BIOCHEMICAL MEDICINE 28, 273–284 (1982)

Evidence for Type V and I Trimer Collagens in Dupuytren's Contracture Palmar Fascia

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Received December 29, 1981

Dupuytren's Contracture, a common connective tissue disease, is characterized by thickening of palmar fascia from the hand. In addition, there is an involuntary flexion deformity usually involving the ring and little fingers which continues until those fingers are held in a clenched position. There is lesser involvement of other digits (1).

It is proposed that the disease is hereditary, since it is prevalent in middle-aged men of Celtic origin. For example, in Australia where there are mostly Scottish and Irish immigrants, 20% of the male population over 60 is affected (2). Another proposal for the cause of this disease is that it originates in palmer fascia in fatty fibrous subcutaneous tissue, as a result of chronic trauma (3).

Though little is certain about the etiology or pathogenesis of this disease, Dupuytren's Contracture has a scar-like appearance. Healing and scar formation are important for host survival, but in Dupuytren's Contracture scarring is detrimental and leads to loss of hand function.

Bailey and co-workers have investigated the collagen composition of palmar fascia from individuals affected with Dupuytren's Contracture (4). They extracted type I and III collagens. Affected fascia had higher proportions of type III collagens compared to normal fascia. Beside type I and III collagens, other collagen types may be present in palmar connective tissues. Type I trimer collagen is a fibrous collagen found in embryonic tissue (5) and inflammed tissue (6). It is composed of three $\alpha 1(I)$ chains and has solubility characteristics different from type I collagen (7). Type V collagen, originally called type AB (8), has been de-

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0006-2944/82/060273-12$02.00/0

scribed in a variety of tissues (9). Type V collagen is composed of $\alpha 1(V)$ and $\alpha 2(V)$ chains in a 2:1 proportion in most tissues (10). It is readily extracted from dermal hypertrophic scar (11). Since a Dupuytren's Contracture affected hand has a scar-like appearance, it may have a collagen composition similar to that of dermal hypertrophic scar.

MATERIALS AND METHODS

Samples of palmar fascia obtained from surgical excisions of hands with Dupuytren's Contracture were collected from 15 patients whose average age was 60 years (range between 40 and 72 years). Three control palmar fascia samples of similar age were collected at autopsy, examined, and found free of disease. Tissues were frozen in liquid nitrogen and lyophilized. Dried material was powdered by milling and stored at −20°C until used. In most cases diseased tissues were pooled because of the limited quantity of material.

Collagens were extracted by limited pepsin digestion. Five milligrams of powdered tissue and 100 μg of pepsin (Boehringer-Mannheim) were added per milliliter of 0.5 M acetic acid. The mixture was stirred for 48 hr at 4°C and centrifuged at 10,000*g* for 15 min, and the supernatant saved. Insoluble residue was pepsin digested for an additional 24 hr under identical conditions, and centrifuged as before. Both soluble extracts were pooled and pH adjusted to 8.0 with NaOH, which inactivates pepsin. Sodium chloride was added to a final concentration of 20% w/v and the mixture stirred overnight at 4°C. Collagen-rich insoluble material was collected by centrifugation at 15,000*g* for 15 min.

Two different salt fractionation methods were used to isolate different collagen types; a bulk salt fractionation method (7) and a column technique called Zone Precipitation Chromatography (12). With the bulk salt technique, 20% sodium chloride-insoluble material was suspended in 2.2 M NaCl, 145 mM potassium phosphate buffer, pH 7.6, and stirred for 4 hr at 4°C. The mixture was centrifuged at 15,000*g* for 15 min and the supernatant saved and labeled "2.2 M NaCl fraction." Insoluble residue was resuspended in 1 M NaCl in the same phosphate buffer used above, stirred, and centrifuged as before. This supernatant was labeled "1 M NaCl fraction." Finally, insoluble residue was resuspended in 145 mM potassium phosphate buffer, pH 7.6, stirred, and centrifuged as before. The supernatant was labeled "PO_4-soluble fraction."

