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Dupuytren's Contracture

AN ELECTRON MICROSCOPIC, BIOCHEMICAL, AND CLINICAL CORRELATIVE STUDY†‡

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ABSTRACT: Fascial specimens were obtained from twenty-four patients treated operatively for Dupuytren's contracture. The nodules and cords were examined by electron microscopic and biochemical techniques. The clinical course and response to operative treatment were then correlated with the tissue findings.

Electron microscopic analysis revealed myofibroblasts in the nodules of seven of twenty-four patients. Fibroblasts containing prominent microtubules were found in ten patients. The fascia contained type-III collagen, increased amounts of collagen per unit of dry weight, and an increase in reducible cross-links of collagen. While the nodules were noted to contain a greater increase in reducible cross-links than the cords, there was little variation in the biochemical findings from patient to patient.

Clinical recurrence was not related to the age of the patient at onset, duration, or severity of disease. Recurrence was related to the electron microscopic findings of myofibroblasts in the nodules and fibroblasts containing prominent microtubules in the fascia of these patients.

The examination of fascia from patients with Dupuytren's contracture for electron microscopic and biochemical abnormalities has provided several new and significant findings over the past decade. A correlation of these findings may yield a better understanding of the pathogenesis of Dupuytren's contracture. In 1972, Gabbiani and Majno first described the presence of an unusual fibroblast, the myofibroblast, in tissue obtained from six patients with Dupuytren's contracture. They proposed that the myofibroblast was an active contractile cell, and implicated it in the process of fascial contraction. Myofibroblasts were consistently located in the nodules of the palmar fascia of their patients. Albin et al., in 1975, found that the collagen of fascia from patients with Dupuytren's contracture was less soluble than the collagen of normal fascia. They also noted a major difference in the amino-acid composition with an increase in hydroxylysine. This was accompanied by a change in the relative concentrations of the intermolecular reducible Schiff base cross-links. An increase in the major reduced aldimesines, hydroxylsinsinoxyronorleucine and hydroxylsinoonorleucine, was confirmed by Brickley-Parsons et al. in 1976. Bailey et al. found that the fascia of patients with Dupuytren's contracture contained type-III collagen, different from the type-I collagen present in normal palmar aponeurosis. They noted the similarity between palmar fasciitis and post-traumatic reparative scar tissue, which also contains type-III collagen.

There has been no previous correlative study of the biochemical and electron microscopic findings in fascia obtained from the same patients with Dupuytren's contracture. A correlation of these findings with the patients' clinical course also has not been done. In order to evaluate the clinical significance of these tissue characteristics, we obtained fascial specimens from patients treated operatively for Dupuytren's contracture, and carried out a comparison of the electron microscopic and biochemical findings with the history and response to treatment. We were particularly interested in the tissue findings relating to clinical recurrence following fasciectomy. The results of this clinical correlation form the basis of this report.

Materials and Methods

Of thirty-six consecutive patients treated operatively for Dupuytren's contracture on the Hand Surgery Service at the University Hospital and Veterans Administration Medical Center, San Diego, California, adequate tissue for combined histological and biochemical analysis was available from twenty-four patients. In the remaining twelve patients, either sufficient tissue for the combined study was not obtained or tissue identifiable as nodule was not present. The principal indication for operation was a digital contracture of more than 30 degrees that was causing the patient significant functional disability. Patients with painful fasciitis were not considered candidates for the procedure. Specimens obtained at operation were separated into two categories -- nodules or cords -- on the basis of their physical characteristics. A nodule was de-
fined as a discrete area of palpable fusiform thickening within the diseased fascia. The firm, band-like tissue that lacked areas of thickening was labeled cord. Samples of each were submitted for study by electron microscopic and biochemical techniques. Patients were followed for a minimum of one year, with an average follow-up of fourteen months. Frequent follow-up was maintained, with careful recording of range of motion and close examination for recurrence of fasciitis at each visit.

