Proteoglycans and glycosaminoglycans

NORMAL PALMAR FASCIA

In the normal, healthy palmar fascia a network of specifically oriented connective tissue fibres consisting of collagen type I is embedded in a ground substance made up of proteoglycans, hyaluronate and proteins. Distributed throughout this substance are the cells that synthesize the macromolecular components of the palmar fascia and keep them functioning via a steady state of synthesis and degradation. The interactions of these macromolecules with each other and the effects of mechanical forces contribute to the formation of the architecture that is typical for this tissue, the basic characteristics of which are already evident in the morphological three-dimensional arrangement of the connective tissue cells at the embryonal stage (Chapter 11).

Proteoglycans are biological macromolecules with a protein core to which at least one glycosaminoglycan chain and oligosaccharides are covalently linked. The glycosaminoglycans are polyanionic chains of different lengths consisting of repeating series of disaccharide units. One component of these disaccharides is always an N-acetylated amino sugar, to which a sulphate group may be attached. The other component is a uronic acid. These macromolecules play an important role in the physical and functional characteristics of the connective tissue, e.g. molecular interactions, aggregation, viscosity, permeability, water-attracting capacity and swelling (Donoff & Schweidt 1982; Flint et al 1982).

The type and pattern of the glycosaminoglycans is specific for the individual types of connective tissue. Figure 5.1 shows the types and amounts of the different sulphated and non-sulphated glycosaminoglycans in healthy human palmar fascia (Tunn et al 1988). Characteristically there is a high percentage of the non-sulphated hyaluronate, and among the sulphated glycosaminoglycans dermatan sulphate predominates, as also shown by Flint et al (1982). Heparan sulphate occurs in trace amounts and is probably a component of the cell surface structure.

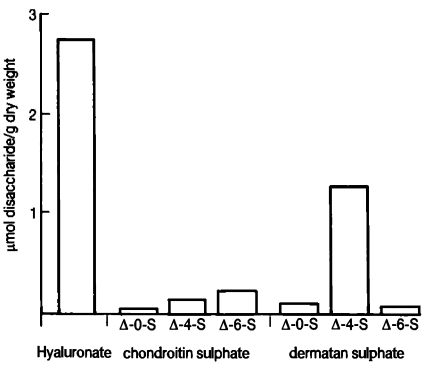

Fig. 5.1 Glycosaminoglycan pattern of human healthy palmar fascia. Combined enzymatic–high performance liquid chromatography analysis according to Gurr et al (1985). Biopsy.
Δ–0–S = non-sulphated disaccharide metabolite of glycosaminoglycan;
Δ–4–S = C–4–sulphated disaccharide metabolite of glycosaminoglycan;
Δ–6–S = C–6–sulphated disaccharide metabolite of glycosaminoglycan.
Total glycosaminoglycans = 4.45 µmol/g dry weight.

In: McFarlane RM, McGrouther DA, Flint MH (eds):
Dupuytren's Disease Biology and Treatment
Churchill Livingstone Edinburg 1990.

