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# Biochemical Changes in the Collagen of the Palmar Fascia in Patients with Dupuytren's Disease*

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ABSTRACT: **The palmar fascial tissues of more than 400 patients with Dupuytren's disease were studied biochemically and compared with normal tissue obtained from more than 100 patients who were undergoing hand surgery for other reasons. No alterations of the molecular structure or the state of macromolecular aggregation of the collagen in Dupuytren's disease were detected by wide or low-angle x-ray diffraction studies or by transmission electron microscopy. Major biochemical changes in the palmar fascia affected by Dupuytren's disease included increased collagen and hexosamine contents and the presence of galactosamine in the most severely involved tissue. Type-III collagen, which is virtually absent from normal adult palmar fascia, was abundant in the tissue of patients with Dupuytren's disease. Post-translational modifications included a very elevated hydroxylysine content, an increase in the total number of reducible cross-links, and the appearance of hydroxylysinohydroxynorleucine (virtually absent from normal palmar fascia) as the major reducible cross-link. Even palmar fascia from patients with Dupuytren's disease that appeared grossly and histologically normal showed the same biochemical changes, albeit to a lesser extent. All of these biochemical changes are similar to those that occur during the active stages of connective-tissue wound repair. This includes the rapid synthesis and turnover of collagen which leads to newly synthesized, immature collagen being more abundant in the involved tissue than in normal tissue.**

**There is no evidence that the gross, macroscopic contracture of the palmar fascia in Dupuytren's disease is due to shortening, plication, or contraction of the collagen fibrils or fibers present in the tissue at the onset of the disease or synthesized during its development. Instead, we propose that the gross contracture (shortening) of the palmar fascia in Dupuytren's disease is due to an active cellular process that progressively draws the distal extremities of the affected tissue closer together at the same time that the original tissue is being replaced. The result of these two processes is simply a shorter, smaller piece of tissue fabric containing collagen molecules, fibrils, and fibers of normal length and organization, but with pretranslational and post-translational modifications similar to those observed in collagens during the active stages of connective-tissue repair in general.**

CLINICAL SIGNIFICANCE: **Compositional and post-translational modifications of the collagen in Dupuytren's disease are *not* the underlying basis for the gross shortening of the tissue fabric; rather they simply represent the usual changes that occur in rapidly synthesized, new collagen during the active stages of repair and healing of connective tissues. The fact that grossly and histologically normal palmar fascia in patients with Dupuytren's disease shows the biochemical signs of repair may account for the relatively high rate of recurrence after surgical excision of the clinically and grossly affected tissue.**

Dupuytren's disease (Dupuytren's contracture) results in progressive and irreversible flexion of the fingers

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due to alteration of the palmar fascia. The pathogenesis of these changes has not been determined despite many excellent histological and clinical studies. Although the lesion principally involves the palmar aponeurosis[38,43], Hueston[32] suggested that the initial pathological changes begin in the prefascial spaces between the skin and the palmar aponeurosis. The major clinical features of the changes in the palmar fascia are discrete nodules and contracted longitudinal bands[38]. The nodules contain tissues with a high cellular density and develop during the early stages of the disease[38,43]. The longitudinal bands consist principally of collagen fibers and represent a more advanced and less biologically active phase of the disease[38,43]. In the late and most biologically quiescent states of the disease, few if any nodules are present, and the predominant clinical and histological feature of the disease is a shorter, densely packed, tough, inelastic, and fibrotic palmar fascia[38,43].

Early morphological studies of Dupuytren's disease investigated possible changes in the macromolecular organization of the structural components[15,24,35,44]. As no abnormalities were noted, attention soon was focused on the cells, especially on those in the nodules. Many of these cells were found to have some of the ultrastructural features of contractile smooth-muscle cells[8] and were termed *myofibroblasts*[12,24,26]. The presence of such cells in Dupuytren's disease, and in granulating wounds in general[26,40], led to the problematic suggestion that contraction of these cells was the underlying basis for the gross contractures of the palmar aponeurosis[12,24] and for the contractures of wound defects and scars in general[25,26].

More recently, attention has been centered again on biochemical changes of the collagen in Dupuytren's disease. Preliminary studies have clearly indicated major differences between the collagen of normal palmar fascia and that of the palmar fascia of patients with Dupuytren's disease[1,5,11]. In the present study, we report principally biochemical data detailing these changes and correlate these findings with the morphological and histological appearance of the tissue. Based on these laboratory findings and on others that have been reported, we concluded that the gross contracture (shortening) of the palmar aponeurotic tissues in Dupuytren's disease does not simply represent a shortening or plication of the collagen fibrils or fibers that are present in the tissue when the disease begins or that are synthesized during its development; that is, no denaturation or other intrinsic changes in the length of the collagen fibrils or fibers *per se*, and no external force — such as a force attributable to myofibroblast contraction — *directly* accounts for the clinically observable palmar contractures of Dupuytren's disease. The biochemical changes noted in the collagen component of the fascia in Dupuytren's disease show that the active stage of the disease is characterized by a rapid remodeling of the tissue and consequently by the presence of rapidly synthesized new collagen with post-translational modifications and genetic composition similar to those observed in the active stages of wound repair in general. Based on these observations and on those in the literature, we propose a mechanism for the production of the macroscopic palmar contractures in this disease.

Preliminary results of these studies have been presented elsewhere[1,11].

## Materials and Methods

Specimens of palmar fascia were obtained from patients with Dupuytren's disease and from patients who had no evidence of the disease as well as no history of hypertrophic scarring or keloid formation. The patients who provided this normal palmar fascial tissue had no history of any of the collagen diseases and were undergoing surgical treatment for unrelated conditions, such as non-rheumatoid carpal-tunnel syndrome, acute tendon and nerve injuries, and so forth. None of the so-called normal specimens were taken from areas of previous surgical treatment or scarring.

The specimens from patients with Dupuytren's disease were divided into four categories clinically. *Nodules* were spherical or globular masses of fibrous tissue that appeared to have longitudinal orientation and often were intimately adherent to the overlying skin. Nodules were found most frequently in the distal part of the palm or between the proximal and middle flexion creases of the fingers. In some patients the nodules represented the only abnormal portion of the palmar fascia, as the surgeon could palpate no contracted longitudinal bands. In these patients, the nodules lay principally in the distal part of the palm over the fourth and fifth metacarpal heads. When there was tissue that was seen clinically to have a longitudinal orientation of its fibers, this tissue was termed a *contracted longitudinal band*. These bands were occasionally as long as six to seven centimeters and were two centimeters wide. The bands could be palpated preoperatively and felt like tight, thickened cords that would bowstring across the metacarpophalangeal or interphalangeal joints. When the surgeon thought that portions of the palmar fascia were abnormal but were neither nodular nor as condensed and inelastic as longitudinal bands, and did not appear to be causing contracture, such tissue was termed *mildly involved bands or tissue*. In addition to the three types of pathological tissue, the surgeon removed samples of what appeared to be perfectly normal palmar fascia. These tissue specimens were termed *apparently uninvolved fascia*.