With Zone Precipitation Chromatography, pepsin-soluble collagen preparations were neutralized as before and precipitated with 20% w/v NaCl. Salt-precipitated material was resuspended in 50 mM Tris–HCl, pH 7.5, (referred to as Tris 7.5), containing 0.15 M NaCl. This mixture was applied to a column packed with Sephadex G-25 Medium resin (Pharmacia Laboratories, Piscataway, N.J.) having a total bed volume

of 150 ml. The column was first equilibrated with 100 ml of 30% NaCl in Tris 7.5, which made up its lower two-thirds, followed by 50 ml of 25% NaCl in Tris 7.5, which made up the top one-third of its volume. The sample was then applied and the column eluted with a stepwise gradient of 50 ml each of 20% NaCl, 15% NaCl, 10% NaCl, and 150 ml 1% NaCl all in Tris 7.5. Ten-milliliter fractions were collected and the optical density of each fraction was monitored at 230 nm. The salt concentration of each fraction was measured with a Radiometer conductivity meter (London Company). Appropriate fractions were pooled and dialyzed against 0.1 M acetic acid and concentrated by dialysis against Aquacide (Calbiochem–Behring Corp.).

Collagens fractionated by these two techniques were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), using the method of Miner (13). This system is a moving-boundary discontinuous voltage gradient which uses 0.1% SDS with 0.375 M Tris–SO_4 pH 9.0 buffer and an Ortec Pulsed Power Supply (Oak Ridge National Laboratory, Tenn.). Samples for electrophoresis were in 0.1% SDS, 0.1% mercaptoethanol, 5% glycerol, 0.1% bromophenyl blue, and 50 mM Tris–SO_4, pH 9.0. Following electrophoresis, gels were stained with 0.5% Coomassie Brilliant Blue R-250 in a propanol, acetic acid, and water solution (1:2:7). Gels were destained in the same solution without stain. Relative intensities of stained protein bands were measured with a Helena Quick Scan Junior Densitometer. Relative densities of stained α-chain protein bands were electronically integrated by the densitometer and used for quantitative analysis.

Nonfibrous collagens such as type V do not form heat gels. The heat gelling technique of Trelstad and Lawley was used to confirm the presence of type V collagen (14). The bulk salt "2.2 M NaCl fraction" from Dupuytren's collagen preparation was dialyzed against 145 mM potassium phosphate buffer, pH 7.6. The dialysate was incubated at 37°C for 18 hr. Polymerized collagens were collected by centrifugation at 15,000*g* for 10 min at 37°C. Supernatant, heat gel-soluble material was precipitated with the addition of NaCl to a final concentration of 20% w/v, centrifuged, and analyzed by SDS–PAGE. Insoluble material, heat-gelled collagen, was analyzed by CM-cellulose ion-exchange chromatography following the procedure of Bornstein *et al.* (15). The heat-denatured sample in 4 M urea, 40 mM sodium acetate was applied to a CM-cellulose (Whatman CM-52) column which had been equilibrated in the same buffer at 42°C. The column was eluted with a 400-ml linear gradient from 0 to 0.1 M NaCl in urea–sodium acetate buffer. The optical density of each fraction was measured at 230 nm. Appropriate fractions were pooled, dialyzed, concentrated, and examined by SDS–PAGE.

Hydroxyproline content of acid hydrolysates was determined by the colorimetric method of Bergman and Loxley (16).

RESULTS

Limited pepsin digestion released greater than 70% of the hydroxyproline contained in palmar fascia. Over 90% of the pepsin-solubilized material, containing hydroxyproline, was insoluble in 20% NaCl w/v.