Electron Microscopy

Tissue submitted for electron microscopic study was cut into one-millimeter cubes in the operating room and immediately placed in 4 per cent glutaraldehyde and 5 per cent paraformaldehyde buffered with 0.1-molar sodium cacodylate, pH 7.3 to 7.4, at room temperature (21 to 22 degrees Celsius). After four hours of fixation, the tissue was rinsed three times with 0.1-molar cacodylate buffer and post-fixed with 2 per cent cacodylate-buffered osmium tetroxide at 4 degrees Celsius. Blocks of tissue were then progressively dehydrated with increasing concentrations of ethanol followed by propylene oxide and then propylene oxide-Epon mixtures, proceeding to pure Epon. Blocks were placed in flat embedding molds with fresh Epon and cured overnight at 60 degrees Celsius. The blocks were trimmed and sections one micrometer thick were stained with methylene blue. Thin sections were stained with uranyl acetate and lead citrate and were examined in a Zeiss-10 electron microscope at sixty kilovolts, at a magnification of 4,000 to 16,000 times.

All electron microscopic specimens were examined in the same way by the same microscopist to assure uniformity of examination. Blocks of tissue were identified by code so that the microscopist would not know the history or diagnosis. Constant reference was made to the thick sections for identification of cells, and usually ten to fifteen fibroblasts were studied from each thick section. Immediately after each grid was studied, the microscopist recorded the percentage of cells that contained classic myofibroblast features: sixty to eighty-angstrom electron-dense bodies, convoluted nuclei, basal laminae, and intercellular junctions.

Biochemistry

The specimens of nodule and cord submitted for biochemical analysis were dried in several changes of acetone for four days at 4 degrees Celsius and then were defatted with a mixture of 50 per cent acetone and ether. Next, the tissue was lyophilized and ground in a Wiley mill to pass through a twenty-mesh screen.

Solubilization with Pepsin

The tissue was washed with 0.5-molar acetic acid for two days at 4 degrees Celsius, and the insoluble residue was lyophilized. The washed tissue was suspended in 2 per cent acetic acid. The pH was lowered to 2.0 with 1N hydrochloric-acid solution. (Collagen concentration was approximately two milligrams per milliliter.) After addition of pepsin (from pig, twice crystallized, 3290 Anson units per milligram — Calbiochem, California) in a collagen-to-pepsin ratio of ten to one, the suspensions were stirred for sixty hours at 4 degrees Celsius. The suspensions were then centrifuged at 15,000g for thirty minutes to eliminate the insoluble residues from the pepsin-solubilized fraction. The solubilized collagen was precipitated by the addition of sodium chloride to a concentration of 0.9 molar.
TABLE I

<table>
<thead>
<tr>
<th>Duration of Contracture (Yrs.)</th>
<th>No. of Patients</th>
<th>Average Age (Yrs.)</th>
<th>Other Fasciitis* (No.)</th>
<th>Recurrences (No.)</th>
<th>Collagen Type</th>
<th>Myofibroblasts (No.)</th>
<th>Microtubules (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>6</td>
<td>65</td>
<td>2</td>
<td>0</td>
<td>III</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3-7</td>
<td>9</td>
<td>60</td>
<td>4</td>
<td>1</td>
<td>III</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>7-12</td>
<td>4</td>
<td>59</td>
<td>1</td>
<td>0</td>
<td>III</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&gt;12</td>
<td>5</td>
<td>66</td>
<td>2</td>
<td>2</td>
<td>III</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

* Other hand, foot, or penis.

total collagen in both cords and nodules as determined by hydroxyproline content.

**Differential Salt Precipitation of Pepsin-Solubilized Collagen**

According to the method of Chung and Miller, collagen samples treated with pepsin were dissolved in 1.0-molar sodium chloride and 0.05-molar Tris, pH 7.5, at a concentration of three milligrams per milliliter with stirring for two days. The salt concentration was increased slowly by the addition of 4.0-molar sodium chloride to 1.7-molar sodium chloride. The solution was stirred overnight and the material (type-III collagen) that had precipitated was collected by centrifugation for one hour at 35,000g. The precipitated type-III collagen was washed three times with 1.7-molar sodium chloride and 0.04-molar Tris, pH 7.5, suspended and dialyzed against 1 per cent acetic acid, and subsequently lyophilized. The supernatant was used to recover type-I collagen. The type-I collagen was precipitated overnight by increasing the sodium chloride concentration of the supernatant from 1.7 to 2.5 molar, collected by centrifugation, dialyzed for twenty-four hours against 1 per cent acetic acid, and lyophilized.

