to determine its histochemical nature.

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