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The Distribution of Unsulphated and Sulphated Glycosaminoglycans in Palmar Fascia from Patients with *Dupuytren*'s Disease and Healthy Subjects[1])

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Summary: Eighty specimens from 20 patients with *Dupuytren*'s disease and 7 biopsies of healthy palmar fascia were analysed for their glycosaminoglycan isomer patterns with a combined enzymatic/HPLC method. The diseased portions of palmar fascia tissue were characterized by elevated total glycosaminoglycans together with a relative increase in the sulphated fractions. The macroscopic stages of nodules, bands and unaffected tissue could be classified very well by multivariate statistical analysis on the basis of their glycosaminoglycan patterns. The biochemical analysis provided evidence of the pathological process even in those specimens that did not yet show any clinical symptoms of the disease.

Introduction

The mechanisms involved in the pathogenesis of *Dupuytren*'s disease are still unknown (1). Recent studies on the morphological changes in diseased tissue have demonstrated occlusion of capillaries by cells that migrate into the perivascular space. These cells are thought to be the origin of dedifferentiated cells that proliferate and metabolize in such way that they cause contracture of the aponeurosis (2, 3). As to the pathobiochemistry, an increase in collagen type III protein has been reported (4–7), as has an increase in the proportion of chondroitin sulphate (4, 8–11).

Because of the role played by sulphated glycosaminoglycans in the interaction with collagen (12), the question arises of whether there are changes in the proportions of glycosaminoglycans and their unsulphated and sulphated isomers in affected palmar fascia that may have an influence on the collagen pattern and the macromolecular structure. The present study was conducted to determine the types and amounts of the various sulphated and unsulphated glycosaminoglycans and to reassess the data published so far on the glycosaminoglycan content of both healthy human palmar fascia and tissue from *Dupuytren*'s contracture, by employing a method of determination more specific than any used previously. The new method also enabled differentiation of the unsulphated, C4-sulphated and C6-sulphated components (11).

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Materials and Methods

Eighty biopsy specimens from 20 patients (aged 18 to 76 years; 2 women, 18 men) with *Dupuytren*'s disease of the hand were taken during surgery and classified according to their macroscopic appearance as
(1) apparently normal,
(2) tissue adjacent to bands or nodules,
(3) bands, or
(4) nodules.

For reference, palmar fascia from healthy persons was taken during reconstruction surgery following accidental injury (group 0, n = 7, male) and during autopsies performed within 15 hours post mortem (n = 8, female). The specimens were freed from adjacent tissue and blood and stored at −20 °C until processing. A representative sample of each specimen was set aside for microscopic examination.

The total glycosaminoglycans were isolated and the various fractions identified by specific enzymatic degradation, followed by a quantitative determination of the respective disaccharide metabolites by high performance liquid chromatography as described elsewhere (11). This method is specific and reliable, with an overall coefficient of variation of 6.8% at a substrate concentration of 100 nmol per assay and a sensitivity of < 10 nmol per assay. Uronic acid was determined with the method described by *Bitter & Muir* (13), hydroxyproline with that of *Stegemann* (14) and DNA with that of *Burton* (15).

The microscopic examination of the specimens was performed on samples taken from the tissue portions that were analysed biochemically. Samples were stained with hematoxylin and by the *van Gieson* technique. The classification was made according to *Millesi* (16):
Stage 1A = thickening and stretching of fibres,
Stage 1B = development of fibre bands,
Stage 2 = cell proliferation, and
Stage 3 = few cells only, unoriented thick fibre bundles (fig. 1).

A discriminant analysis according to *Schneider* (17) was performed with the SPSS-X program "Discriminant"-Release 2.