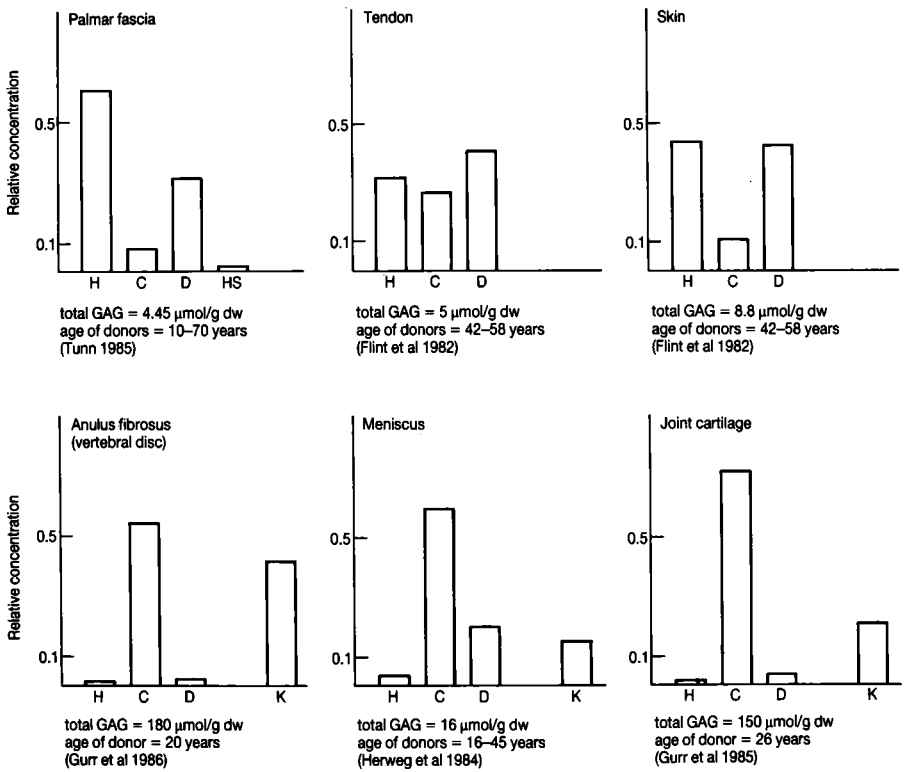

Fig. 5.2 Glycosaminoglycan (GAG) pattern from healthy human palmar fascia in comparison with other human connective tissues in relative concentrations. H = hyaluronate; C = chondroitin sulphate; D = dermatan sulphate; HS = heparan sulphate; K = keratan sulphate. dw = Dry weight.

Figure 5.2 shows that in spite of limitations in comparability related to methodology, the profiles for other types of connective tissue are clearly different from that found in the palmar fascia. Skin and tendon tissue also have relatively large proportions of dermatan sulphate, whereas anulus fibrosus, meniscus and joint cartilage all contain very small proportions of hyaluronate and large amounts of chondroitin sulphate and keratan sulphate. The large amount of hyaluronate in the palmar fascia cannot be explained solely by the formation of complexes with proteoglycans such as occurs in cartilage. Rather, we must assume that the physical and chemical properties of hyaluronate are of importance for the function of the tissue as such, as for example Viidik et al (1982) have discussed regarding the function of the tendons. The characteristic glycosaminoglycan content is paralleled by an equally typical collagen profile consisting almost exclusively of collagen type I; there is less than 5% collagen type III in healthy palmar fascia (Menzel et al 1979).

The structures of the proteoglycans from palmar fascia have not yet been studied. The best we can do is to make assumptions on the basis of our knowledge about proteoglycans isolated from tis-

sues related to palmar fascia. Proteoglycans extracted from tendon consist of two different subpopulations (Vogel & Heinegard 1985). One of these, which contains about 12% of the total proteoglycan content, has a high molecular weight and chondroitin sulphate side chains. In areas where the tendons are subject to relatively high compressive loads, the percentage of large proteoglycans containing chondroitin sulphate increases to about 50%, and this difference is maintained when the tendinocytes are placed in cell cultures and synthesize proteoglycans under culture conditions (Vogel et al 1986).

In contrast to the small proteoglycans containing dermatan sulphate, the large proteoglycans from tendon containing chondroitin sulphate bind to hyaluronate in the same way as the proteoglycans in cartilage. In contrast, Scott (1984) found that the low molecular weight dermatan sulphate proteoglycan of tendon binds specifically to the surface of the collagen type I fibrils. Vogel et al (1984) showed that it binds via the protein core. From skin it was possible to isolate a subpopulation of the proteoglycans containing chondroitin sulphate with a high molecular weight and another subpopulation containing dermatan sulphate with a lower molecular weight.

In contrast to tendon and cartilage, the proteoglycans in the skin that contain chondroitin sulphate do not bind to hyaluronate. Moreover, the proportions of the two types of proteoglycans in the skin are age-dependent; the percentage of proteoglycans containing dermatan sulphate increases with age (Habuchi et al 1986). Studies on human rectus sheath revealed the presence of three types of proteoglycans. In addition to a high molecular weight fraction, two low-molecular weight proteoglycans are present, distinguished by a difference in their electrophoretic mobility (Gurr et al, unpublished observations). If hypotheses about the structure of the extracellular matrix of the palmar fascia are based on these findings, one would expect to find (at least) two different types of proteoglycans. The glycosaminoglycan pattern of healthy palmar fascia suggests that the main portion of proteoglycans in this tissue belongs to the dermatan sulphate-containing fraction of low molecular weight.

