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Histochemical Localization of Hyaluronan in Psoriasis, Allergic Contact Dermatitis and Normal Skin

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In suction blister fluid from active psoriatic lesions we have previously found elevated concentrations of hyaluronan. The aim of this investigation was to study the localization of hyaluronan with a histochemical method, in biopsy specimens from lesions of 13 patients with progressive psoriasis. Ten normal subjects and seven patients with allergic contact dermatitis were also studied.

In normal epidermis the highest intensity of hyaluronan staining was found in the intercellular spaces in the middle and upper spinous layer, whereas the staining was much weaker in the basal layer. No hyaluronan was detected in the granular layer or in the orthokeratotic stratum corneum. In the dermis there was pronounced staining of the papillary dermis and around the sebaceous glands, sweat glands, hair follicles and blood vessles.

In six of the 16 specimens from psoriatic lesions the normal epidermal meshwork of hyaluronan was partly absent and replaced by diffuse staining of both the spinous and the basal layer. In the remaining ten of these 16 specimens the same type of meshwork was found in stratum spinosum as in normal skin. The parakeratotic stratum corneum contained hyaluronan, in contrast to the normal stratum corneum, where no hyaluronan was present. The pattern of hyaluronan staining in the dermis of the psoriatic lesions did not differ from that in normal dermis.

In the majority of the allergic patch test reactions the junction was less distinct than in normal skin between dermis and epidermis and the normal hyaluronan pattern of the basal layer was abolished and replaced by a diffuse staining throughout the layer. The importance of the changes in the hyaluronan pattern in the inflammatory reactions of psoriasis and contact dermatitis is unknown. ***Key words: Hyaluronic acid; HYA; Biotinylated hyaluronan binding region.***

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Hyaluronan (hyaluronic acid, HYA) is a polysaccharide which is found in tissues and body fluids in varying amounts, mainly as an extracellular component of the connective tissue matrix. Reed et al. have previously measured the total HYA content in the rat and found that the major portion of HYA is present in the skin (1). At least part of the HYA seems to leave the skin by entering the blood circulation via the lymphatics (2).

In psoriasis, particularly in an active stage, we have previously observed elevated levels of HYA in the serum (3). Suction blister fluid from lesions in active psoriasis was found to contain 5–6 times higher concentrations of HYA than blisters from non-involved skin (4). The relevance of the increased HYA in active psoriasis lesions is not known.

Until recently it has been difficult to detect HYA in tissue sections (5). Histochemical techniques for location of HYA have recently been improved, however, and the distribution of HYA in normal human epidermis has been described (6), whereas details of the dermal pattern of HYA were not visible in that report. We have employed a histochemical method based on the use of purified and biotinylated hyaluronan binding region (HABR) which gives further information about the distribution of dermal HYA including its relation to hair follicles, sebaceous glands and sweat glands. Psoriatic lesions and allergic patch test reactions were also studied.

MATERIAL AND METHODS

Psoriasis

This group comprised 13 patients (8 men and 5 women, age range 37–90 years; mean 51.7 years). Two patients had guttate psoriasis which had developed during the last weeks. One patient was erythrodermic but had no pustules. Six patients had nummular and/or plaque psoriasis with slow deterioration during the last month. Four had more active psoriasis and an increasing number of nummular lesions had appeared during the last weeks. Emollient was the only topical treatment and 12 of the 13 patients had no systemic treatment. Biopsy specimens were taken from the margin of the lesions. One biopsy was taken from each of 11 of the 13 patients. In one patient, who was erythrodermic and had been treated with cyclosporin, 200 mg daily, for 3 months, two biopsies were taken, one before the start of treatment and the other 3 months later, when she still