Results

The results of the glycosaminoglycan determinations on the normal palmar fascia tissue and the four groups of diseased tissue are summarized in table 1 and figures 2 and 3. In the most advanced stage of the disease the glycosaminoglycan content was double that found in the unaffected palmar fascia. In the early phases of the disease the hyaluronate content remained almost constant, with a decrease in the later stages, whereas the chondroitin sulphate and the dermatan sulphate fractions increased with the severity of the disease, being 10 times greater in nodules than in healthy fascia. It should be noted that the autopsy

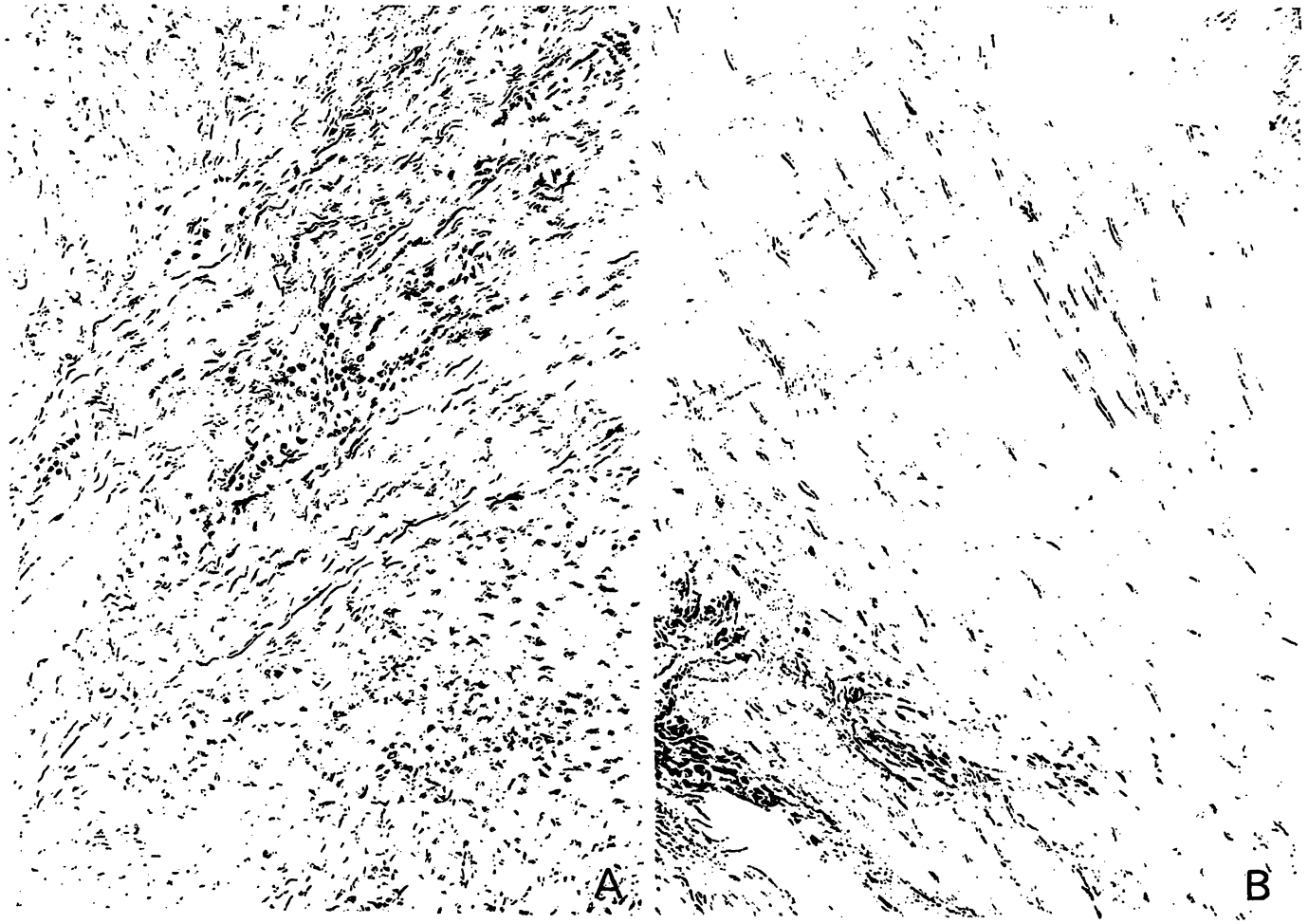

Fig. 1. *Dupuytren*'s disease according to *Millesi* (16). The examples illustrate the proliferating stage 2 (A) and the stage 3 (B, few cells only, unoriented thick fibre bundles). HE, 10x.