The steady state of the extracellular matrix of healthy palmar fascia is maintained by the metabolism of the fascia cells. Specific enzymes catalyse the different steps in the synthesis and degradation of the matrix components and the intermediary and energy metabolism. The extracellular matrix provides a pathway for the metabolic exchange with the vascular system, and therefore factors affecting metabolism produced in specific organs or tissues also reach the cells of the palmar fascia. Information on the activity of the enzymes involved in the intermediary and energy metabolism of the palmar fascia can be found in Hoopes et al (1977) and Delbrück et al (1981). Although differences in the methods employed make it difficult to compare the findings, the two studies are in agreement that fascia cells, which synthesize the specific components of the palmar fascia matrix from basic components such as glucose and amino acids and make available the necessary energy (Delbrück et al 1981), have a high metabolic potential. Energy production is mainly via glycolysis, as is evident from the activity of lactate dehydrogenase (EC no. 1.1.1.27) and glucose-6-phosphate dehydrogenase (EC no. 1.1.1.49) in the enzyme profile (Delbrück et al 1959). An enzyme profile which is almost identical to that of the palmar fascia in vivo can be found in fibroblast cultures of cells from human palmar fascia (Delbrück et al 1981). The authors could also show that the activity of lysosomal enzymes in vivo behaved in the same manner as in fibroblast cultures of cells from the palmar fascia.

Studies on the synthesis of extracellular matrix components in which [^{35}S]-sulphate and [^{3}H]-proline were incorporated into glycosaminoglycans and collagen respectively have shown the ability of these cells to synthesize the specific matrix components of the palmar fascia in a defined in vitro system (Fig. 5.3). The fact that the rates of synthesis depend on the cell density in the cultures indicates that the interaction of the cells with each other and/or with the surrounding matrix has a regulatory influence on cell metabolism (Delbrück & Schröder 1982). Handley et al (1985) hypothesized a feedback regulation of the synthesis of the proteoglycans for cartilage, and this would appear to hold for the palmar fascia, too. Although there is no evidence so far that any hormones play a role in the regulation of metabolism

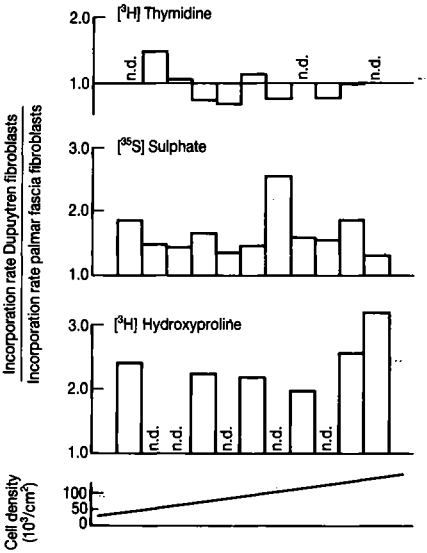

Fig. 5.3 Incorporation of labelled precursors into DNA, glycosaminoglycans and collagen by cultured fibroblasts from DD and normal human palmar fascia. Determinations in quadruplicate cultures, Dupuytren and palmar fascia lines are matched according to the cell density. The incorporation rates of palmar fascia are set at 1.0
n.d. = not determined.

in the cells of the palmar fascia, within the framework of the overall control of metabolism in the human body such influences must be assumed to exist. Another factor involved in the regulation of the metabolic equilibrium and the types and amounts of the extracellular matrix components was identified by Gillard et al (1979), Merrilees & Flint (1980), and Gurr et al (1985a), namely the mechanical forces which act on tissues and cells, which can, among other things, lead to a change in the glycosaminoglycan content of rabbit tendon.

Although there are many gaps in our knowledge about the biochemistry of the palmar fascia, it is evident that complex metabolic processes produce and maintain the biochemical and morphological structure of healthy palmar fascia, enabling the mechanics of hand movements to function smoothly.

THE PALMAR FASCIA IN DD

Under pathological conditions, various factors can have inhibitory or activating effects on the complex metabolic processes that take place in the cells of the palmar fascia, resulting in an imbalance, with consequences for the structure and function of this tissue. To do justice to the dynamics of the pathological process in DD, a careful treatment is required of the individual stages seen in the development of the disorder. In agreement with Flint et al (1982), a classification according to macroscopic appearance is recommended: apparently normal fascia; fascia adjacent to bands or nodules (termed fibrous bands by Flint et al); bands (called fleshy bands by Flint et al) and nodules.