Normal tissue was obtained from more than 100 patients without Dupuytren's disease. Not all histological, electron microscopic, x-ray diffraction, or biochemical determinations were carried out on each sample of tissue. The number of samples, representing different patients, is listed separately in the Results section for each of the biochemical analyses. An attempt was made to compare the normal tissue with that from age-matched patients with Dupuytren's disease, although on the average the normal patients were somewhat younger than the patients with Dupuytren's disease. The ages of the patients with Dupuytren's disease ranged from forty to seventy-five years, with an average of approximately sixty years. About 90 per cent of the patients with Dupuytren's disease were men, whereas 50 to 60 per cent of the patients without Dupuytren's disease who served as normal controls were women. Preliminary analyses, however, showed no significant sex-linked differences in the biochemical and ultrastructural characteristics of either normal or pathological tissues. Therefore the results from both men and women were pooled.

### *Collagen and Hexosamine Contents and Over-All Amino-Acid Analyses*

Samples of fresh tissue, quickly rinsed of blood with doubly distilled water and carefully dissected free of any fat or extraneous tissue, were dried to constant weight. Samples were hydrolyzed in 6N hydrochloric acid at 105 degrees Celsius for twenty-two hours and subjected to complete amino-acid analysis using either a Beckman 121-M automatic amino-acid analyzer (Beckman Instrument Company, Fullerton, California) or a Phoenix automatic amino-acid analyzer (Phoenix Precision Instrument Division, Gardiner, New York) adapted to the single-column method of Piez and Morris[46]. Other aliquot samples of the 6N hydrochloric acid hydrolysate were analyzed for hydroxyproline by the method of Stegemann[55]. Approximately seventy-five samples of each of the categories of palmar fascia from patients with Dupuytren's disease and fifty control samples were subjected to complete amino-acid analysis. In the remaining normal and Dupuytren's disease samples, hydroxyproline and hydroxylysine contents were determined by ion-exchange chromatography[48].

The total hexosamine content and the concentrations of galactosamine and glucosamine were determined by ion-exchange chromatography as described by Eyre et al.[20].

### *Solubility of Collagen as Undenatured and Denatured Protein (Gelatin)*

Freshly removed tissue was washed quickly with doubly distilled water, dissected free of gross subcutaneous fat, and minced over ice into approximately one-millimeter pieces. Approximately ten to fifteen milligrams (wet weight) of this tissue was suspended in fifteen milliliters of one-molar sodium chloride, pH 7.4, and stirred at 4 degrees Celsius for forty-eight hours, and a second extraction was carried out for an additional forty-eight hours with fresh buffer. The supernatant was separated by low-speed centrifugation at 4 degrees Celsius for twenty minutes

and the residue was washed three times with water. The pooled supernatant of the two extracts is considered the one-molar salt-soluble collagen. The acid-soluble collagen fraction was obtained by placing the salt-insoluble residue in 3 per cent acetic acid at 2 degrees Celsius for forty-eight hours, repeating the extraction once with fresh acetic acid, and pooling the centrifuged supernatants. The amount of collagen that could be solubilized as gelatin from the salt-insoluble and acid-insoluble residues was determined by extraction of the residue in four-molar calcium chloride, pH 7.5, and 0.1-molar Tris buffer at 25 degrees Celsius for one week, repeating the procedure once with fresh buffer and pooling the centrifuged supernatant. All of the supernatants were dialyzed extensively against water at 4 degrees Celsius and freeze-dried. Aliquot samples then were used for total amino-acid analysis and hydroxyproline determinations, as described.

### *Reduction of Tissues with Tritiated Sodium Borohydride and Analysis for Reducible Collagen Cross-Links*[17]

Cleaned tissue samples were reacted with tritiated sodium borohydride (ten curies per mole, 0.01 millimole per ten milligrams of tissue) in 0.1-molar phosphate buffer, pH 7.4, for one hour at 25 degrees Celsius. Excess borohydride was destroyed by drop-by-drop addition of glacial acetic acid to pH 3.0. The samples were washed with water and dried in a vacuum desiccator over phosphorus pentoxide. Samples of known weight (approximately ten milligrams) were placed in two milliliters of 3N hydrochloric acid, sealed under nitrogen, and hydrolyzed at 105 degrees Celsius for forty-eight hours. Hydrolysates were dried on a Buchler Evapo-mix. The samples were dissolved in 0.2-molar sodium citrate, pH 2.2, and analyzed by elution from a sixty-centimeter column of an automatic amino-acid analyzer[17]. Using a split-stream device, $^3$H-activity could be monitored continuously by a scintillation counter with a specially constructed flow-coil (Intertechnique model SL 20) which mixed the column effluent with scintillation fluid according to the method of Eyre[17]. The reducible cross-linking compounds were identified on the basis of their elution position[17].

### *Determination of Glycosylated Hydroxylysine Residues*

Samples of known weight (approximately twenty-five to fifty milligrams) were placed in two milliliters of 2N potassium hydroxide, sealed under nitrogen in alkali-resistant tubes, and hydrolyzed at 105 degrees Celsius for twenty-four hours. The hydrolysate was neutralized by the addition of cold three-molar perchloric acid, and the precipitate was separated by low-speed centrifugation and washed three times with small amounts of water. Supernatant and washings were pooled and dried on a Buchler Evapo-mix. Hydroxylysine and glycosylated hydroxylysine were eluted from a P-2 column and analyzed and chromatographed on an amino-acid analyzer[47].

### *Cyanogen Bromide Cleavage and SDS Gel Electrophoresis*

Aliquot samples of native tissue were suspended in cyanogen bromide in 70 per cent formic acid, flushed with nitrogen, stoppered, and incubated at 30 degrees Celsius for four hours with constant stirring[18]. The samples were centrifuged at 50,000 times gravity for thirty minutes and washed with water. The supernatant and washings were pooled, diluted eight to ten times with water, and lyophilized. Aliquot samples were electrophoresed in acrylamide gels containing 7.5 per cent sodium dodecyl sulphate and stained with Coomassie brilliant blue[49].

