Biochemical Characterization and Tissue Distribution of the Scleredema in a Case of Buschke’s Disease

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Biopsies from a patient with a longstanding form of scleredema adultorum Buschke were analysed for morphological and biochemical changes in the dermal connective tissue. By light microscopy the tissue changes were located to the deep part of the reticular dermis. Therefore dermal tissue was separated into a superficial and a deep part and analysed biochemically. By this procedure it was possible to show that the concentration of hyaluronan in the deep part of the dermis was increased. The urinary excretion of methylimidazole acetic acid, an indicator of the mast cell mass in the body, was also elevated. Key words: Scleredema adultorum Buschke; Biochemistry; Histopathology.

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Laboratory data
Morning blood sugar was approximately 10 mmol/l (normal 3.9-5.8 mmol/l). The concentration of serum cholesterol was slightly elevated, 13 mmol/l (normal 3.2-9.6 mmol/l) as well as that of serum triglycerides, 9.1 mmol/l (normal 0.2-2.8 mmol/l).

Immune electrophoresis and analysis of IgE were within normal limits.
Liver scintigraphy showed a liver of normal size. The ECG was abnormal with sinus rhythm and left anterior hemiblock. X-ray of the cervical spine was normal.

Histamine turnover
The urinary excretion of the main metabolite methylimidazole acetic acid (MelmAA) is a good indicator of the total histamine turnover in the body (4). Urine was collected on two consecutive days. To avoid bacterial growth, hydrochloric acid was added to obtain a pH of less than 2. During the days of urine collection, a standardized diet with a defined low histamine content was given (5). MelmAA was measured by high performance liquid chromatography (6). The result is given as the mean value of the two days of urine collection and expressed as mg/24 h.

Histological examination
Punch biopsies from normal and lesional skin and a knife biopsy from lesional skin were available for examination. Tissue specimens were fixed in a formalin-sublimate-acetic acid solution according to Stieve (7) and stained with haematoxylin-eosin, PAS-Alcian blue, Giemsa and toluidine blue at pH 4 and with Alcian blue at pH 0.5 and 2.5. The last two stainings were also applied after digestion with bovine testicular hyaluronidase (8).

Biochemical analysis
Punch biopsies, 4 mm in diameter, were taken at different occasions from the same lesional skin area on the upper back. Control biopsies were also taken from a clinically uninvolved skin area below the involved skin. The fresh biopsy material was trimmed of subcutaneous fat. The punches were cut in two pieces, one of which approximately represented the superficial two thirds of the biopsy and the other the remaining deep one third. The biopsies were weighed directly after sampling.

Each cylindrical piece was divided into two symmetrical halves along its long axis. The half destined for biochemical analysis was weighed wet and immediately thereafter frozen in isopentane-liquid nitrogen and kept at -70°C until used. The other half was processed as above for control histological examination.

Before the biochemical analysis the biopsies were freeze-dried until constant weight was obtained. The dry samples were delipidated by extraction with 5 ml chloroform-methanol (3:1) at 22°C. The extraction was repeated three times. Water and fat content was calculated as the loss of weight during freeze-drying and extraction, respectively. The punches were subjected to digestion with 20 units of papain (EC 3.4.22.2) in 0.05 M phosphate buffer pH 7.0 containing 0.01 M cystein hydrochloride and 0.01 M Na₂-EDTA at 65°C for 6 h. The digest was applied to a column of DEAE-cellulose in acetate form. The column was washed with 7 volumes of 0.2 M and 0.4 M acetate-pyridine buffer pH 5.0. Hyaluronan was eluted with 7 volumes of 1.2 M acetate-pyridine buffer pH 5.0 and sulphated glycosaminoglycans with 7 volumes of 3.0 M acetate-pyridine buffer pH 5.0 (9). The amounts of