Tab. 1. Concentration of DNA, hydroxyproline, glycosaminoglycans (GAG) and GAG-fractions per g dry weight in palmar fascia from *Dupuytren*'s contracture and healthy palmar aponeurosis. Grouping of specimens according to their macroscopic appearances. GAG isolation by proteolysis, β-elimination and sodium acetate/ethanol precipitation. Enzymatic differential degradation and HPLC determination of the disaccharide metabolites. Total GAG estimation by uronic acid assay in the isolated total GAG fraction. Hydroxyproline determination after *Stegemann* (14), DNA determination according to *Burton* (15). p = barrier of significance between the values of the respective groups. *Student*'s t-test. ns: no significance, Δ−0−S, Δ−4−S, Δ−6−S: unsulphated, C-4 sulphated and C-6 sulphated isomers of chondroitin sulphate and dermatan sulphate, resp., dw = dry weight.

	DNA mg/g dw	Hydroxy-proline µmol/g dw	Total GAG uronic acid µmol/g dw	Hyaluronate disaccharide µmol/g dw	Chondroitin sulphate disaccharide				Dermatan sulphate disaccharide			
					total µmol/g dw	Δ-0-S µmol/g dw	Δ-4-S µmol/g dw	Δ-6-S µmol/g dw	total µmol/g dw	Δ-0-S µmol/g dw	Δ-4-S µmol/g dw	Δ-6-S µmol/g dw
	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$
Group 0												
Normal palmar fascia biopsies n = 7 for total GAG n = 4 for GAG fraction	1.02 ± 0.32	64.1 ± 2.87	4.45 ± 0.46	2.75 ± 0.62	0.33 ± 0.16	<0.01	0.13 ± 0.05	0.20 ± 0.17	1.37 ± 0.22	0.06 ± 0.01	1.27 ± 0.25	0.04 ± 0.02
$p_{[1-2]}$ =	0.001	0.005	0.001	n s	n s	0.01	n s	n s	0.025	n s	0.001	n s
Group 1												
Apparently normal fascia n = 12	3.88 ± 1.00	96.8 ± 20.6	6.59 ± 1.55	3.34 ± 0.74	0.47 ± 0.28	0.12 ± 0.14	0.17 ± 1.12	0.25 ± 0.15	2.59 ± 0.96	0.04 ± 0.04	2.48 ± 0.88	0.07 ± 0.06
$p_{[2-3]}$ =	0.001	n s	n s	0.003	n s	n s	n s	n s	n s	n s	n s	n s
Group 2												
Fascia adjacent to bands or nodules n = 20	1.79 ± 0.83	101 ± 18.1	5.44 ± 1.85	2.62 ± 0.87	0.55 ± 0.41	0.11 ± 0.17	0.14 ± 0.08	0.26 ± 0.16	2.54 ± 0.89	0.04 ± 0.06	2.45 ± 0.82	0.11 ± 0.08
$p_{[3-4]}$ =	n s	n s	0.01	0.001	0.001	0.001	0.001	0.001	0.001	0.01	0.001	0.001
Group 3												
Bands n = 19	2.37 ± 0.83	100 ± 13.9	6.94 ± 1.16	1.42 ± 0.33	1.74 ± 1.08	0.50 ± 0.25	0.23 ± 0.09	0.71 ± 0.27	3.95 ± 1.2	0.10 ± 0.06	3.81 ± 0.71	0.20 ± 0.07
$p_{[4-5]}$ =	0.001	0.025	0.001	n s	n s	0.001	0.001	0.001	0.005	0.001	0.001	0.002
Group 4												
Nodules n = 11	6.23 ± 1.85	87.7 ± 11.3	10.9 ± 1.73	1.68 ± 1.14	2.85 ± 1.06	1.02 ± 0.35	0.59 ± 0.26	1.70 ± 0.63	6.18 ± 0.74	0.26 ± 0.12	5.59 ± 0.62	0.34 ± 0.11