### *Collagen Polymorphism*

Peptides generated by cyanogen bromide digestion of native tissue were chromatographed on a column (0.9 × 15 centimeters) of CM-cellulose at 43 degrees Celsius[36], as follows. Samples (fifty to 100 milligrams) were dissolved in five milliliters of starting buffer (0.02N sodium citrate per 0.02-molar sodium chloride, pH 3.8) and applied to the column equilibrated in the same buffer. Peptides were eluted with a linear gradient formed between 250 milliliters each of the starting buffer and limiting buffer (0.02N sodium citrate per 0.16-molar sodium chloride, pH 3.8). A flow rate of forty-eight milliliters per hour was used and the effluent was continuously monitored at 230 nanometers. The fractions representing the CB8 peptide peak were pooled and lyophilized. Separation of this peptide into its constituents — $\alpha$1(III)CB8, $\alpha$1(I)CB8, aud $\alpha$1(I)CB8-3 — was achieved by molecular sieve chromatography on calibrated columns (2 × 120 centimeters) of agarose beads (Bio-Gel A-1.5m) in 1.0-molur calcium chloride per 0.05-molar Tris, pH 7.4, at room temperature[45]. All samples were heated to 43 degrees Celsius to ensure denaturation before being applied to the column. The molar ratio of type-I and type-III collagen was calculated from the molar ratios of $\alpha$1(I)CB8 plus $\alpha$1(I)CB8-3 and $\alpha$1(III)CB8, determined quantitatively by the hydroxyproline contents of each of the peptide peaks[50].

### *Histological Studies*

Immediately after removal from the patients, small pieces of tissue were fixed in 10 per cent neutral buffered formalin for light microscopy or in 2.5 per cent glutaraldehyde for electron microscopic studies, as described previously[52]. In addition to regular six-micrometer sections embedded in paraffin, one-micrometer-thick sections also were prepared for light microscopy from the tissue fixed for electron microscopy[52].

TABLE I

COLLAGEN CONTENT OF NORMAL AND DUPUYTREN'S FASCIA

| Fascia | Collagen (Hydroxyproline) Content* (*μg/mg Dry Wt. of Tissue*) | No. of Samples |
|---|---|---|
| Normal | 60.4 ± 2.4 | 56 |
| Dupuytren's disease | | |
| Apparently uninvolved | 73.4 ± 2.5 | 84 |
| Mildly involved | 87.6 ± 3.2 | 83 |
| Longitudinal bands | 91.3 ± 1.7 | 97 |
| Nodules | 108.6 ± 2.1 | 78 |

* Mean ± standard deviation.

### *X-Ray Diffraction Studies*

Wide and low-angle x-ray diffraction studies were carried out on thin longitudinal strips of tissue both dry and in the hydrated state, as previously described[29].

## Results

### *Chemical Composition*

The collagen and hexosamine contents of normal palmar fascia and of the palmar fascia of patients with Dupuytren's disease are shown in Tables I and II. There is a clear increase in the contents of collagen and hexosamine in the tissues from patients with Dupuytren's disease compared with normal fascia. Moreover, in general, the more active the disease process the more prominent are these changes. Thus, nodules contain the most collagen and hexosamine; contracted longitudinal bands, somewhat less; mildly involved fascia, still less; and apparently uninvolved fascia contains the least. In every sample examined, the apparently uninvolved tissue was clearly different biochemically from normal tissue, although it appeared free of the disease by gross and clinical standards.

In addition to the fourfold increase in the hexosamine content of the nodules of patients with Dupuytren's disease, this tissue was found to contain significant amounts of galactosamine, suggesting the presence of the chondroitin sulphates in the more severely involved tissues of Dupuytren's disease and their absence from less involved and normal tissues.

A marked increase of hydroxylysine content was the most significant divergence from normal in the amino-acid composition of tissues from patients with Dupuytren's disease. The extent of this increase again paralleled the se-

TABLE II

HEXOSAMINE CONTENTS IN NORMAL AND DUPUYTREN'S FASCIA*

| Fascia | Galactosamine | Glucosamine | Total |
|---|---|---|---|
| Normal | Not detected | 0.16 | 0.16 |
| Dupuytren's disease | | | |
| Apparently uninvolved | Not detected | 0.16 | 0.16 |
| Longitudinal band | Trace | 0.24 | 0.24 |
| Nodule | 0.32 | 0.32 | 0.64 |

* Values expressed as the per cent of the dry weight of the tissue. Each figure is the mean result of three samples.

TABLE III

HYDROXYLYSINE AND GLYCOSYLATED HYDROXYLYSINE CONTENTS IN NORMAL AND DUPUYTREN'S FASCIA

| Fascia | Hydroxylysine Residues/ 100 Hydroxyproline Residues*† | Glycosylated Hydroxylysine Residues*† *(Per cent)* | Glucosylgalactosyl-hydroxylysine/ Galactosylhydroxylysine Ratio |
|---|---|---|---|
| Normal | 5.3 ± 0.7 (100) | 34.9 ± 1.7 (38) | 1.53 |
| Dupuytren's disease | | | |
| Apparently uninvolved | 8.3 ± 1.1 (115) | 34.6 ± 1.3 (42) | 1.55 |
| Mildly involved | 10.3 ± 1.8 (200) | 35.4 ± 1.9 (81) | 1.57 |
| Longitudinal bands | 11.5 ± 1.7 (137) | 34.8 ± 1.4 (80) | 1.54 |
| Nodules | 13.9 ± 1.5 (107) | 35.5 ± 1.8 (64) | 1.55 |

* Mean ± standard deviation.
† Number of samples tested is in parentheses.

verity of tissue involvement (Table III). The increase in the number of hydroxylysine residues was accompanied by a parallel increase in the number of glycosylated hydroxylysine residues, so that the ratio of glycosylated hydroxylysine to non-glycosylated hydroxylysine residues is approximately the same in normal and diseased tissues (Table III). The ratio of glucosylgalactosylhydroxylysine to galactosylhydroxylysine also is unchanged despite the marked elevation in the total number of glycosylated hydroxylysine residues (Table III). However, until homogeneous preparations of type-I and type-III collagen are prepared from these palmar fascial tissues, one cannot tell whether the increased hydroxylysine content occurs predominantly in type-I or in type-III collagen, or is equally distributed between the two. Such studies are now under way.

*Nature and Amount of Reducible Cross-Links*

The number of reducible cross-links per milligram of collagen in the tissue in Dupuytren's disease was increased compared with normal tissue and, like the hydroxylysine content, was dependent on the degree of pathological involvement (Table IV). However, even more striking was the change found in the chemical nature of the major intermolecular cross-links. The predominant intermolecular cross-link of normal human palmar fascia is hydroxylysinonorleucine, with little or no hydroxylysinohydroxynorleucine being detectable. In contrast, the major reducible aldimine cross-link in all the tissues from patients with Dupuytren's disease is hydroxylysinohydroxynorleucine. The data are presented as percentages for all of the reducible components in Table V and for just the major reducible cross-linkages in Table VI.