Assessed in terms of DNA concentration, fascia tissue affected by Dupuytren's process has a much larger cellular component than normal fascia (Hoopes et al 1977; Delbrück et al 1981, Tunn 1985; Fig. 5.4). Morphologically, cell proliferation begins in the perivascular space (Millesi 1981). Mohr & Vossbeck (1985) found an increase in [^{3}H]-thymidin uptake by the cells which Kischer & Speer (1984) have termed pericytes and that they consider to be the initial manifestation and

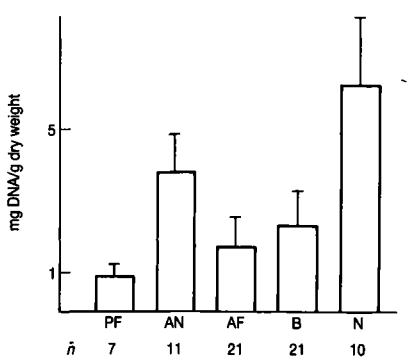

Fig. 5.4 DNA content in specimens of DD in comparison with normal palmar fascia (PF). AN = Apparently normal fascia; AF = fascia adjacent to bands or nodules; B = bands; N = nodules. n = number of biopsies (patients). Bars indicate standard deviation. From Tunn (1985).

starting point of the pathogenetic chain. In DD these authors found perivascular cell proliferation even in palmar fascia tissue which had not yet shown any macroscopic changes. This morphological picture is consistent with the finding that the increase in DNA concentration as a measure of the cell count is particularly marked in specimens of diseased fascia that have been classified as 'apparently normal' (Fig. 5.4).

No differences were observed in the growth rates of isolated cells from healthy palmar fascia and DD tissue in cell culture (Delbrück & Schröder 1982), however. Furthermore, there was no activation of [^{3}H]-thymidin incorporation into the cells from DD tissue.

Azzarone et al (1983) also found identical cell growth kinetics for cells from tissue of DD and healthy palmar fascia. The number of DNA-synthesizing cells was unaltered in the plateau phase of growth. However, the authors were able to show that in contrast to skin fibroblasts, the cells from DD tissue grow and form colonies in an agar culture medium. In monolayer cultures they reach a higher final cell density than healthy skin fibroblasts.

The findings were similar in a study by Rüssel & Witt (1976) on cell cultures of human fibroblasts from keloid and scar tissue. As in DD tissue in vivo, the cells in these tissues are apparently stimulated to proliferate by factors outside the cells such as connective tissue-activating peptide (Castor et al 1979) and growth factors from platelets (Dresow et al 1986) which stimulate cell proliferation in vivo and, after substitution, also in vitro. In addition to cell proliferation, the rates of synthesis of the glycosaminoglycans and collagens can be increased in vitro by the action of the growth factor from platelets on cells of healthy palmar fascia (Dresow et al 1986).

In tissue of DD the total glycosaminoglycan content and the proportions of the different glycosaminoglycans are different from those in healthy palmar fascia. In the papers published on this subject over the last 15 years there has been general agreement about the increase in the total glycosaminoglycans and the shifts in proportions of different glycosaminoglycans, as Table 5.1 shows. The few contradictory findings (Hunter et al 1975) are probably due to methodological problems, which in the past have severely limited the usefulness of the experimental findings. Characteristic differences in the total and fractional content at different stages of DD have been identified, and they suggest associated structural and quantitative changes in the proteoglycans.

Figure 5.5 shows the glycosaminoglycan profile in the palmar fascia at different stages of the disease (Tunn 1985). There is a decrease in the concentration of hyaluronate, with a 50% reduc-