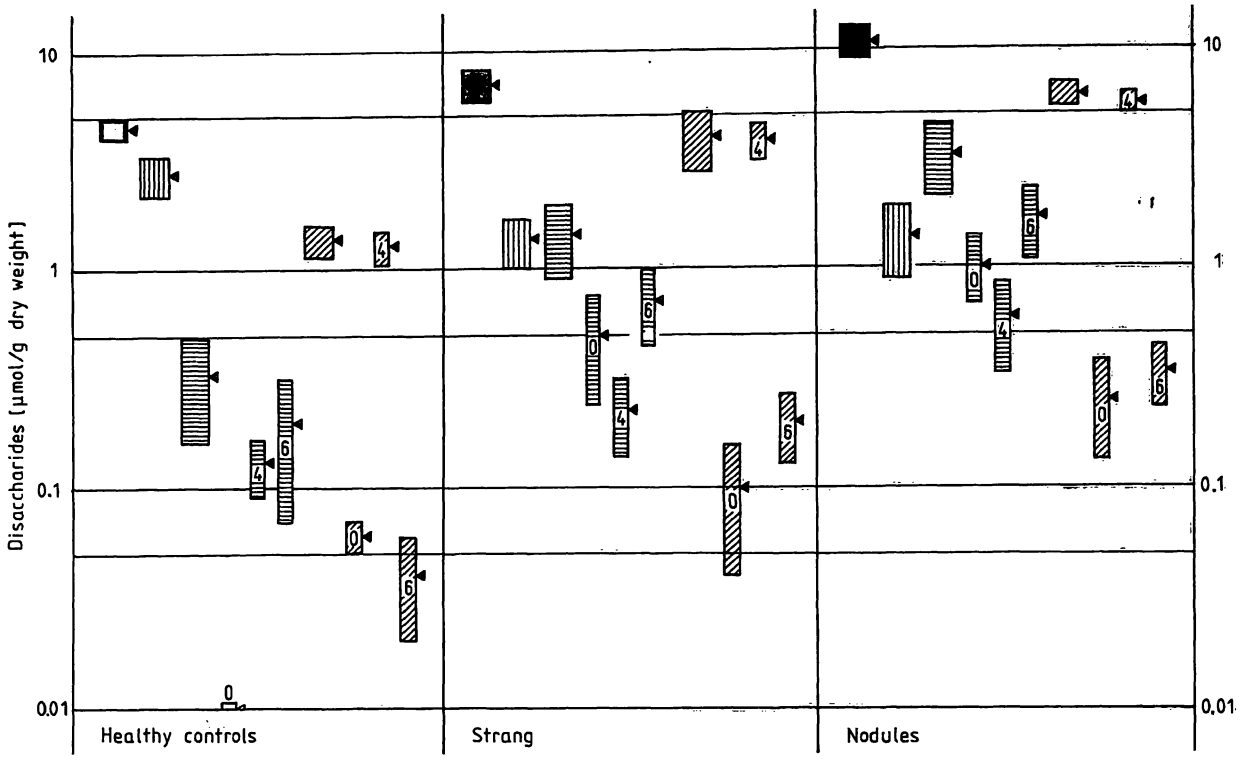

Fig. 2. Glycosaminoglycan patterns of healthy palmar fascia and in specimens from *Dupuytren*'s disease (bands, nodules). Combined specific-enzymatic-HPLC analysis.