Typical cross-link profiles are shown in Figure 1. From Tables V and VI and Figure 1, it is clear that while the concentration of hydroxylysinonorleucine remains relatively constant, the amount of hydroxylysinohydroxynorleucine increases as a function of pathological activity; consequently, the ratio of hydroxylysinohydroxynorleucine to hydroxylysinonorleucine also increases.

The relative concentrations of hexosyllysine and hexosylhydroxylysine *decrease* in the fascia in Dupuytren's disease (Table V), the decrease being directly related to pathological activity: the two peaks eluting in positions corresponding to standard hexosyllysine and hexosylhydroxylysine were observed to be present in much higher concentrations in normal fascia than in tissue from patients with Dupuytren's disease (Table V).

*Solubility*

The solubilities of the collagens in the palmar fascia, both as the undenatured protein and as the gelatin in a denaturant solvent, are shown in Table VII. Very little collagen was soluble from either the normal or involved fascia, although the latter showed a slight but statistically significant decrease in the amount of collagen that could be extracted as the gelatin at room temperature. This con-

TABLE IV

RELATIVE CONTENTS OF REDUCIBLE INTERMOLECULAR CROSS-LINKS IN COLLAGEN AFTER REDUCTION WITH TRITIATED SODIUM BOROHYDRIDE

| Fascia | Total of Reducible Intermolecular Cross-Links*† |
|---|---|
| Normal | 575 ± 104 |
| Dupuytren's disease | |
| Apparently uninvolved | 810 ± 115 |
| Mildly involved | 945 ± 85 |
| Longitudinal bands | 1172 ± 106 |
| Nodules | 1588 ± 105 |

* Mean ± standard deviation of seventy-five samples.
† Values expressed as counts per minute per milligram of hydroxyproline recovered in hydroxylysinohydroxynorleucine, hydroxylysinonorleucine, and hydroxymerodesmosine.

TABLE V

COMPLETE DISTRIBUTION OF REDUCIBLE CROSS-LINK COMPONENTS OF $^3$H-LABELED COMPONENTS FROM NORMAL AND DUPUYTREN'S FASCIA AFTER REDUCTION WITH TRITIATED SODIUM BOROHYDRIDE*

| Fascia | No. of Samples | Aldehyde Region | Hexosyllysine | Hexosyl-hydroxylysine | Hydroxylysino-hydroxynorleucine | Hydroxylysino-norleucine | Hydroxymero-desmosine | Scatter† | Hydroxylysinohydroxy-norleucine/Hydroxy-lysinonorleucine Ratio |
|---|---|---|---|---|---|---|---|---|---|
| Normal | 36 | 18.6 ± 2.2 | 18.2 ± 2.0 | 16.1 ± 1.7 | Not detected | 10.3 ± 1.6 | 26.4 ± 3.5 | 10.4 | — |
| Dupuytren's disease | | | | | | | | | |
| Apparently uninvolved | 44 | 21.0 ± 2.5 | 10.1 ± 2.3 | 11.6 ± 1.2 | 15.8 ± 1.8 | 10.5 ± 1.3 | 21.3 ± 3.3 | 9.7 | 1.5 |
| Mildly involved | 83 | 34.7 ± 2.7 | 6.3 ± 1.1 | 6.7 ± 0.9 | 16.9 ± 1.6 | 10.4 ± 1.5 | 17.0 ± 3.9 | 8.3 | 1.6 |
| Longitudinal bands | 87 | 39.8 ± 2.4 | 5.1 ± 1.0 | 5.9 ± 1.0 | 21.6 ± 1.5 | 9.9 ± 1.7 | 13.6 ± 3.1 | 8.8 | 2.2 |
| Nodules | 94 | 36.9 ± 2.3 | 1.7 ± 0.4 | 2.2 ± 0.3 | 31.4 ± 1.2 | 10.2 ± 1.5 | 6.9 ± 2.2 | 10.7 | 3.1 |

* Values are expressed as the percentage of the total counts per minute per milligram of hydroxyproline recovered in the cross-link region of the chromatograph. The average and standard deviation were determined for each set of samples.

† Total of all other small peaks in the chromatograph.

tributed to the slight over-all decrease in the total amounts of collagen solubilized from the tissue of the patients with Dupuytren's disease.

*Collagen Polymorphism*

A major finding was the presence of a significant amount of type-III collagen in tissues from patients with Dupuytren's disease, the amount paralleling the degree of pathological severity (Table VIII). Type-III collagen was not initially identified in normal fascia (Table VIII), although our more recent (unpublished) studies using very large samples of tissue have indicated the probable presence of very small, trace amounts of type-III collagen.

*Correlation of Biochemical Changes with Histological Appearance*

Histological sections of normal palmar fascia and of tissue removed from patients with Dupuytren's disease showed the characteristic changes that have been described well in the literature[38,43].

It is important to note that *in every patient with Dupuytren's disease the palmar fascial tissue that was considered both grossly and histologically normal demonstrated biochemical changes*. Electron microscopic examination of this apparently uninvolved fascia showed no abnormalities in the individual collagen fibrils or in their organization. Either no changes in the cells could be observed or only small changes were seen, and the detailed morphological ultrastructure of the cells was consistent with the changes that occur when fibroblasts are more actively synthesizing protein[58]. This latter possibility is consistent with studies of these tissues *in vitro* utilizing [$^3$H]proline[11,58] and [$^{14}$C]lysine[11] to monitor the rate of protein synthesis and reducible cross-link formation. No cells having the characteristic ultrastructure of myofibroblasts were observed in the grossly and histologically normal uninvolved tissue in patients with Dupuytren's disease, all of which showed the characteristic biochemical changes.

A complete description of the histological changes and the ultrastructural cellular changes in all of the various tissues in Dupuytren's disease and of the *in vitro* characteristics of the tissues in organ culture will be published separately.