Table 5.1 Biochemical characteristics in specimens from Dupuytren's contracture

Component	Concentration in Dupuytren's contracture	References
Glycosaminoglycans	Exceeding normal tendon	Viljanto et al (1971)
	Exceeding normal palmar fascia (autopsy)	Flint et al (1982)
	Exceeding normal skin (autopsy)	Bazin et al (1980)
	Exceeding palmar fascia (biopsy carpal tunnel syndrome)	Carr et al (1970)
Total collagen	Exceeding normal tendon	Bazin et al (1980); Gelbermann et al (1980)
	Exceeding normal palmar fascia (biopsy)	Brickley-Parson et al (1981)
DNA	Exceeding normal palmar fascia (autopsy)	Delbrück et al (1981)
Water	Exceeding normal tendon	Bazin et al (1980)
Hyaluronate	Lower than normal palmar fascia and tendon (autopsy)	Flint et al (1982)
Dermatan sulphate	Exceeding normal palmar fascia and tendon (autopsy)	Flint et al (1982)
Chondroitin sulphate	Exceeding normal palmar fascia and tendon (autopsy)	Flint et al (1982) Bazin et al (1980)

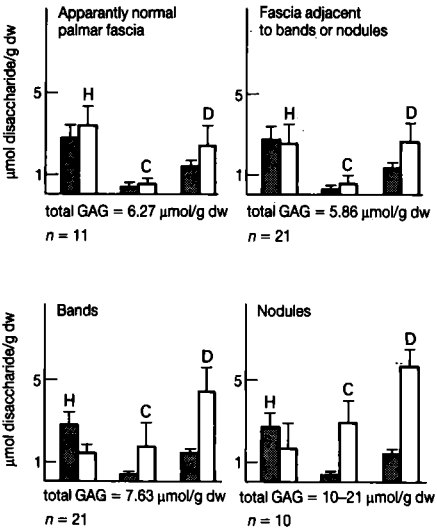

Fig. 5.5 Glycosaminoglycan (GAG) concentrations in different stages of DD. Specimens are grouped according to macroscopic appearances. H = hyaluronate; C = chondroitin sulphate; D = dermatan sulphate. Shaded boxes indicate the respective reference values from healthy palmar fascia biopsies (n = 7; 4.45 µmol/g dry weight total GAG; see Fig. 5.1). Bars indicate standard deviation; dw = dry weight. From Tunn et al (1988).

tion in bands and nodules compared with healthy palmar fascia after a slight initial increase in apparently normal fascia material. Parallel to these changes there is an increase in the proportions of chondroitin sulphate and dermatan sulphate, reaching a concentration three times the normal level. As in healthy palmar fascia, the concentration of dermatan sulphate exceeds that of chondroin sulphate. There is thus an overall shift from the non-sulphated to the sulphated glycosaminoglycans; the quotient of non-sulphated divided by sulphated glycosaminoglycans drops from 1.62 to 0.20.

The findings of Flint et al (1982) on the total and fractional glycosaminoglycan content of these three components are generally consistent with those of Tunn (1985) and Tunn et al (1988), in spite of differences attributable to different methods. Tunn et al used a method that enables differentiation of the individual sulphated subfraction (Gurr et al 1985b). As Figure 5.6 shows, they found the greatest relative increase in the chondroitin sulphate fractions, and within that fraction a substantial increase in chondroitin-6-sulphate. The overall concentration of the dermatan sulphate fraction increased by a factor of five compared with normal fascia, but there was no change in the proportions of the various subfractions.

Characteristic changes in the composition of glycosaminoglycans and their subfractions (see above) in specimens from diseased portions of palmar fascia allow classification of the biopsy material according to the stage of the disease. With multivariate analysis (Schneider 1970) it was possible to group exactly the bands and nodules and the normal palmar fascia. As expected, apparently normal tissue and fascia adjacent to bands and nodules were not significantly different from each other but were significantly different from the other groups (Fig. 5.7). Microscopic examinations using the Millesi (1981) classification yielded poorer agreement, however. The discrepancies between morphological (microscopical) and biochemical classifications resulted from the morphological inhomogeneity of the diseased tissue examined and the consequent effect of sample selection on the classification.