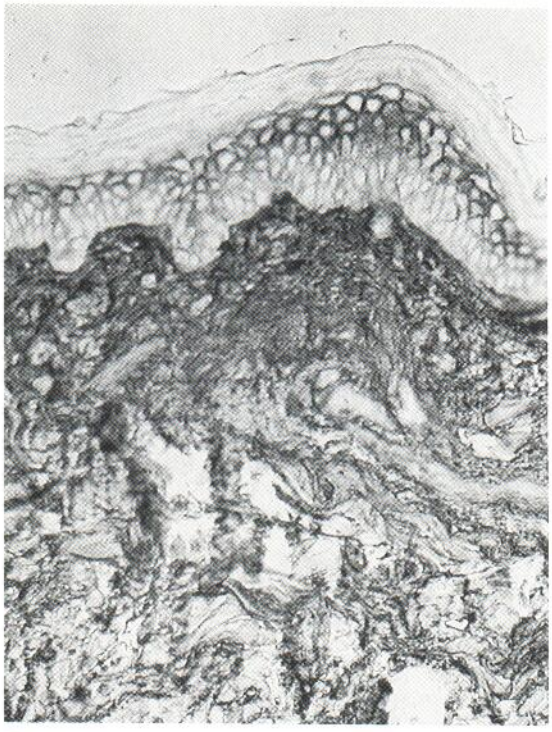

Fig. 1. HYA staining of epidermis and dermis in normal human skin.

had intensely erythematous lesions. From another patient three biopsies were taken: one from the margin of a stationary plaque, one from a small new papule and the third from the border of an almost healed plaque one month later. Her psoriasis was of the slowly progressive type.

Contact dermatitis

Seven patients (1 man and 6 women, aged 33–63 years; mean 51.7 years) with a history of eczema, in whom contact allergy was suspected, were patch tested when the eczema had disappeared. They had no topical or systemic treatment.

In six of the seven patients the patch test procedure was performed with the TRUE test (Pharmacia, Uppsala, Sweden) (7). The standard series recommended by the International Contact Dermatitis Research Group (ICDRG) was used. In one patient patch tested with chrysanthemum paludosum, the test was performed with the Finn chamber method. All patch tests were applied to the upper part of the back. They were removed after 48 h and the readings were made after 72 h. Reactions were recorded according to the scoring system of ICDRG (8) (+ weakly positive reaction – erythema and infiltration, possibly discrete papules; ++ strongly positive reaction – erythema, infiltration, papules and vesicles).

The biopsy specimens were taken from positive patch test reactions at the time of reading of the test. Six patch tests showed ++ reactions for chrysanthemum paludosum, colophony, nickel sulphate, benzocaine and formaldehyde (2 patients), respectively, and two patients showed a + reaction for disolidinuria and carbamix, respectively.

Reference group

This group consisted of 10 healthy subjects (9 women and 1 man, age range 40–78 years; mean 51.7 years, without any medical treatment). The biopsy specimens were taken from the dorsal aspect of the forearm.

None of the patients nor the reference subjects had been sunbathing or had used any type of artificial UV irradiation during the last 2 months before the study.

Preparation of biotinylated hyaluronan binding region (HABR) from cartilage proteoglycans

Biotinylated HABR was a gift from Dr Anders Tengblad (Pharmacia, Uppsala, Sweden). HABR had been prepared as previously described (9). Briefly, cartilage proteoglycans were extracted from bovine nasal cartilage, reaggregated with free HYA, dialyzed and lyophilized. This lyophilized material was resuspended and digested with trypsin (Sigma, St.Louis, Mo, USA). The HABR was purified by dissociating the HYA-protein complex with 4 M guanidinium chloride (GndCl). An affinity column of HYA coupled to Sepharose (Pharmacia, Sweden) was used to bind the proteins and was eluted with 4 M GndCl and 0.5 M NaAc (pH 5.8). The HABR was separated from the link proteins using a Sepharose-6B chromatography column (Pharmacia, Sweden). The purified HABR was biotinylated after aggregation with HYA in order to protect the binding site. Finally, the HABR-biotin was affinity-purified on an HYA-Sepharose column, dialyzed in 0.15 M NaCl, and stored at −20°C.

Staining procedures

The specimens were fixed in 4% neutral buffered paraformaldehyde containing 1% cetylpyridinium chloride (CPC) and embedded in paraffin. A serie of 4 μm thick consecutive sections were stained for HYA (see below). In addition, staining for collagen was performed with the van Gieson technique.

Histochemical staining for HYA

The staining procedure for HYA has recently been described by us (10) and is derived with some modification from the procedure of Ripellino et al. (11). After deparaffinization, the slides were incubated with 1% bovine serum albumin for 30 min to block non-specific binding sites. The slides were then washed in phosphate buffered saline (PBS) and incubated with a fresh solution of 3% H_2O_2 for 10 min in the dark to destroy any endogenous peroxidase activity. After washing again in PBS, the slides were incubated with 100 μl of biotinylated HABR (17 μg/ml) at 4°C overnight. Following a PBS wash, they were incubated with Vectastain-Elite avidin-biotin complex (ABC) reagent (Vector Labs, Burlingame, Ca, USA) for 1 h. Next, the slides were incubated with a 0.1 % solution of diaminobenzidine tetrahydrochloride (Sigma) for 5 min. Finally, they were washed in water and coverslipped. One set of slides was counterstained with Mayer's hematoxylin.