*X-Ray Diffraction Studies*

Both the wide and low-angle x-ray-diffraction studies of wet and dried samples of tissue showed no detectable differences between the normal tissue and tissue from patients with Dupuytren's disease. Wide-angle x-ray dif-

TABLE VI

REDUCIBLE CROSS-LINKS IN COLLAGEN AFTER REDUCTION WITH TRITIATED SODIUM BOROHYDRIDE*

| Fascia | No. of Samples | Hydroxylysino-hydroxynorleucine | Hydroxylysino-norleucine | Hydroxy-merodesmosine | Hydroxylysinohydroxynor-leucine/Hydroxylysino-norleucine Ratio |
|---|---|---|---|---|---|
| Normal | 36 | 0 | 28.0 ± 1.6 | 72.0 ± 3.5 | — |
| Dupuytren's disease | | | | | |
| Apparently uninvolved | 44 | 33.1 ± 1.8 | 22.1 ± 1.3 | 44.7 ± 3.3 | 1.5 |
| Mildly involved | 83 | 41.8 ± 1.6 | 24.5 ± 1.5 | 33.6 ± 3.9 | 1.7 |
| Longitudinal bands | 87 | 44.0 ± 1.5 | 21.2 ± 1.7 | 34.7 ± 3.1 | 2.1 |
| Nodules | 94 | 64.7 ± 1.2 | 21.0 ± 1.5 | 14.0 ± 2.2 | 3.1 |

* Values expressed as percentage of counts per minute per milligram of hydroxyproline recovered.

TABLE VII

SOLUBILITY OF COLLAGEN AS NATIVE AND DENATURED PROTEIN FROM NORMAL AND DUPUYTREN'S FASCIA*†

| Fascia | Solvents: 1M Sodium Chloride, pH 7.4, 4°C | 3% Acetic Acid, 4°C | 4M Calcium Chloride, pH 7.4, 4°C | 4M Calcium Chloride, pH 7.4, 25°C | Total |
|---|---|---|---|---|---|
| Normal | 0.13 ± 0.02 | 0.18 ± 0.03 | 1.43 ± 0.16 | 4.6 ± 0.60 | 6.4 |
| Dupuytren's disease | | | | | |
| Apparently uninvolved | 0.13 ± 0.02 | 0.17 ± 0.03 | 1.44 ± 0.16 | 3.19 ± 0.44 | 4.9 |
| Mildly involved | 0.14 ± 0.02 | 0.18 ± 0.03 | 1.37 ± 0.19 | 2.67 ± 0.50 | 4.4 |
| Longitudinal bands | 0.13 ± 0.02 | 0.17 ± 0.03 | 1.38 ± 0.21 | 2.88 ± 0.63 | 4.6 |
| Nodules | 0.12 ± 0.02 | 0.16 ± 0.02 | 1.48 ± 0.23 | 2.03 ± 0.38 | 3.8 |

* Average values ± standard deviation of triplicate analyses of twenty samples of each tissue.
† Values expressed as percentage of total collagen content of tissue.

fraction showed no evidence of the kinds of changes in orientation and organization of the collagen that necessarily would accompany plication or bunching of the collagen fibrils, fibers, and fiber bundles. The axial periodicity of the collagens of all the wet tissues was approximately 690 ± 10 angstroms.

TABLE VIII

AMOUNT OF TYPE-III COLLAGEN IN NORMAL AND DUPUYTREN'S FASCIA

| Fascia | Type-III Collagen in Total Collagen Content* (Per cent) | |
|---|---|---|
| Normal | None detected | (N = 6) |
| Dupuytren's disease | | |
| Apparently uninvolved | 11.4 ± 0.54 | (N = 6) |
| Mildly involved | 19.8 ± 0.80 | (N = 5) |
| Longitudinal bands | 24.6 ± 1.1 | (N = 6) |
| Nodules | 27.9 ± 0.86 | (N = 8) |

* N = number of separate determinations on different *pooled* samples of normal and involved tissue.

## Discussion

The chemical composition of the collagens of the palmar fascia in patients with Dupuytren's disease is similar to that of the collagens of embryos and of very young animals, or of newly synthesized collagens formed during the healing of wounds and tissue defects in postnatal animals[2,4-10,22,23,25,26,30,42,50,53,54,56]. Compared with normal adult tissue, these tissues show: (1) a markedly elevated concentration of hydroxylysine with a corresponding increase in the total number, but not in the percentage, of glycosylated hydroxylysine residues, and with no change in the glycosylgalactosylhydroxylysine-galactosylhydroxylysine ratio; (2) an increase in the number of reducible aldimine intermolecular cross-links; (3) the appearance of hydroxylysinohydroxynorleucine, which is virtually absent from normal adult palmar fascia, as the major intermolecular cross-link; and (4) the appearance of significant amounts of type-III collagen, which also is virtually absent

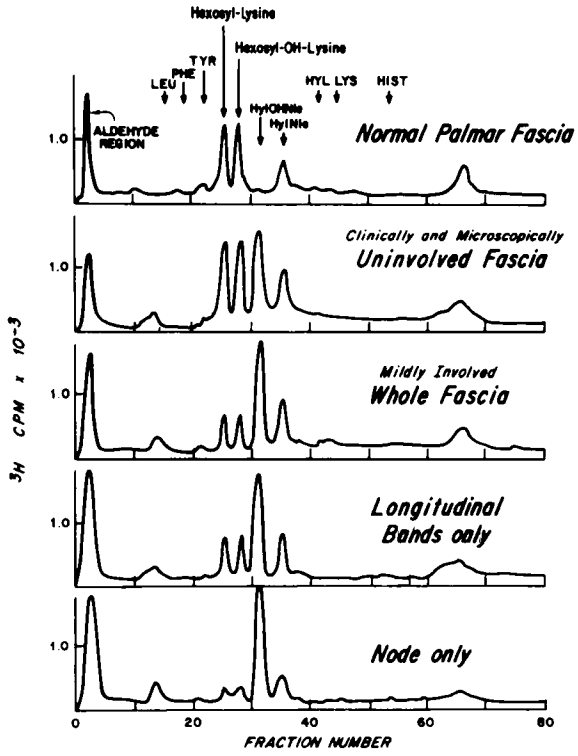


FIG. 1

Typical chromatographic profiles of reducible cross-links from normal palmar fascia and from the palmar fascia of patients with Dupuytren's disease. The chromatographic conditions are described in the text and in the literature[17]. Note the virtual absence of hydroxylysinohydroxynorleucine in the normal palmar fascia and its presence as the major reducible intermolecular cross-link in the palmar fascia of patients with Dupuytren's disease, including the tissue that was considered grossly and histologically normal.

from normal adult palmar fascia. The findings noted in the present study are similar to those reported by others[5,37]. The presence of significantly increased amounts of type-III collagen also has been observed during the active, rapidly healing phase of granulating wounds and scars[4,5,22]. In contrast, less type-III collagen has been found in relatively inactive, chronic fibrous lesions such as cirrhosis of the liver and pulmonary fibrosis[50].

Thus, the chemical modifications observed in the collagens of the palmar fascia of patients with Dupuytren's disease have many of the characteristics associated with newly and rapidly synthesized collagen and especially resemble those of collagen formed during the active phase of wound-healing. *During its active period,* when the tissue fabric is shortening and the clinical features of the disease are developing, Dupuytren's disease behaves biologically like a local, active repair of the palmar fascia with a sustained rapid turnover of the collagen. As a consequence, the involved contracted palmar fascia contains, proportionately, a great deal more young, newly and rapidly synthesized collagen than does normal palmar fascia.