The distinct glycosaminoglycan patterns in the various stages of DD lead us to expect similar findings for the proteoglycans in the afflicted tissue portions. However, no data have been published so far on the types and amounts of proteoglycans in DD tissue. Gurr & Borchert (1988) were able to isolate proteoglycans from DD. Gel permeation chromatography and electrophoresis in agarose or polyacrylamidegel enabled identification of at least two proteoglycan subpopulations differing in size and electrophoretic mobility. Based on the proportion of chondroitin sulphate and dermatan sulphate in DD, one can assume that in the course of this disease the high molecular weight proteoglycans increase to 30–40% while the low molecular weight proteoglycans decrease to about 60% of the total proteoglycans (Table 5.2). The low molecular weight proteoglycan population seems to be com-

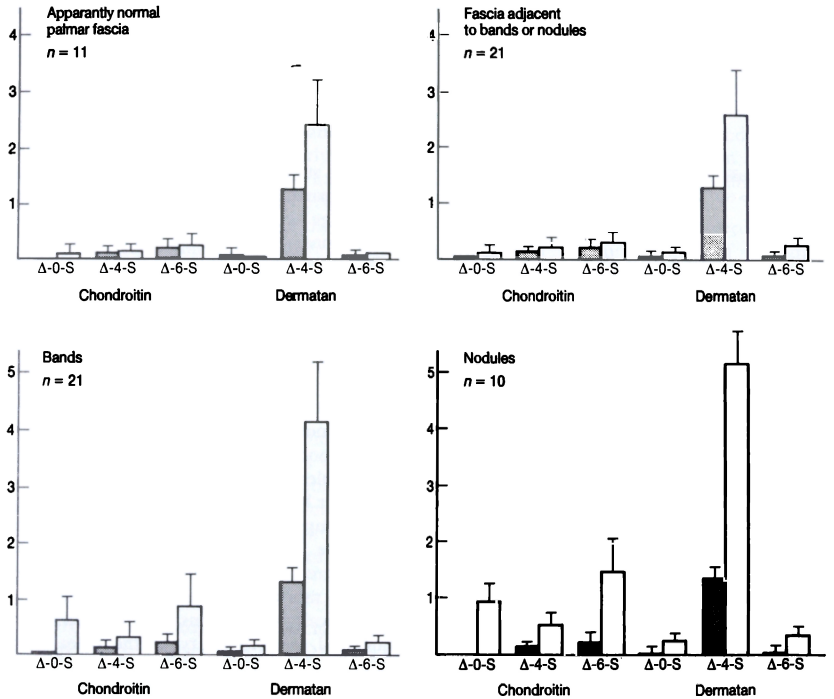

Fig. 5.6 Glycosaminoglycan patterns in Dupuytren's contracture specimens, grouped according to macroscopic appearance. Shaded boxes indicate the pattern of healthy palmar fascia biopsies for reference (n = 7). Bars indicate standard deviation. From Tunn et al (1988).

Table 5.2 High and low molecular weight (HMW, LMW) proteoglycans in palmar fascia and DD calculated on the basis of the proportion of chondroitin sulphate or dermatan sulphate

Proteoglycan	Normal palmar fascia	Dupuytren's disease
HMW proteoglycan (chondroitin sulphate)	19.4%	31.6%
LMW proteoglycans (dermatan sulphate)	80.6%	68.4%

posed of two subpopulations. The importance of these changes in the proteoglycan pattern for the fibrillogenesis and the collagen or dermatan sulphate proteoglycan interaction (Scott & Hughes 1986) is still unknown.

The altered steady state of matrix components in DD tissue makes it likely that there are metabolic aberrations in the cells of the palmar fascia or in those cells invaded from foreign tissues. In a study of the types and amounts of main metabolic pathway enzymes it was found that the increases in enzyme activity correlated with the increase in the amount of DNA in specimens of healthy palmar fascia and DD tissue (Delbrück et al 1981). Furthermore, when the activity of glycerinaldehyde-phosphate dehydrogenase (EC no. 1.2.1.12), one of the key enzymes of the