<table>
<thead>
<tr>
<th></th>
<th>Punch weight (ww mg)</th>
<th>Water (% ww)</th>
<th>Fat (% ww)</th>
<th>Hydroxyproline (µg/mg dw)</th>
<th>Hexosamine in hyaluronan (µg/mg dw)</th>
<th>Hexosamine in sulphated glycosaminoglycans (µg/mg dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scleredema</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superficial layer</td>
<td>22.0</td>
<td>69</td>
<td>2.0</td>
<td>113</td>
<td>3.3</td>
<td>1.0</td>
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<tr>
<td>Deep layer</td>
<td>13.4</td>
<td>69</td>
<td>2.2</td>
<td>140</td>
<td>5.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Normal skin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superficial layer</td>
<td>13.4</td>
<td>73</td>
<td>0.7</td>
<td>104</td>
<td>3.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Deep layer</td>
<td>5.8</td>
<td>63</td>
<td>2.2</td>
<td>118</td>
<td>2.3</td>
<td>2.2</td>
</tr>
</tbody>
</table>

The figures are the mean values of two biopsies. The superficial layer constitutes two-thirds of the biopsy and the deep layer one third. ww = wet weight, dw = dry weight.
Hyaluronan and sulphated glycosaminoglycans were expressed as hexosamine contents in the fractions as determined by Elson-Morgan procedure after hydrolysis in 6 M hydrochloric acid for 8 h at 100°C and subsequent removal of the acid by freeze-drying (10).

Collagen was estimated from the hydroxyproline concentration in the digest. This was determined according to Stegemann (11) after hydrolysis in 6 M hydrochloric acid at 100°C for 17 h.

Hyaluronan was also determined by a specific radioassay (12). Biopsies taken at a separate occasion were not divided into a superficial and deep part. They were kept in 80% ethanol until analysed. After drying they were suspended in 0.5 ml of 0.1 M phosphate buffer pH 6.0 containing 0.005 M cystein hydrochloride, 0.005 M Na₂-EDTA and 1 M sodium chloride. They were digested for 24 h at 60°C with 12 units of papain and dialysed for 48 h against 0.025 M phosphate buffer, pH 7.0, containing 1.5 M sodium chloride and 0.0067 M iodoacetic acid. The latter was added to inactivate the papain. Small precipitates were removed by centrifugation before hyaluronan was determined on the supernatant (12).

RESULTS

Physical observations

The punch biopsies from lesional skin demonstrated an about two-fold increased thickness of the dermis and a two-fold increase in wet weight (Table I). Moreover, the biopsied tissue cylinders from the lesioned skin did swell noticeably when removed.

Light microscopic examination of skin biopsies revealed a thickened reticular dermis, where the collagen bundles in the deep part of the biopsy were markedly separated by faintly stained basophilic material (Figs. 1 and 2). The superficial two-thirds of the reticular and papillary dermis, on the other hand, appeared essentially normal in structure. The collagen fibres stained normally throughout the dermis with the haematoxylin-eosin and Alcian-blue methods. There was no obvious remodelling of the collagenous fibre framework so as to suggest the formation of new collagen. The interfibrillar material in the deep dermis stained positively with Alcian blue at pH 2.5 but not at pH 0.5. The staining with Alcian blue at 2.5 was extinguished by testicular hyaluronidase digestion. A sparse perivascular lymphocytic infiltration was noticed in the superficial dermis. There was no inflammatory reaction in the deep dermis but the number of mast cells was possibly increased.

The control histological examination of the punch biopsies confirmed a satisfactory division of the tissue cylinders in a superficial and a deep part and that the deep part reached subcutis.

Biochemical analysis

The results of the biochemical analysis are presented in Table I. The concentrations of water, fat and collagen (hydroxyproline) were essentially the same in the affected and unaffected areas of the skin. The concentrations of hyaluronan and sulphated glycosaminoglycans expressed on a dry weight basis were also the same in normal and diseased skin with one exception. There was approximately twice as much hyaluronan in the deep layer of the dermis in scleredema compared to the control.