SDS–gel electrophoresis patterns and densitometry tracings after bulk salt fractionation are shown in Fig. 1. In the "2.2 M NaCl fraction," there were α1(V) and α2(V) collagen chains. With normal fascia, α1(V) and α2(V) chains were found only in the "2.2 M NaCl fraction" (see Fig. 1A). In Dupuytren's fascia, α1(V) and α2(V) chains were in both the "2.2 M NaCl fraction" (Fig. 1D) and the "1 M NaCl fraction" (Fig. 1E). The α1(V) to α2(V) densitometry ratio for normal and Dupuytren's collagen extracts was 2.1. Equal volumes of normal and affected palmar extracts were applied to each gel well. Dupuytren's fascia extract appears richer in type V collagen. (Compare Figs. 1A and D.)

In addition to α1(V) and α2(V) collagen chains in the "2.2 M NaCl fraction" (see Fig. 1D), the α1(I) to α2(I) ratio for Dupuytren's fascia was 9. With normal fascia no α1(I) material was seen (see Fig. 1A). Presence of α1(I) chains in Dupuytren's "2.2 M NaCl fraction" indicates the possibility of type I trimer collagen.

The "1 M NaCl fraction" from normal fascia has α1(I) and α2(I) chains; its densitometry ratio is 2.3 (see Fig. 1B). Dupuytren's "1 M NaCl fraction" (Fig. 1E) shows α1(V), α2(V), α1(I), and α2(I) chains. Unlike normal fascia, Dupuytren's Contracture fascia has type V collagen in this 1 M NaCl bulk salt fraction. In normal fascia, the α1(I) to α2(I) ratio was 2.3. In Fig. 1E, Dupuytren's "1 M NaCl fraction," α1(I) to α2(I) ratio was 2.4.

Figure 1C shows a "PO_4-soluble fraction" from normal fascia. The presence of a single α1 chain represents the α1 chain's type III collagen, α1(III). Dupuytren's "PO_4-soluble fraction" (Fig. 1F) has both α1(I) and α2(I), with a density ratio of 3.8. Since type I collagen has two α1(I) chains for every α2 chain, the excess α1 chain is attributed to type III collagen, α1(III) chain. Dupuytren's "PO_4-soluble fraction" is a mixture of both type I and III collagens, at approximately a 1:1 ratio.

By bulk salt fractionation techniques, normal and Dupuytren's fascia demonstrate some similarities and differences. Both preparations have type I, III, and V collagens. The α1 to α2 ratio in type V collagen is 2 for both. Normal palmar fascia collagens are more completely separated by bulk salt techniques, as compared to affected collagens.

To substantiate the presence of type V collagen, the "2.2 M NaCl fraction" from Dupuytren's fascia was dialyzed into 145 mM potassium