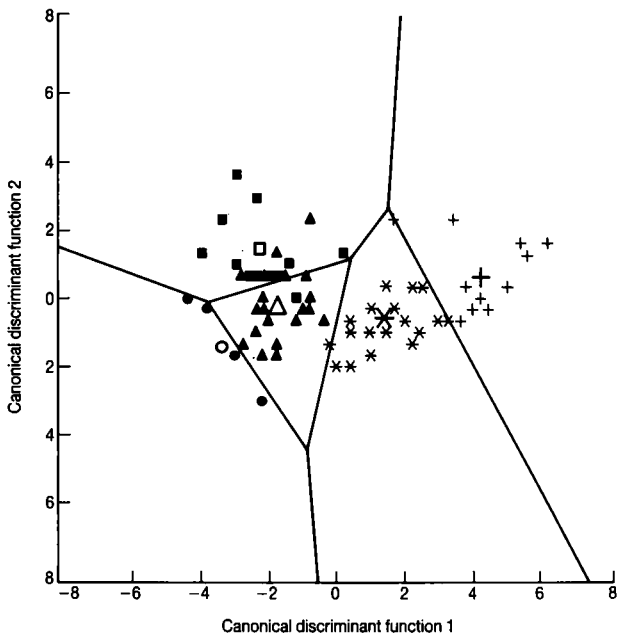

Fig. 5.7 Classification of specimens from healthy palmar fascia and DD by multivariate statistical evaluation (18) of the glycosaminoglycan patterns: nine variables (hyaluronate, total chondroitin sulphate, total dermatan sulphate, and the respective unsulphated, C4 and C6-sulphated isomers). Open symbols indicate group centres. ● = Healthy controls; ■ = apparently normal fascia; ▲ = fascia adjacent to strands and nodules; ★ = strands; ◆ = nodules. From Tunn et al (1988).

Embden–Meyerhof pathway, was used as a point of reference (Delbrück et al 1959), no changes were found in the relative amounts of activity of the different enzymes in fascia tissue at different stages of DD (Delbrück et al 1981). Moreover, even in isolated cultured cells from healthy and pathological palmar fascia tissue there were no changes in the proportions or in the overall activity of the enzymes. The contradictory findings of Hoopes et al (1977) can be explained by methodological differences, which have been discussed elsewhere (Delbrück et al 1981).

In contrast to the main metabolic pathway enzymes, the activities of those enzymes involved in the synthesis of the specific extracellular matrix components show a marked increase in the cells of DD tissue, as could be demonstrated in a study on the incorporation rates of marked precursors of glycosaminoglycan and collagen synthesis in isolated cells in vitro (Delbrück & Schröder 1982; Fig. 5.3). It must be assumed that there has been a modulation of synthesis metabolism and that it has been transferred to the culture.

The findings have been similar in studies on isolated cells from other fibromatous tissues such as keloid tissue (Diegelmann et al 1977), cirrhotic liver (Galambos et al 1977) and arteriosclerotic vessel wall (Mey et al 1980). On the other hand, there are no changes in the types and amounts of lysosomal enzymes involved in the degradation of the matrix components in the cells of DD tissue as compared with healthy palmar fascia (Delbrück et al 1981). This is true for the hexosaminidases, glucuronidases and sulphatases just as it is for the

collagen peptidases. No data are available on the collagenases. For fibroblasts from normal palmar fascia and DD tissue there are no differences in vitro in the activity levels of the lysosomal enzymes (Delbrück et al 1981). The differences between these findings and those of Hoopes et al (1977) are probably of a methodological nature (see above).

The site of action of these enzymes is sometimes extracellular, and intracellularly the lysosomes are only required to the extent that pieces of the extracellular matrix components are internalized. Therefore, the enzymes involved in degradation are probably subject to regulatory mechanisms which activate only a portion of the enzyme activity available from a constant pool of lysosomal total enzyme activity. In the case of cartilage, for example, it is known that interleukin I, as the mediator of cartilage degradation, acts by stimulating the release of proteinases, proteoglycanases and other lysosomal enzymes from the chondrocytes (Tyler 1985). Another possibility is that lysosomal enzymes of different origins, e.g. from macrophages or granulocytes that have migrated into the matrix, may initiate the degradation of the matrix outside the cell. Similar mechanisms need to be discussed with reference to the pathogenesis of DD; as yet, no experimental data are available to support this hypothesis.

On balance, the metabolic rates of the synthesis and degradation of the extracellular matrix components in Dupuytren's disease tissue are shifted toward synthesis. Therefore, an accumulation of both proteoglycans and collagen would be expected; in fact this does occur. In addition to this increase in collagen in DD tissue there is also an enormous increase in the collagen type III fraction, which is extremely small in the healthy palmar fascia (Menzel et al 1979; Bazin et al 1980; Gelbermann et al 1980; Brickley-Parson et al 1981).