**Disc Electrophoresis**

Electrophoresis on 5 per cent acrylamide gels containing sodium dodecylsulphate was performed essentially as described by Furthmayr and Timpl. Samples of both reduced and non-reduced extracted collagen were run. For reduced collagen, one milligram of sample was dissolved in 0.5 milliliter of 0.02-molar phosphate buffer (pH 7.2) containing 0.5 per cent sodium dodecylsulphate and 1 per cent 2-mercaptoethanol. This mixture was incubated for two hours at 37 degrees Celsius prior to application on gels. The non-reduced collagen was treated similarly, with the exception that 2-mercaptoethanol was omitted. Fifty-microliter treated samples were applied to the gels.

**Analysis of Reducible Cross-Links**

**Preparation of Sample**

Dissected specimens from the cords and nodules of the palmar fascia were analyzed for the characterization and quantification of the reducible cross-links of collagen. The tissues were minced into approximately one-millimeter cubes and suspended in 0.2-molar potassium phosphate buffer, pH 7.4. The tissues were reduced with [3H]NaBH₄ (250 millicuries per millimole), as described by Tanzer and Mechanic.

The reduced collagen was dialyzed exhaustively against cold water and lyophilized. An aliquot of each reduced sample was hydrolyzed in vacuo with two milliliters of 3N p-toluene sulfonic acid for twenty-four hours at a temperature of 110 degrees Celsius. The acid hydrolysates of the reduced collagen were filtered with suction through a fine, porous, sintered glass funnel and chromatographed on a two-column system for the complete resolution of the intermolecular cross-links (Fig. 1).

The detailed biochemical methods used for the chromatography and counting technique of the radioactive elution profile of a 3N p-toluene sulfonic-acid hydrolysate of [3H]NaBH₄-reduced collagen has been reported previously.

Electron micrograph of myofibroblast from a nodule within the palmar fascia of a patient with Dupuytren’s contracture. Microfilament bundles with electron-dense bodies (asterisks) are prominent, along with microtubules (arrows) (× 40,000). Nucleus = N.
Quantitative Alteration in Collagen Cross-Links

Elution profiles of the \([\text{PH}]\text{NaBH}_4\)-reducible components in collagen from cords and nodules of the palmar fascia from patients with Dupuytren's contracture show the presence of two main reducible cross-links, dihydroxylysinonorleucine and hydroxylysinonorleucine. The quantitative determination of each of these reducible cross-links was obtained by standardizing the \([\text{PH}]\text{NaBH}_4\) by the method of Paz et al. and of Gallop et al. using p-nitrobenzoyl amidobutanol. The mean average of the specific activity from four determinations for the standardization of the \([\text{PH}]\text{NaBH}_4\) was found to be $4.05 \times 10^7$ counts per minute per micromole. Hydroxyproline content was determined on aliquots of the hydrolysate to calculate the amount of collagen applied to the columns for the separation of the cross-links. A Woessner modified colorimetric procedure on an automated auto-analyzer was used for the determination of hydroxyproline content.

Total Collagen

For the hydroxyproline determination, five milligrams of fat-free dry tissue from the cord and nodule of the patients with Dupuytren's contracture was hydrolyzed without preliminary purification by adding hydrochloric acid to a final concentration of 6N. The sample was hydrolyzed for three hours at 130 degrees Celsius. The hydrolysate was treated according to the procedure of Woessner and the hydroxyproline content was determined spectrophotometrically at 557 nanometers.