glycosaminoglycan content
hyaluronate content
total condroitin sulphate fraction
0 4 6 unsulphated, C4- and C6-sulphated isomers
total dermatan sulphate fraction
0 4 6 unsulphated, C4- and C6-sulphated isomers
$\bar{x} \pm 2\,s$

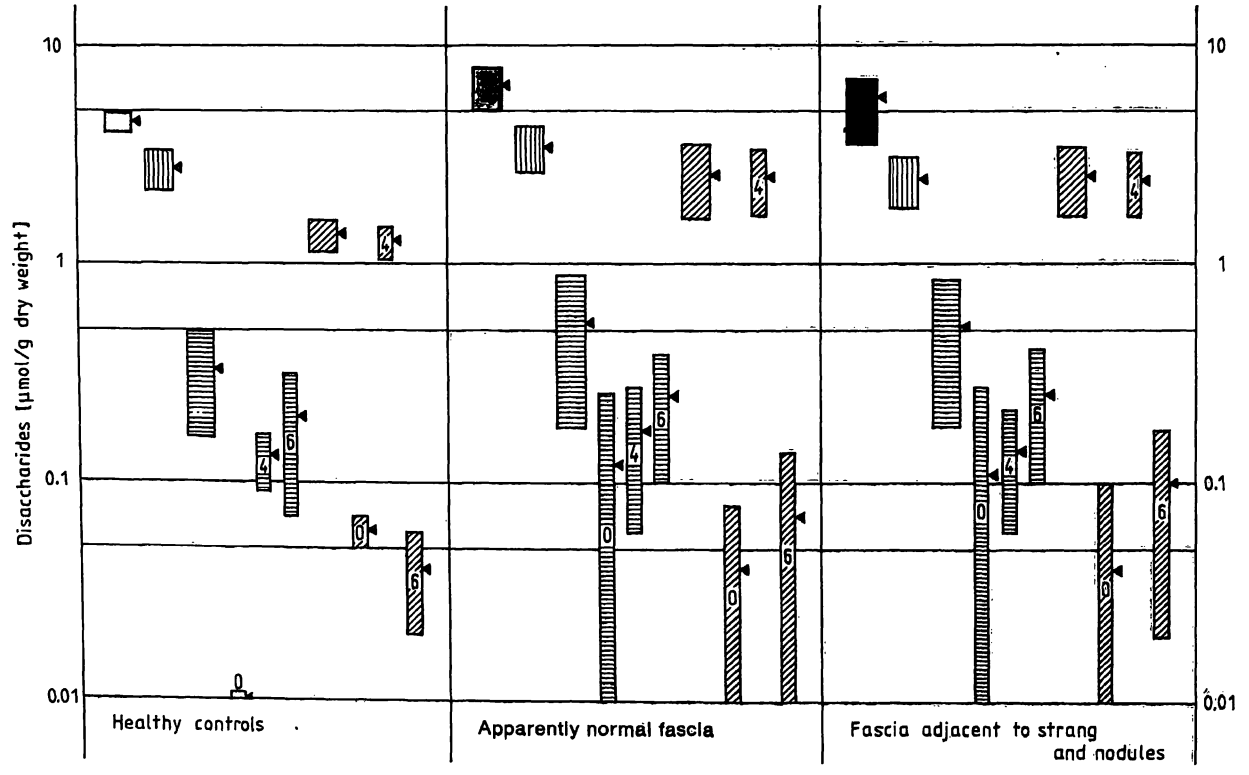

Fig. 3. Glycosaminoglycan patterns of healthy palmar fascia and in specimens from *Dupuytren*'s disease (apparently normal tissue, tissue adjacent to bands and nodules). Combined specific-enzymatic-HPLC analysis. Explanation see figure 2.