Although a good number of detailed studies have been conducted on biochemical changes in DD, the causes of the disease are still unknown. Furthermore, the pathogenesis of the disorder is still only poorly understood. The heterogenous morphological picture of the disease at different stages (Mohr & Vosbeck 1985), which leads to a much wider range of findings than in healthy palmar fascia, is a complicating factor in the interpretation of findings obtained in biochemical studies. However, biochemical and microscopic investigations have clearly demonstrated that the onset of the pathological changes in the palmar fascia occurs before the afflicted tissue is diseased by clinical observation. The cell population in the diseased palmar fascia is not uniform. Mohr & Vosbeck (1985) differentiate between the tendinocytes, i.e. the cells of the healthy palmar fascia, and the proliferating endothelial cells and pericytes as well as the macrophages and lymphocytes that have migrated into the fascia. All types of cells found in a tissue sample can contribute in differing degrees to the metabolism of the diseased palmar fascia. Therefore, it is not surprising that in DD the composition of the proteoglycans indicated by their glycosaminoglycan patterns does not resemble any known pattern for other types of connective tissue (Fig. 5.8). The same holds for a calculated pattern of glycosaminoglycans produced by a mixed tendinocyte and pericyte population present in an assumed ratio of 1:4 (see Fig. 5.8). The most likely explanation for this aberration is atypical synthesis of matrix components caused by a modulated or uncontrolled metabolism in the cells of the DD tissue. In this case there may be a lack of differentiation of cells of the palmar fascia or of those cells which invaded the fascia from other tissue sources.

In as much as no changes of any consequence are apparent in the activity levels of the enzymes involved in the energy and intermediary metabolism in the direction of what is found in malignant tumours, from a biochemical perspective the presence of a malignant process is rather unlikely. This view is supported by the studies of Azzarone et al (1983), in which Dupuytren cells were compared with healthy skin fibroblasts and the tumour cell lines for a number of cell physiology criteria. From the criteria selected the authors found that the Dupuytren's cells were in a middle position between the healthy fibroblasts and the tumour cells. It is interesting to note that these phenomena of modulation of cell behaviour could be observed even in cells isolated from macroscopically normal fascia tissue taken from an individual with DD. The aetiology of this modulation of metabolism and of the proliferation

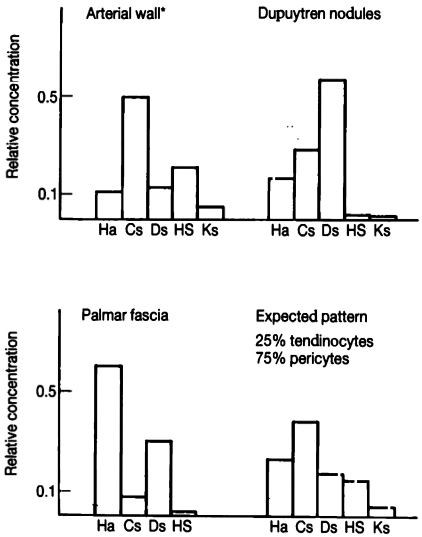

Fig. 5.8 Glycosaminoglycan patterns of arterial wall, Dupuytren's contracture (nodules) and palmar fascia in relative concentrations. Calculated pattern of a diseased palmar fascia containing 25% normal tendinocytes and 75% pericytes. H = Hyaluronate; C = chondroitin sulphate; DS = dermatan sulphate; HS = heparan sulphate; K = keratan sulphate. * = Stuhlsatz et al (1980).

behaviour of the cells in the diseased palmar fascia is still unclear. The explanation will probably be found on the level of the genome, the translation or the post-translational modifications of the synthesis of the matrix components. On all three levels exogenous, still unknown factors could be the precipitating factors. If there is an alteration in the genetic code or if exogenous factors affect metabolism constantly, then the modulation is perpetuated, and it can be increased through feedback mechanisms in the production of atypical matrix components. The latter also alter the characteristics of the pathway for the metabolites of the cells in DD tissue, thus contributing to the vicious circle. The result is the predominant symptom of DD — the contracture with thickening and shrinking of the palmar fascia.

In spite of marked progress, our understanding of the pathobiochemistry of DD is still limited, and this prevents a detailed characterization of the factors directly responsible for the development of the contracture. It is conceivable that in addition to the altered physical chemistry, i.e. the pathological content of the extracellular matrix, external mechanical forces or the contractile elements of atypical cells (tractofibroblasts; Tomasek et al 1986) may be involved.