To check the binding specificity of the probe, one section from each biopsy was digested with 50–100 units/ml Strep-

Fig. 2. Intense HYA staining along the outermost layer of the sweat glands in normal human skin.

tomyces hyaluronidase (Seikagaku Kogyo Co., Tokyo) for 4 h at 37°C and then treated as described above (except for counterstaining).

RESULTS

Normal skin

In all specimens from normal skin the staining for hyaluronan was positive in both the epidermis and dermis (Fig. 1).

In the epidermis the HYA staining was concentrated in the intercellular spaces around the keratinocytes in the middle and upper part of the stratum spinosum, so that a meshwork was formed. In the basal layer the intercellular HYA staining was much weaker and in some specimens virtually no HYA was found around the lower parts of the basal cells. There was no staining either of the stratum granulosum or of the stratum corneum.

HYA was visible throughout the entire dermis. In the papillary dermis intense HYA staining was observed. In the reticular dermis there was sparse staining of HYA around the coarser fibers.

The outer lining of the blood vessels showed strong staining. There was also marked HYA staining along the outermost layer of the sweat glands

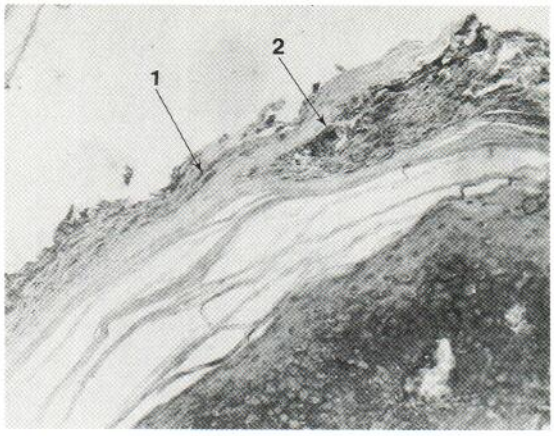

Fig. 3. 1) Indicate grains and streaks of HYA in the parakeratotic stratum corneum of a psoriatic lesion 2) indicate grains of HYA within a microabscess.

(Fig. 2) and the hair follicle wall facing the dermis, as well as of the lining around the sebaceous glands.

Psoriatic lesions

Hematoxylin staining. Three types of inflammatory pattern were observed among the biopsy specimens from psoriatic lesions, differing from each other with regard to the presence of granulocytes in the epidermis: 1) Small groups of granulocytes and microabscesses in the stratum corneum and subcorneally (9 specimens). 2) No granulocytes in the stratum corneum and only a few in the stratum spinosum (3 specimens). 3) Inflammatory cells present only in the dermis (4 specimens). All biopsies displayed acanthosis and varying numbers of inflammatory cells in the dermis. Parakeratosis was noted in all specimens except one (from an almost healed psoriatic lesion) with pattern 3.

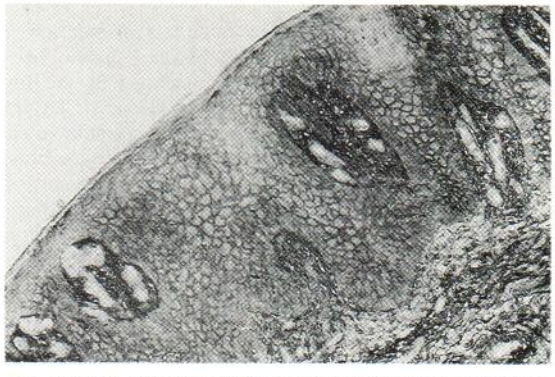

Fig. 4. HYA staining of the intercellular spaces in stratum spinosum and a diffuse staining of the basal layer in psoriasis.