Further support for the proposal that the changes observed in the collagen of the palmar fascia in Dupuytren's disease are similar to those of a repair response comes from biochemical analyses of the palmar fascia of a thirty-one-year-old man who sustained an injury to the palm of the hand while playing handball, and whose fascia, a sample of which was obtained at operation eight months later, showed the same changes in the collagen as those observed in Dupuytren's disease.

Despite the clear-cut biochemical changes observed in the collagen and in the proteoglycan components of the connective tissue of the palmar fascia in Dupuytren's disease, and the ultrastructural changes observed in certain of the connective-tissue cells[12,24,26], it is not an easy matter to relate these changes to the major clinical feature of the disease, namely the contracted palmar fascia. Indeed, there is no general agreement in the literature on what the gross clinical contracture of the palmar fascia represents or how it develops.

A commonly held belief is that the fascial contracture is simply a physical shortening of the collagen, the forces being generated either *intrinsically* due to changes in the collagen *per se* (for example, denaturation) or *extrinsically* on the collagen or the distal extremities of the tissue[12,32,39] (Fig. 2). Although collagen fibers do shorten when the triple helical configuration of the molecules is lost during denaturation (Fig. 2), none of the biochemical, morphological, or structural observations[15,24,35,44,58] support the proposal that grossly contracted fascia consists principally of contracted denatured collagen (gelatin). Similarly, there is no evidence that the same volume and mass of collagen that originally is present in the fascia before the onset of the disease is simply folded and plicated in order to fit into a shortened span between the opposite edges of the tissue (Fig. 2). The bunching and balling-up of the tissue which necessarily would have to accompany such crimping is not observed clinically, nor is there any evidence for such gross distortions of the collagen from light microscopy, transmission electron microscopy, or scanning electron microscopy[21,33]. Indeed, both the wide and low-angle x-ray-diffraction studies showed that the collagen in Dupuytren's fascia was better oriented and organized than the collagen in normal fascia.

How then to explain what the macroscopic contracture of the palmar fascia in Dupuytren's disease is, how it develops, and what, if any, are the relationships between the palmar fascial contracture and the biochemical changes in the collagen of the contracted fascia? On the basis of the x-ray diffraction, electron microscopic, and other studies described in this report, and on a variety of observations by other investigators[15,24,33,34,44,58], we concluded firstly that the gross contracture of the palmar fascia in Dupuytren's disease consists of a smaller piece of otherwise *struc-*

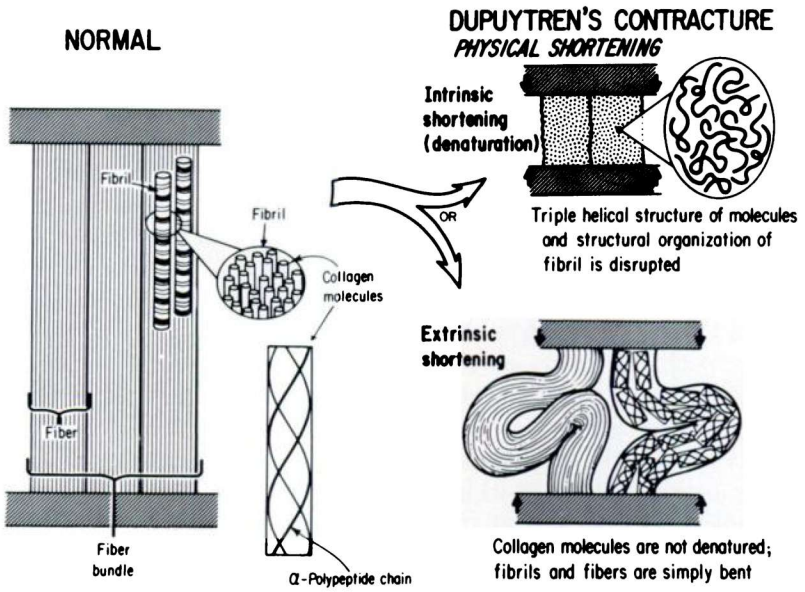


FIG. 2

Schematic drawing illustrating two possible ways in which a physical shortening of collagen might occur and thus account for the shortening of the palmar tissue fabric in Dupuytren's disease: (a) by intrinsic changes within the collagen — that is, by denaturation, and (b) by plication or folding of the fibrils, fibers, fiber bundles, and higher-ordered structural elements. Neither of these explanations accounts satisfactorily for the gross shortening or contracture of the palmar fascia in Dupuytren's disease.

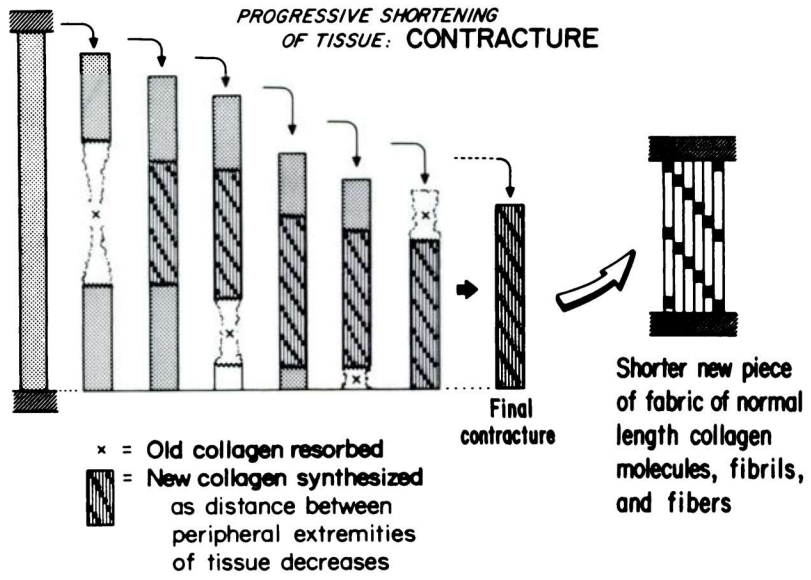


FIG. 3

Illustration of the proposal that the gross shortening of the palmar fascia tissue fabric (the contracture *per se*) in Dupuytren's disease represents a newly synthesized, shorter piece of palmar fascia containing structurally normal collagen that has replaced the original palmar fascia. In brief, as the distal ends of palmar fascia are brought closer together (possibly the role of myofibroblasts), the old collagen is resorbed and replaced by progressively less collagen; that is, by a smaller, shorter, new piece of tissue fabric. The configuration of the collagen molecules and their macromolecular organization in the fibrils are normal. There is no gross distinction or crimping of the higher-ordered fibers in this new tissue fabric.