Results

Clinical Correlation

The twenty-four patients studied were all men, ranging in age from forty-five to seventy-six years. The average duration of the disease was eight and one-half years (range, six months to thirty-five years). Six patients had bilateral disease. Associated fasciitis was present in the plantar fascia in two patients and Peyronie's disease, in two patients. The contracture was classified as mild if it was 30 to 45 degrees at the metacarpophalangeal or proximal interphalangeal joint; moderate, if it was 45 to 60 degrees; and severe, if it was more than 60 degrees. If more than a single joint was involved, the classification of contracture was based on the more severely involved joint. The deformity was mild in eight patients, moderate in seven, and severe in nine.

The results of operation were based on the degree of correction of the contracture. Correction was good if no more than 15 degrees of extension was lacking at either the metacarpophalangeal or the interphalangeal joint and flexion to the palm was complete; fair, if there was between 15 and 25 degrees of loss of extension and the patient could flex to within a centimeter of the distal palmar crease; and poor, if the patient had greater loss of flexion or extension. Results were considered good in eleven patients, fair in twelve, and poor in one.

During the initial eighteen months of follow-up, three recurrences were noted. In each instance, the first appearance of recurrent fasciitis was discovered by the third postoperative month and became more significant over the next twelve to eighteen months. The final digital contracture in each of these three patients was equal to or greater than the original contractures. Recurrence was defined as the appearance of new fascial bands, determined by appearance and palpation, in an area where fasciectomy had been previously performed. While the recurrence of digital contracture occasionally resulted from postoperative subcutaneous scar and recurrent joint contracture, these recurrences, called false recurrences by Iselin, were carefully distinguished from true recurrent Dupuytren's fasciitis in our patients. None of the false recurrences led to the degree of recurrent contracture noted in the patients with true fascial recurrence.

The duration of the disease averaged twelve years in patients with recurrences. No relationship between age of the patient, duration of the contracture, and recurrent fasciitis was identified (Table I). There was no correlation between bilateral incidence, presence of plantar fasciitis or Peyronie's disease, occupation, alcohol ingestion, cirrhosis, seizure disorders, barbiturate and diphenylhy-
dantoin sodium usage, or severity of the contracture and the recurrence of fasciitis.

Electron Microscopy

Characteristic myofibroblasts were identified in the tissues of seven of twenty-four patients. Identification of myofibroblasts was based on the presence of characteristic microfilaments, measuring sixty to eighty angstroms, and electron-dense bodies (Figs. 2 and 3). These features were present in every cell that was considered to be a myofibroblast. Convoluted nuclei and typical basal laminae were occasionally seen, while intercellular junctions were common (Fig. 3). In every instance, only the nodule contained myofibroblasts. In no case were myofibroblasts discovered in the cord (Figs. 4 and 5). Ten of the nodules contained fibroblasts with a significant number of intercellular microtubules, while three of the specimens of cord contained cells with prominent microtubules. These microtubules were found in fibroblasts of three specimens that lacked myofibroblasts.

Comparison of electron microscopic data with the clinical course revealed several findings. Each patient with a recurrence had characteristic myofibroblasts in the nodule and had fibroblasts with prominent microtubules. The incidence of recurrence in patients with myofibroblasts was three of seven, while the incidence in patients with prominent microtubules was three of ten. Of the fourteen patients who lacked both myofibroblasts and prominent microtubules on electron microscopy, none had a recurrence.

Biochemistry

Tissue from both the nodule and the cord was analyzed for total collagen content, collagen type, and reducible cross-links. The total collagen content is demonstrated in Table II. In each patient the cord contained significantly more collagen than the nodule per unit of fat-free dry weight (p < 0.005).

Disc electrophoresis revealed type-III collagen in the cords and nodules of each patient. Confirmation of the identity of type-III collagen was obtained by fractional precipitation with sodium chloride and elution position on gel electrophoresis (Fig. 6). The presence of large amounts of 4-hydroxyproline, proline, and glycine and the appearance in the nodule and not the cord supports the conclusion that the nodule collagen is type-III.

| Table II |
|------------------|------------------|
| TOTAL COLLAGEN CONTENT (MILLIGRAMS PER GRAM OF FAT-FREE DRY TISSUE) |
|                  | Cord             | Nodules          |
| 24 patients      | 688.9 ± 13.7     | 573.8 ± 25.8     |
| t test           |                  | p < 0.005        |

Fig. 4
Electron micrograph of resting fibroblast from a cord within the palmar fascia of a patient with Dupuytren's contracture. The cell is surrounded by dense collagen (C) (× 40,000).