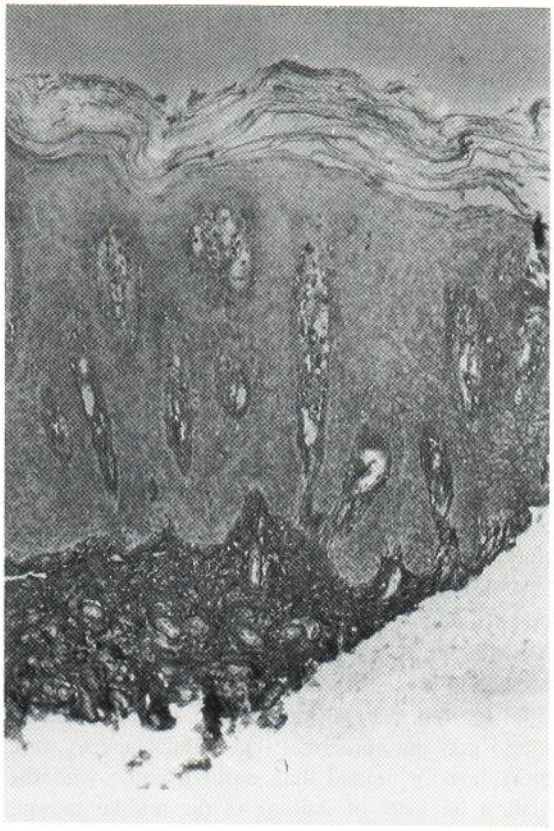

Fig. 5. One area of diffuse staining and one with a meshwork of HYA staining in epidermis of a psoriatic patient.

HYA staining. In the parakeratotic stratum corneum, grains or streaks of HYA were found (Fig. 4). Grains of HYA were often also observed around small groups of granulocytes and within microabscesses in the stratum corneum (Fig. 3) and subcorneally. The stratum granulosum was usually absent, but when it was present it never showed positive HYA staining.

In the stratum spinosum 10 of the 16 specimens displayed a meshwork of HYA around the cells. In contrast to normal epidermis where this meshwork usually is present only in the upper half of stratum spinosum, the HYA meshwork in the specimens from psoriatic lesions was often observed throughout the whole stratum spinosum (Fig. 4). In six specimens a more diffuse staining without meshwork was observed between areas with meshwork (Fig. 5). Only one specimen, which clinically was almost healed, showed a tendency to have the same pattern in the basal layer as in that of normal skin. The dermal pattern of HYA staining did not differ from that of normal dermis in 15 of 16 specimens from psoriatic lesions. In one specimens the papillary dermis seemed less stained than in normal skin (Fig. 4). All control sections treated with hyaluronidase were negative (Fig. 6).

With the exception of the almost healed lesion, there was no obvious relationship between the pattern of HYA staining and the activity of the disease, as judged by the clinical appearance and the duration of deterioration of the disease. The patterns in the two biopsies taken before and after 3 months of treatment with 200 mg of cyclosporin were of the same appearance. There was no clear relationship between the three histological types of psoriasis and the HYA staining pattern.

Contact dermatitis

Hematoxylin staining. Three different patterns were observed in the epidermis: 1) multiple intraepidermal vesicles which were partly confluent, spongiosis, and reticular degeneration (2 specimens, both ++ reactions); 2) a few vesicles and spongiosis (2 specimens, both ++ reactions); and 3) mild spongiosis but no vesicles (2 specimens with ++ and 2 with +

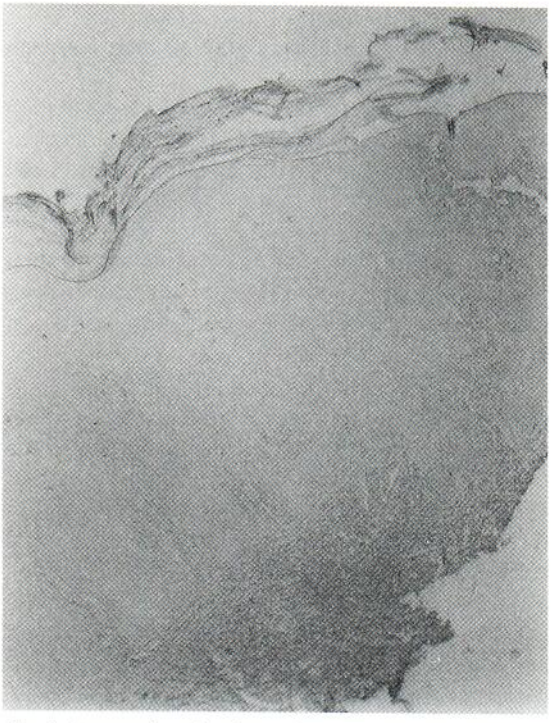

Fig. 6. A control section from the same specimen as Fig. 5, digested with hyaluronidase before staning with HABR.