*turally normal* tissue and does *not* represent a folding, plication, or shortening of the structural elements of collagen within this tissue[12,32] (Figs. 2 and 3). Secondly, we think that the most likely chain of biological events leading to the gross contractures of the palmar fascia in Dupuytren's disease probably parallels what occurs during the healing of wound defects and the subsequent formation of scar contractures in general. During the healing and repair of tissue defects, the size of the defect is continuously and progressively diminished by the centripetal movement of the wound edges. As the opposing edges of a granulating and healing wound are drawn together, the defect, progressively and continuously diminishing in size, is continuously being filled with newly synthesized collagen while at the same time the old collagen is being resorbed[11,13,14,58]. The progressive decrease in the size of the original defect together with the simultaneous resorption of the collagen in the wound and its replacement with lesser amounts of new collagen eventually result in a smaller wound defect covered by a smaller piece of tissue fabric in which the molecules, fibrils, fibers, and fiber bundles of the collagen are of normal length. If one views Dupuytren's disease biologically as a relatively continuous process of active repair, then the process of drawing together the extremities of the involved fascia along with rapid turnover and synthesis of new collagen in the intervening space gradually lead to a macroscopic shortening of the length of the palmar fascia. The cells continue to resorb existing collagen and replace it with smaller amounts of structurally normal collagen. In effect, as the distance spanned by the tissue progressively decreases, the cells simply reweave a progressively shorter or smaller piece of fabric containing collagen molecules, fibrils, and fibers of normal length and organization (Fig. 3).

However, as is true of wound and scar contracture in general, the cellular mechanisms responsible for the progressive and continuous centripetal movement of the peripheral extremities of the tissue are unknown. There are, however, several plausible explanations. Recent studies have described the presence of specialized cells (myofibroblasts) in granulating wounds in general and in the palmar fascia of patients with Dupuytren's disease[12,24,26]. These cells appear to have the ability to contract and on this basis have been implicated in the genesis of wound contracture in general and in Dupuytren's disease specifically. One could postulate that these or other specialized cells in the repair tissue migrate centripetally, pulling the edges of the affected tissue progressively closer together; the cells' motility possibly may be related to their ability to contract[31]. Alternatively, cellular contraction itself may cause the edges of a wound to draw closer together without any concomitant migration toward the center of the tissue (Fig. 4). To accomplish the task of bringing the wound edges closer together by either of these mechanisms, a number of specific conditions must be met. First, there must be adhesions by means of which contraction of these cells can be transmitted to each other and to other extracellular components, such as collagen. Second, such adhesions must be strong enough so that the tissue fabric can be tugged and thereby moved. If there were no physical connection or adhesion between the cells and the tissue fabric, or if the adhesion between the cells and the extracellular structures were not strong enough, the microscopic contraction of the cells would not be able to effect a

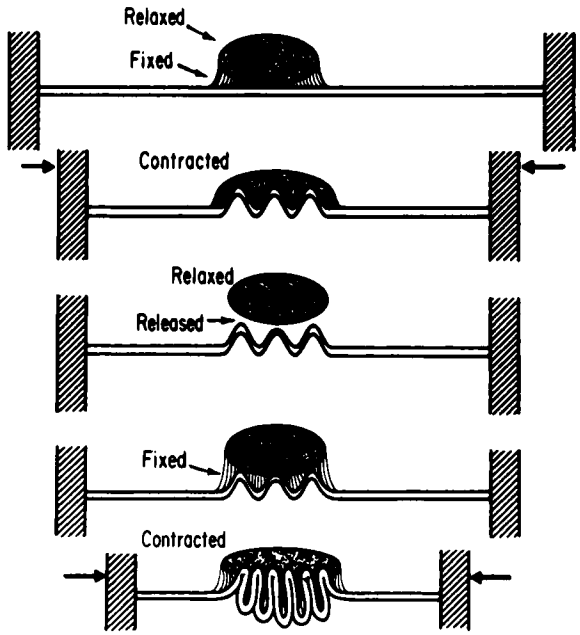


FIG. 4

Schematic drawing demonstrating how simple contraction and relaxation of cells could shorten the distance between the distal extremities of the wound or of a tissue fabric. The cell contracts, producing minute wrinkles in the underlying tissue and shortening the distance between the distal edges of the tissue. When the cell then relaxes, its adhesion to the tissue is released but the tissue remains shortened. The cell then reattaches itself at the same site, wrinkling the substratum still further. Repeated contractions of the cells would serve to progressively draw the edges of the affected tissue together. The minute wrinkles in the tissue substratum are not meant to imply that there is a permanent gross plication or shortening of the collagen elements in the palmar fascia proper. The drawing merely depicts how repeated cell contraction and relaxation could progressively draw the ends of a tissue together. Indeed, the cells need not be within the main body of the fascia at all but may be, for example, at one end of the tissue only. In any event, the collagen that is temporarily plicated by either cell motion or cell contraction, or both, would be quickly resorbed and replaced by new collagen of normal structure, forming a new and shorter piece of tissue.

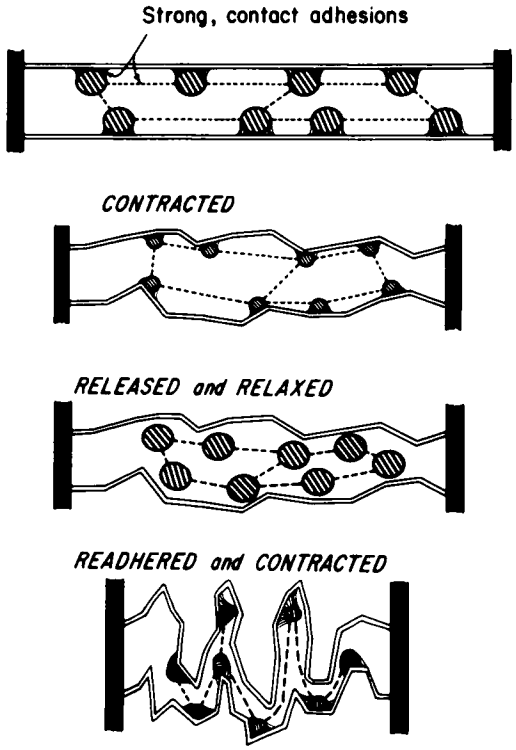


FIG. 5

Schemata showing how the alternative contraction and relaxation of a cell such as a myofibroblast could eventually shorten the distance between the distal ends of a tissue fabric without the cells migrating, provided that the cells released their adhesion on the substratum between successive contractions. As noted earlier, the plicated collagen is resorbed and replaced with new collagen spanning the shorter distance between the distal ends of the tissue. The shorter tissue containing the newly synthesized, structurally normal collagen and other tissue elements represents the gross contracture observed clinically.