Tab. 2. Comparison of biochemical data from specimens of palmar fascia from healthy persons taken by biopsy or autopsy (within 15 h post mortem). Glycosaminoglycan (GAG) isolation by proteolysis, β-elimination and sodium acetate/ethanol precipitation. Enzymatic differential degradation and HPLC determination of the disaccharide metabolites. GAG estimation by uronic acid assay in the isolated total GAG-fraction. Hydroxyproline determination after *Stegemann* (14), DNA determination according to *Burton* (15). p = barrier of significance between the values of the respective groups. *Student*'s-test.
Δ −0−S, Δ −4−S, Δ −6−S: unsulphated, C-4-sulphated and C-6-sulphated isomers of chondroitin sulphate and dermatan sulphate, resp., dw = dry weight

Palmar fascia of healthy persons	DNA mg/g dw	Hydroxy-proline µmol/g dw	Total GAG uronic acid µmol/g dw	Hyaluronate disaccharide µmol/g dw	Chondroitin sulphate disaccharide				Dermatan sulphate disaccharide			
					total µmol/g dw	Δ-0-S µmol/g dw	Δ-4-S µmol/g dw	Δ-6-S µmol/g dw	total µmol/g dw	Δ-0-S µmol/g dw	Δ-4-S µmol/g dw	Δ-6-S µmol/g dw
	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$	$\bar{x} \pm s$
Biopsy specimens n = 7	1.02 ± 0.32	64.1 ± 2.87	4.45 ± 0.46	2.75 ± 0.62	0.33 ± 0.16	<0.01	0.13 ± 0.04	0.20 ± 0.14	1.37 ± 0.22	0.06 ± 0.01	1.27 ± 0.22	0.04 ± 0.02
p =	0.25	0.25	0.00005	0.005	0.001	–	0.01	0.01	0.005	0.05	0.05	0.10
Autopsy specimens n = 8	0.77 ± 0.07	64.4 ± 3.49	2.75 ± 0.46	1.60 ± 0.37	0.13 ± 0.06	<0.01	0.07 ± 0.03	0.07 ± 0.04	1.02 ± 0.25	0.04 ± 0.01	0.96 ± 0.23	0.03 ± 0.01

specimens of normal fascia contained smaller amounts of glycosaminoglycans than the biopsy specimens. The autopsy material proved to be unsuitable for reference because of a post mortem drop in glycosaminoglycans, mainly hyaluronate (tab. 2). However, both collagen and DNA contents remained unchanged in the autopsy specimens, compared with the biopsy material. The chondroitin sulphate isomer pattern found in *Dupuytren*'s contracture tissue was characterized by a very marked increase in unsulphated chondroitin, which was 500 times higher than in healthy fascia (fig. 2). The main portion of the chondroitin sulphate fraction was the 6-sulphated component, which showed a concentration 10 times higher than in healthy fascia. However, in the dermatan sulphate fraction the elevation in diseased palmar fascia was due entirely to the 4-sulphated compound, which is the predominant isomer in the healthy palmar fascia (fig. 2). Regarding the unsulphated and 6-sulphated compounds, it must be kept in mind that the concentrations of these substances in palmar fascia are at the lower limit of the range of measurement of the method applied. Therefore, no conclusive statement can be made as to whether changes in the dermatan sulphate isomer pattern occur during the course of the disease (fig. 2).

Although less pronounced, the changes in the glycosaminoglycan patterns in tissue adjacent to nodules