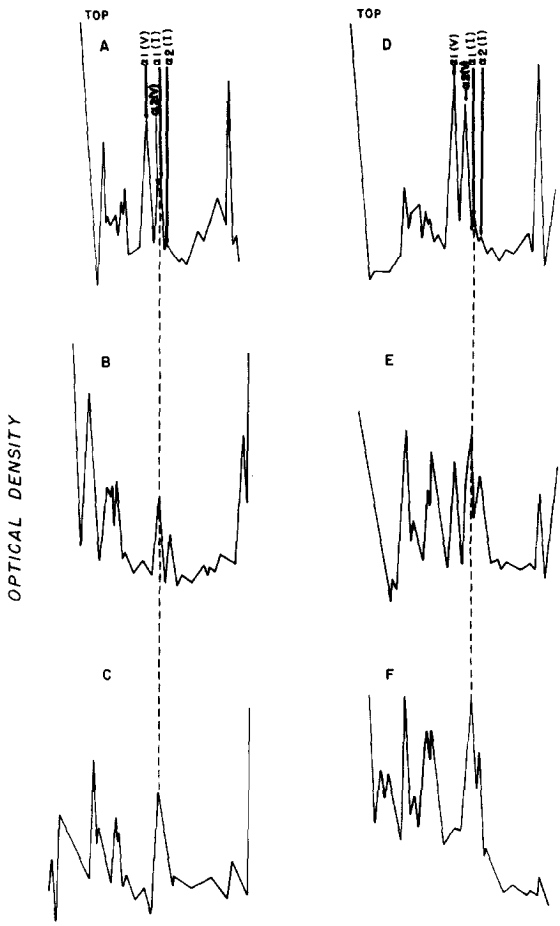


FIG. 1. Electrophoretic patterns and densitometry scans of salt fractionated collagens from normal and Dupuytren's Contracture palmar fascia. Collagens were extracted and solubilized from palmar fascia by limited pepsin digestion. Different collagen types were separated by bulk salt fractionation. Aliquots from each salt fraction were electrophoresed in SDS–polyacrylamide slab gels. Gels were stained and then scanned by densitometry. Stained gel appears in bottom of each panel and its densitometry scan appears above it. (A) The "2.2 M NaCl fraction" from collagen extract of normal fascia is presented showing two α chains. The α1(V) to α2(V) ratio is estimated by densitometry to be 2.1. (B) The "1 M NaCl fraction" shows two β chains, the β_{12} moves just ahead of β_{11}. The two α chains have an α1 to α2 ratio of 2.3. (C) The "PO_4-soluble fraction" shows two β chain; the β_{11} chain migrates just behind the β_{12}. There is only α1(III) chain present. (D) The "2.2 M NaCl fraction" from a collagen extract of Dupuytren's fascia has α1(V) and α2(V) with a ratio of 2.1. There is some α1(I) chain present. Type V makes up more than 90% of this fraction and type I trimer the remainder. (E) The "1 M NaCl fraction" has two β chains. The β_{11} migrates just above the β_{12}. There is type V collagen present as indicated by the α1(V) chain. The shoulder on the slower migrating edge of α1 represents α2(V) chains. This fraction is a mixture of type I and V collagens. (F) The "PO_4-soluble fraction" has two β chains. The β_{12} runs just ahead of the β_{11}. The α1 to α2 ratio is 3.8.

phosphate buffer, pH 7.6. This dialysate was incubated overnight at 37°C. The heat gel-polymerized collagen was recovered by centrifugation. The supernatant was examined by SDS–PAGE (see Fig. 2). The α1(V) to α2(V) ratio was 2.2 with no detectable α1(I) or α2(I) bands. Type V collagen was native under heat gelling conditions, since it was precipitable by 20% NaCl.

The presence of type I trimer in Dupuytren's tissue was confirmed with CM-cellulose ion-exchange chromatography of the "2.2 M NaCl fraction" heat-gelled residue. A single prominent optical density peak which coeluted with α1(I) chains was resolved (see Fig. 3). There was no evidence for the α2(I) chain. The presence of α1(I) and the absence of α2 confirm the presence of type I trimer collagen which is composed of three α1(I) chains.

To demonstrate differences between normal and Dupuytren's fascia collagens, another salt fractionation technique was used. Similar amounts of pepsin-soluble, 20% NaCl-precipitated collagen extracts from normal and Dupuytren's fascia were chromatographed by Zone Precipitation Chromatography. In Fig. 4A normal fascia eluted with five peaks. Fractions 1 and 2 were unresolved by SDS–PAGE. These fractions appear to be made up of noncollagen proteins, denatured collagen, small peptides, and salt. Fraction 3 is type V collagen, as shown by SDS–PAGE, with α1(V) and α2(V) chains (see Fig. 5(A3)). Fraction 4 is type I trimer collagen, having only α1(I) (see Fig. 5(A4)). It represents 2.5% of collagen