Hyaluronan was also analysed separately by a radioassay on whole biopsies. Two analyses on skin lesions gave 1.7 and 2.0 µg/mg wet weight while control biopsies on normal skin gave 0.8 and 0.9 µg/mg wet weight. The biopsies from the pathological skin were two-fold increased in thickness and weight and therefore the total amount of hyaluronan was about four times greater in the pathological case.

A slight increase of the main histamine metabolite MelmAA, 6.9 mg/24 h, was found. Normal values are 2.6 mg±1.3 mg (mean ± SD).
DISCUSSION

This patient has a skin lesion typical for scleredema adultorum Buschke. When studied, he had a two-fold increase in thickness and in the wet weight of the skin of the involved area compared to normal areas. The morphological analysis indicated that accumulation of polysaccharide especially in the deep dermis could be responsible for the observed tissue change. This seemed to be at variance with those of most other authors who claim, on the basis of morphological results, that the changes occur both in superficial and deep dermis (3, 13, 14, 15).

When analysed biochemically the superficial layer of the lesion had essentially the same composition as normal skin (Table 1), corroborating the morphological observations. However, taking into account the increase in volume of the superficial layer it must contain twice as much polysaccharide as normal skin. The large biochemical change in the skin lesion was found in the deep zone, where the hyaluronan concentration was doubled and the total amount of hyaluronan was four-fold that of the normal skin. This local variation between superficial and deep layers of the dermis has not been described before. As far as we know, nobody has previously compared the biochemical composition of deep
and superficial layers in scleroderma, nor compared sclerodematous skin with unaffected skin in the same patient. In the light of our results earlier reports on slight or absent histological and biochemical changes in clinically evidently affected skin might be explained by insufficient depth of the biopsy.

The data on increased hyaluronan concentration in the sclerodematous skin was confirmed by independent measurements using a completely different technique, which is now available (12). The results are not directly comparable because the latter analyses were made on whole biopsies taken at a different time and the results were related to wet weight of the samples rather than the dry weight. However, if one recalculates the data in Table 1 to a wet weight basis, the hyaluronan concentration becomes approximately two-fold higher than that obtained by the radioassay. This discrepancy, whether methodological or due to biological variation, does not invalidate the conclusion that the sclerodematous skin biopsies have a higher hyaluronan concentration than the normal ones.

Hyaluronan confers an osmotic swelling pressure to the tissue (16). This is resisted by the collagen framework. In cases when the concentration of hyaluronan is increased one should expect an increased swelling pressure. This was demonstrated when the punches were extracted and the involved skin swelled quite markedly. The increased pressure could influence the pain receptors and be the reason for the sensation of pain noted by the patients.

The mechanism behind the relative increase in hyaluronan concentration is unknown. Scleroderma often starts with an infection or a trauma. The resulting inflammatory response stimulates fibroblast growth as well as synthesis. Fibroblast cultures established from involved areas show a considerably higher synthesis of hyaluronan than those from uninvolved areas (17). It is noteworthy that the increased accumulation is retained even after subculture. This suggests that the fibroblasts even in the absence of inflammatory stimulation are capable of an increased synthesis of hyaluronan (17). The patients often have diabetes. Fibroblasts from diabetics secrete a different proportion of heparan sulphate and dermatan sulphate (18). It is therefore possible that the diabetes of these patients influence the composition of the connective tissue.

In our study only a weak inflammatory reaction was noted in the otherwise normal superficial dermis. However, histological examination indicated a possible increase in the number of mast cells in the lower part of the dermis. This corroborated with an increased urinary excretion of MelMAA (4). This may point towards a relationship between mast cells and the metabolism of hyaluronan. It is therefore interesting to note that Bjermer et al. (19) found a relationship between number of mast cells and hyaluronan concentration in bronchoalveolar lavage fluid from patients with sarcoidosis.

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REFERENCES