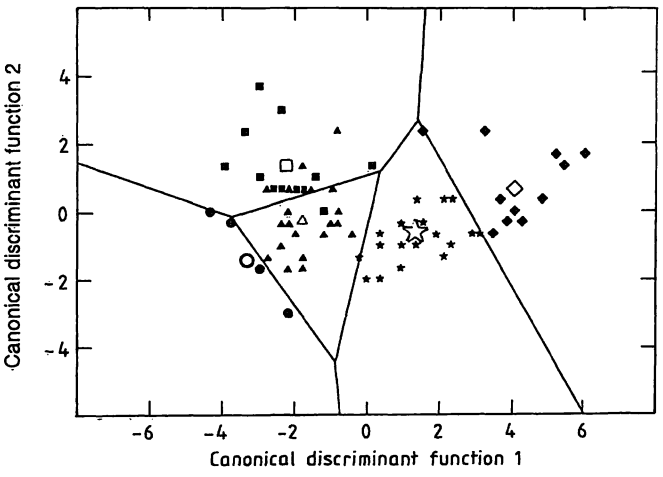

Fig. 4. Classification of specimens from healthy palmar fascia and *Dupuytren*'s disease by multivariate statistical evaluation (18) of the glycosaminoglycan patterns: 9 variables (hyaluronate, total chondroitin sulphate, total dermatan sulphate, and the respective unsulphated, C4- and C6-sulphated isomers).
● healthy control
■ apparent normal fascia
▲ fascia adjacent to strang and nodules
★ strang
◆ nodules
The open symbols indicate the respective group centroid.

Tab. 3. Clinical, histomorphological and biochemical classification of 46 specimens from *Dupuytren*'s disease. Clinical classification according to the macroscopic appearance of the specimen. Microscopic staging according to *Millesi* (16). Biochemical classification by multivariate statistical analysis (17) of the glycosaminoglycan patterns. Each number in the morphologic stage areas represents one classified specimen. Its size expresses the respective biochemical classification (stage 0: healthy palmar fascia, diseased fascia stages 1 through 4).

Clinical classification \ Microscopic staging	1 A	1 B	2	3
Group 1 Apparently normal	1 1	1 2		
Group 2 Tissue adjacent to bands and nodules	0 2 2 2 2 2 2 2 2	0 1 1 1 2 2 2 2		
Group 3 Bands		3 3 3 3 3 3 3 3	3 3 3 3 3	2 2
Group 4 Nodules		3	3 4 4 4 4 4 4 4	4

Tab. 4. Classification of results of specimens from healthy palmar fascia and *Dupuytren*'s disease by multivariate statistical analysis (18) on the basis of 9 variables of biochemical parameters (see fig. 3). Definitions of groups predicted according to the macroscopic appearance of the specimens.

Classification of results							
Actual group		No. of cases	Predicted group membership				
			1	2	3	4	5
Group 0 Healthy controls	1	4	4 *100.0%*	0 0.0%	0 0.0%	0 0.0%	0 0.0%
Group 1 Apparently normal fascia	2	12	0 0.0%	8 *66.7%*	3 *25.0%*	1 *8.3%*	0 0.0%
Group 2 Adjacent fascia	3	20	1 *5.0%*	4 *20.0%*	15 *75.0%*	0 0.0%	0 0.0%
Group 3 Band	4	19	0 0.0%	0 0.0%	0 0.0%	18 *94.7%*	1 *5.3%*
Group 4 Nodules	5	11	0 0.0%	0 0.0%	0 0.0%	2 *18.2%*	9 *81.8%*

Percent of "Grouped" cases correctly classified: *81.82%*

and bands as well as in apparently healthy portions of diseased palmar fascia were still great enough to classify these tissue as clearly diseased (fig. 3, tab. 1). These findings are compatible with the elevation in DNA and in hydroxyproline concentrations (tab. 1) as well as with the increase in the number of cells (2, 18) reported for these specimens of diseased palmar fascia.

A multivariate statistical analysis (17) of the data yielded a classification that clearly distinguished between the specimens taken from nodules or bands and those from apparently normal or adjacent tissue, or from the healthy reference specimens (fig. 4, tab. 3). This biochemical classification was thus in good agreement with the macroscopic classification. A less satisfactory agreement, however, was found with the results of the microscopic examinations (tab. 4).