change in the size of the structural elements in the tissue matrix and in the length of the tissue. The myofibroblasts may be likened to a group of raisins (cells) mixed together with a heap of spaghetti (the collagen fibers). If the raisins contract, nothing will happen to the spaghetti unless the raisins adhere to the spaghetti and also to each other. Electron microscopic studies have suggested that there are indeed physical connections between the individual myofibroblasts and between the myofibroblasts and collagen fibrils in Dupuytren's palmar fascia as well as in granulating wounds[25,26]; thus, the conditions described appear to be met by the myofibroblasts. If cell migration is the important factor, there would have to exist, in addition, some means by which the cell, through repetitive contraction and relaxation, could move centripetally — perhaps in the manner of an inchworm. Another alternative, which entails no cell migration, is that repeated contractions of cells could progressively shorten a tissue if after each contraction, which itself wrinkled and thereby shortened the tissue fabric, the strong bonds between cell and tissue were cleaved, to be reformed before or during the succeeding contraction (Figs. 4 and 5). Otherwise, if adhered cells

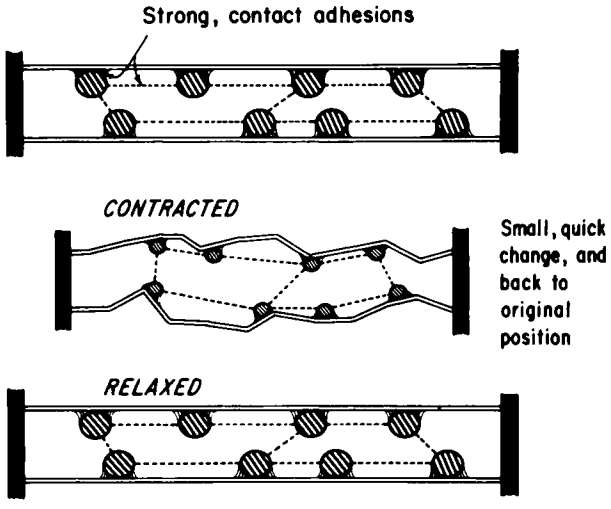


FIG. 6

Schemata showing that even if strong contact adhesions exist from cell to cell and from cell to matrix, simple contraction by the fibroblasts may cause a structural or physical shortening that promptly disappears as soon as the cells return to a relaxed state. For cell contraction to draw the tissue edges closer together, it is necessary for the structural shortening to remain while the cells release themselves from the matrix and then reattach to it, thereby contracting and shortening the tissue still further (see Fig. 5). In all of these schemata, it is understood that the progressive shortening of the tissue over a long period of time is accomplished by the continued proliferation and differentiation of myofibroblasts or other connective-tissue cells.

merely contracted and relaxed repeatedly, this would simply alternately shorten and lengthen the cells and the collagen fibers they adhere to; both would regain their original length and position on relaxation and no net shortening of the tissue *fabric* would result (Fig. 6), as in the active contraction and relaxation of the heart. It is worth noting that myofibroblasts may not be the only cells implicated in drawing the distal edges of the tissue together in Dupuytren's contracture. Recent electron microscopic studies have suggested that fibroblasts in general wrinkle a substratum[31], and thus they too may have the ability to diminish the distance between distal extremities by one or both of the mechanisms just described.

In any event, we would like to stress the point that if the myofibroblasts or other connective-tissue cells play a role in the genesis of the gross contractures of the palmar fascia in Dupuytren's disease, it is their potential ability to bring the distal ends of the fascia together that is crucial.

It is important to note both the clinical and the biological significance of the fact that seemingly uninvolved palmar fascia of Dupuytren patients that appears normal both macroscopically and microscopically nevertheless is undergoing the biochemical changes of repair. Clinically, this may point to the basic reason why in many patients excision of the grossly affected tissue does not eliminate the disease, and why the disease often is seen later in adjacent parts of the palm that earlier appeared clinically normal. To understand the biology of the disease, it is important to note that the biochemically abnormal tissue that seems clinically and histologically normal lacks myofibroblasts and other evident cellular abnormalities. If myofibroblasts indeed contribute to both the genesis of the gross contracture and the biochemical abnormalities of the connective tissue in Dupuytren's disease, it is clear that they are *not* the cells that initiate the biochemical changes, as these changes are already apparent before any fibroblasts present in the tissue have undergone the internal modifications that transform them into myofibroblasts, or, alternatively, before myofibroblasts from adjacent regions are able to spread to the involved tissue.

Superficially the results of the collagen-solubility experiments with the fascia of patients with Dupuytren's contracture seem paradoxical, since with so much newly synthesized collagen present one would expect a high degree of extractability, and yet this is not the case. This paradox can be resolved by considering the fact that the newly synthesized collagen molecules in Dupuytren's disease are stabilized principally by the aldimine cross-link hydroxylysinohydroxynorleucine, which is cleaved much less readily than the aldimine cross-link hydroxylysinonorleucine or the histidinyl hydroxymerodesmosine cross-link present in the collagen of normal fascia[3,16,18,19,27,28]. The decreasing number of hexosyllysine and hexosylhydroxylysine cross-links with increasing severity of the involvement of Dupuytren's disease is what one would expect from a tissue that contains a greater proportion of young, newly synthesized collagen, since hexosyllysine and hexosylhydroxylysine cross-links presumably represent bonds between the collagen and the proteoglycan components[37], and as a general rule these cross-links increase in number with age and maturation[51].

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Note added in proof: Since the submission of this manuscript, two papers have appeared that reported similar biochemical changes in the fasica of patients with Dupuytren's disease:

1. BAZIN, S.; LE LOUS, M.; DUANCE, V. C.; SIMS, T. J.; BAILEY, A. J.; GABBIANI, G.; D'ANDIRAN, G.; PIZZOLATO, G.; BROWSKI, A.; NICOLETIS, C.; and DELAUNAY, A.: Biochemistry and Histology of the Connective Tissue of Dupuytren's Disease Lesions. European J. Clin. Invest., **10:** 9-16, 1980.
2. GELBERMAN, R. H.; AMIEL, DAVID; RUDOLPH, R. M.; and VANCE, R. M.: Dupuytren's Contracture. An Electron Microscopic, Biochemical, and Clinical Correlative Study. J. Bone and Joint Surg., **62-A:** 425-432, April 1980.