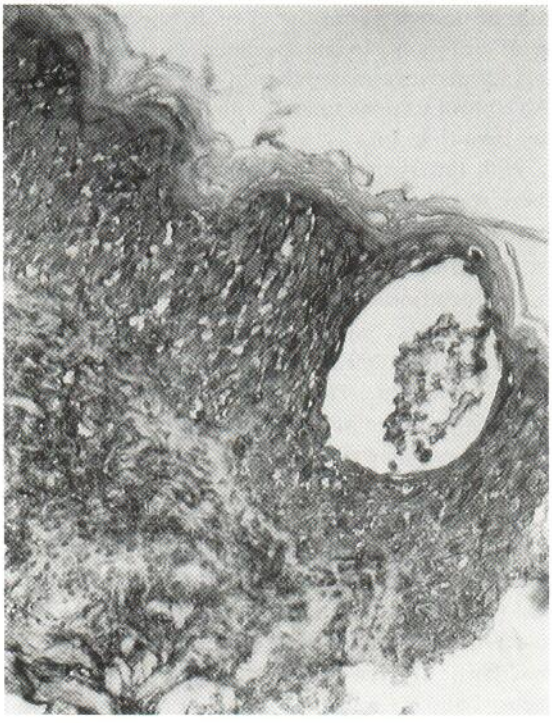

Fig. 7. Diffuse HYA staining throughout stratum spinosum and stratum basale in allergic contact dermatitis. Some streaks of HYA in the vesicle.

reactions). In all specimens the stratum corneum was orthokeratotic.

In the dermis varying degrees of vasodilatation and edema, and groups of perivascular mononuclear infiltrates, were found. The most pronounced cell infiltrates were present in three specimens with ++ reactions in the patch test.

HYA staining. The stratum corneum and stratum granulosum displayed no HYA in any of the specimens. In the vesicles there were some streaks of HYA (Figs. 7 and 8).

In the upper part of the stratum spinosum a meshwork of HYA was observed around the keratinocytes in five out of eight specimens, but not in the remaining three, where diffuse veillike staining of HYA was present throughout the layers (Fig. 7). In the lower part of the epidermis all specimens showed a diffuse staining pattern. Whether HYA is located extracellularly as well as intracellularly in the keratinocytes in the section with diffuse HYA staining is not possible to decide. The border between the epidermis and dermis was less distinct in most specimens (Fig. 8) in contrast to the well defined epidermis-dermis border seen in normal skin. Otherwise the pattern of HYA staining in the dermis was the same as in normal subjects. All control sections treated with hyaluronidase were negative.

DISCUSSION

The method used for visualization of HYA in this study is similar to that described by Ripellino et al. (11). Some modifications were made, however. These concerned the content of the fixative, the fixation time of the fresh biopsy specimens, differences in the hyaluronan binding probe and the incubation time of the probe. The hyaluronan binding protein used by Ripellino el al. contains both hook and link proteins, while the biotinylated HABR used in this study contains only the hook protein, which makes the probe smaller and probably more stable.

With this method HYA, which has not been immobilized in proteoglycan aggregates, is stained. The staining is independent of the molecular weight provided the HYA is above the size of a decasaccharide. In adult normal human skin Tammi et al. (6) found that the epidermal intercellular spaces were more heavily stained than dermal tissue, with the highest intensity of staining in the middle spinous layer and weak staining of the basal cell layer, whereas the granular layer and the stratum corneum were negative. The observations in the two studies concerning the normal epidermal pattern are thus