Discussion

Data on the total glycosaminoglycan content, as well as on the distribution pattern of their fractions of palmar fascia from healthy subjects and from individuals with *Dupuytren*'s contracture are sparse and for methodological reasons of limited value. Using electrophoresis for differentiation of the glycosaminoglycans, *Flint* and coworkers (8) showed that the elevation of the total glycosaminoglycans seen in *Dupuytren*'s tissue is based mainly on the increase in the chondroitin sulphate fraction. The findings in the present study are in general agreement with the observations of *Flint* et al., although no detailed comparisons can be made because the *Flint* findings are based on relative concentrations of glycosaminoglycans and the use of autopsy specimens for reference. In contrast, our data were obtained by specific enzymatic degradation of the glycosaminoglycans and quantitative determination of the respective disaccharide metabolites by high performance liquid chromatography. The specificity, sensitivity, and reliability of this procedure allow quantitative analyses on very small specimens from various parts of the afflicted palmar fascia and differentiation between the sulphated and unsulphated isomers of the individual glycosaminoglycans. The glycosaminoglycan isomer pattern found in the *Dupuytren*'s contracture tissue showed an increase in the 6-sulphated compounds, and thus a change in the ratio of the 4- to the 6-sulphates (fig. 2). With regard to the high synthesis rate of chondroitin-6-sulphate, it seems likely that the concentration of the unsulphated precursors should also increase. The overall ratio of unsulphated to sulphated glycosaminoglycans shifts toward the sulphated compounds because of the decrease in the hyaluronate concentration and the increase in both chondroitin and dermatan sulphate as the disease progresses. *Mori & Honda* (12) presented experimental evidence for the functional importance of the proteoglycan sulphate groups in fibrillogenesis in heart valve fascia. These findings support the hypothesis that changes in the relation of sulphated to unsulphated glycosaminoglycans in *Dupuytren*'s contracture give rise to aberrations in the structure of fibre proteins and the subsequent contracture. The biochemical changes found in the glycosaminoglycans allowed staging of the disease in which stages 3 (band) and 4 (nodules) could be clearly identified, but stages 1 (apparently normal) and 2 (adjacent tissue) could not be separated from each other or from healthy palmar fascia. However, the biochemical changes in stages 1 and 2 did provide clear evidence of the onset of the pathological process in these portions of tissue that appeared to be unaffected when examined macroscopically. The same was true for the microscopic examination of both stage 1 and stage 2 specimens, although the staging of a given specimen was sometimes different from the macroscopic and/or biochemical classification (tab. 4). This difference resulted from the morphological inhomogeneity of the diseased tissue and the consequent effect of sample selection on the classification. In contrast, the biochemical analysis provided a finding that was valid for the whole specimen. In clinically and macroscopically unsuspicious palmar fascia (stage 1 in the present study) *Kischer & Speer* (2) and *Mohr & Vossbeck* (18) demonstrated, by electron microscopy and autoradiography respectively, a proliferation of endothelial cells in capillaries, migration of these cells and perivascular accumulation. *Kischer & Speer* (2) term this early stage of the disease "pre-*Dupuytren*", which may correlate with our biochemically defined stage 1 (apparently normal). Cell culture experiments with cell lines derived from cells isolated from *Dupuytren*'s contracture specimens showed an abnormal capacity to synthesise sulphated glycosaminoglycans and collagen, which the authors attribute to a permanent modulation of metabolic cell characteristics, which can be propagated in cell culture (19). In addition to the dedifferentiation of palmar fascia cells or cells of other origin, continuous stimulation of the fascia cells by abnormal matrix components or exogenous substances such as growth factors may also induce and maintain metabolic malfunctions.

In conclusion, glycosaminoglycan analysis with the combined enzymatic/HPLC method used in the present study allows characterization and classification of biopsy specimens from *Dupuytren*'s contracture. The typical changes in the isomer fractions suggest metabolic aberrations in cells of so far unknown origin and dedifferentiation. Malcomposition of the extracellular matrix carbohydrates suggests that these carbohydrates may interact with the cell surface, thereby inducing and maintaining aberrations of metabolism and proliferation of the cells. The interactions between fibre proteins and proteoglycans are probably disturbed, with subsequent development of the contracture. Regarding the latter process, a study on the role of proteoglycans in the pathogenesis of the contracture is in progress.

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