Fig. 5
Low-power electron micrograph of bands of collagen (C) in a cord. No myofibroblasts are seen (× 5,000).
Sodium dodecylsulphate acrylamide gel electrophoresis of collagen extracted from tissue of a patient with Dupuytren's contracture, demonstrating the alpha and gamma chains of collagen.

- **A**, Collagen type-I control with $\alpha_1$ and $\alpha_2$ chains isolated (2.4-molar sodium chloride precipitate).
- **B**, Collagen type-III control with $\alpha_1$ (III) chains isolated (1.7-molar sodium chloride precipitate).
- **C and E**, Representative specimens of nodule biopsies from two patients, demonstrating the component chains of both type-I and type-III collagen (0.9-molar sodium chloride precipitate).
- **D and F**, Representative specimens of cord biopsies from two patients, confirming presence of type-I and type-III collagen (0.9-molar sodium chloride precipitate).

Differences in the cross-link patterns of the nodules and cords were noted (Table III). The ratio of dihydroxylysinoisonorleucine to hydroxylysinoisonorleucine in the nodule was 2.87 ± 0.10. The ratio in the cord tissue was 1.84 ± 0.49, a consistently lower value than that noted in the nodules.

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**Discussion**

Since the description of the myofibroblast by Gab-
The significant amount of type-III collagen in the fascia of our patients (25 to 30 per cent) contrasts with that in adult dermis (approximately 15 per cent) and with normal palmar aponeurosis, which has no type-III collagen. Bailey et al. suggested that the change in collagen type represents response to injury, as similar changes in collagen type have been observed in granulating wounds. While the nodule and cord are indistinguishable on the basis of collagen typing, the increased content of reducible cross-links in the nodule was greater than that in the cord. These changes imply that both the cord and nodule contain immature, newly formed collagen, with the nodule containing more newly synthesized collagen than the cord.

While myofibroblasts may produce the abnormal collagen found in Dupuytren's fascia, little evidence has been advanced in support of this to date. The biochemical abnormalities in the fascia of patients with Dupuytren's contracture are uniform, in contrast to the sporadic appearance of myofibroblasts. The duration of the biochemical abnormalities with respect to the clinical disease is unknown. It is also unknown whether the changes antedate or follow the disease activity in Dupuytren's contracture, its appearance being more in keeping with these findings. Surgical release during repeated periods of waxing and waning may be more in keeping with these findings. Surgical release during periods of myofibroblast activity may be followed by recurrence in a higher-than-expected number of patients.

The pattern of recurrence of deformity after the operative treatment of Dupuytren's contracture has been well described by several investigators. While a continuing incidence of recurrence over a period as long as ten years following operation was noted by Hakstian and by Millesi, the majority of recurrences have been noted first within the initial few postoperative months. Efforts to find a common denominator among patients with recurrent fasciitis have previously been unsuccessful. Our finding of myofibroblasts in this group of patients may help explain the mechanism underlying clinical recurrence and provide some insight into the basic pathogenesis of this disease.

Previous theories of the pathogenesis of Dupuytren's contracture suggested that the disease has a definable chronological pattern, with predictable stages of development. Recent studies on granulating wounds in animals indicated that myofibroblasts have a defined life cycle and are present during periods of active contraction. They disappear when wound contraction ceases. It appears from our review that while the myofibroblast is a marker of disease activity in Dupuytren's contracture, its appearance is unrelated to the length of time that the contracture has been present. A cyclic pattern of disease marked by repeated periods of waxing and waning may be more in keeping with these findings. Surgical release during periods of myofibroblast activity may be followed by recurrence in a higher-than-expected number of patients.

References


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