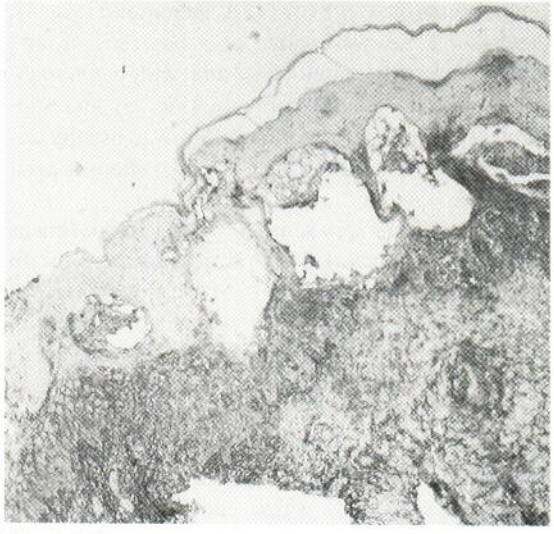

Fig. 8. Diffuse HYA staining in stratum spinosum and the border between epidermis and dermis in allergic contact dermatitis.

the same. In the dermis, however, the results differ. In contrast to the weak, diffuse staining of the dermis described by Tammi et al. (6), we found pronounced staining of the papillary dermis. The same type of intense staining was also seen around the sebaceous glands and sweat glands and along the hair follicles and blood vessels. The heavy staining of the papillary dermis is in accordance with the report by Tajima et al., who found the highest content of HYA in the uppermost part of the dermis (12). Alho & Underhill, in their study on the hyaluronate receptor expression and hyaluronate distribution in normal hamster skin, clearly found a hyaluronate distribution similar to that in normal human epidermis and dermis (13). Interestingly, the HYA receptor was preferentially located on the surface of the basal cells and the lower parts of the spinous layer (13).

We have previously reported that suction blister fluids from active psoriatic lesions contain increased amounts of HYA compared with blister fluid from stable lesions and non-involved skin (4). The blisters are formed between the basal cells and basal lamina (14), and the HYA in the fluid has been assumed to reflect the content of HYA in the papillary dermis.

In view of the finding of increased HYA in blister fluid from active inflammatory psoriasis, it was of interest to compare the distribution and intensity of HYA staining in biopsy specimens from psoriatic lesions of various stages. In the specimens from the most active lesions, intense staining of the papillary dermis might have been expected. However, the staining intensity in the papillary dermis was no different from that in the specimens from more stable lesions and from normal skin. The reason for this is not known. One possible explanation is that during preparation of the slides, some HYA may have been lost from the edematous papillary dermis in active psoriasis. Despite the fact that CPC is added to the fixative to prevent the loss of HYA, it is conceivable that not all of the HYA is retained. Indeed, in more recent experiments we have noted that the use of microwaves (15) to fix the tissue results in the best retention and detection of the polysaccharide*. Another possibility is that the staining is already so intense in normal skin that a stronger reaction cannot be discriminated. Yet another explanation may be that an increase in the dermal HYA level results in more rapid removal by both cells, lymph and blood vessels, as is found in other tissues (1).

The most obvious change in the HYA pattern in the epidermis of the psoriatic lesions compared with normal epidermis was the stronger but also more diffuse staining of the basal cell layer. Interestingly, an almost normal pattern was observed in only one specimen, namely that from the nearly healed lesion where the stratum corneum was orthokeratotic, in contrast to all other specimens, where the stratum corneum was always parakeratotic. The increased content of HYA in suction blister fluid from active psoriatic lesions (4) is probably a result of the inflammation. The diffuse staining of the basal layer may also reflect the increased HYA. Whether these amounts of HYA per se can contribute to the abnormal differentiation of the psoriatic lesion is not known. However, such effects cannot be excluded.

Positive patch test reactions represent an inflammatory process of known duration with erythema, papules and vesicles, later followed by dryness and scaling. The most conspicuous difference in the HYA staining was that the normal pattern of the basal layer was abolished and replaced by diffuse staining throughout the basal layer. In the majority of the patch test biopsies, the junction between the papillary dermis and epidermis was less distinct than in normal epidermis. The relevance of these findings to the development of the inflammatory reaction and the subsequent scaling is not yet known.

Apart from possible direct effects of HYA on epidermal differentiation, it is thought that by virtue of its water-binding capacity HYA helps in the transport of nutrients from the vascularized dermis to the avascular epidermis (16). In suction blister fluid from active psoriatic lesions, an up to tenfold increase in concentration of HYA has been noted compared with that in normal skin (4). It is conceivable that in disorders with increased concentrations of HYA in the skin this may lead not only to edema but also to an imbalance in the transport of nutrients to the skin. In such conditions HYA may also act as a transport medium for inflammatory mediators.

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