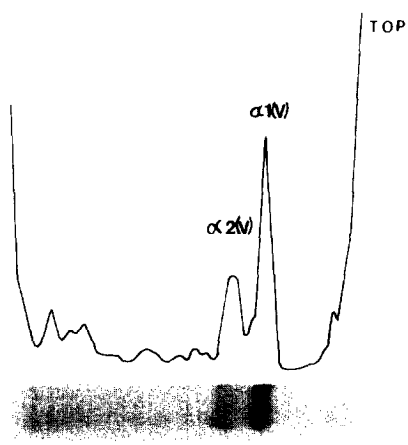


FIG. 2. Electrophoretic gel patterns of "2.2 M NaCl fraction" from heat-gelled supernatant. The "2.2 M NaCl fraction" from Dupuytren's Contracture palmar fascia was heat gelled, see text for details. The nonpolymerized collagen fraction was precipitated with 20% NaCl w/v to collect native collagen. The electrophoretic gel pattern of this fraction shows α1(V) and α2(V) collagen chains. Densitometry demonstrates an α1(V) to α2(V) ratio of 2.2.

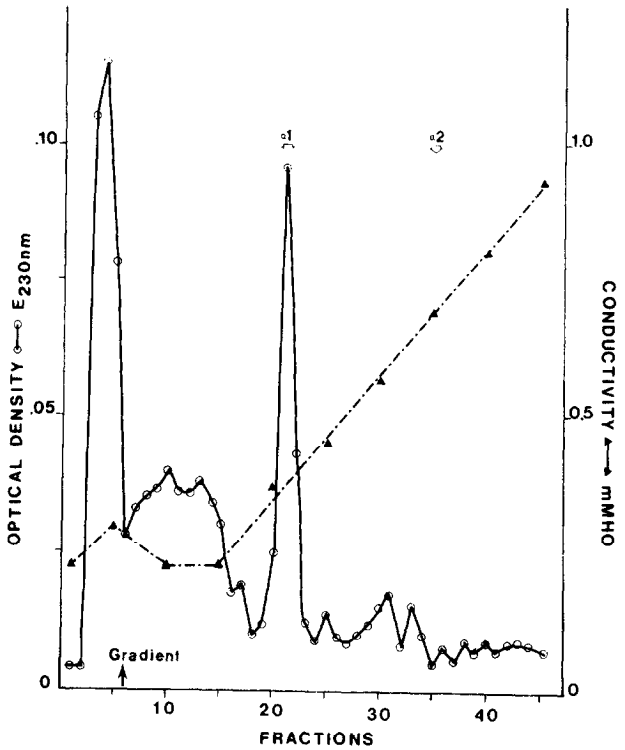


FIG. 3. CM-cellulose chromatograph of "2.2 M NaCl fraction" heat-gelled residue. The "2.2 M NaCl fraction" from Dupuytren's Contracture palmar fascia was heat gelled; see text for details. Polymerized collagen fraction was prepared for CM-cellulose chromatography. The (○) solid line is the optical density and the (▲) interrupted line represents the salt concentration. The initial material which eluted from the column was not resolved by gel electrophoresis. Material that coeluted where α1 chain was expected electrophoresed as a single α1 chain. Little material eluted in the α2 region.

eluted from the column. It demonstrates that normal fascia have type I trimer collagen (18). Figure 5 (A5) represents the major collagen fraction from pooled normal palmar fascia. It represents 93% of the pepsin-solubilized collagen. Its electrophoretic gel pattern (Fig. 5(A5)) shows an α1 to α2 ratio of 2.6, which is a mixture of type I and III collagens. The amount of type III collagen estimated by densitometry is 20%, which agrees with that reported for normal palmar fascia (4).

Figure 4B shows elution of pepsin-solubilized collagen extracted from Dupuytren's fascia. Five fractions were separated. Fractions 1 and 2 were unresolved by SDS–PAGE. Fraction 3, a shoulder from fraction 2, was type V collagen (see Fig. 5 (B3)). The α1(V) to α2(V) ratio was 2.0 with no detectable α1 or α2 bands. Fraction B4 is type I trimer

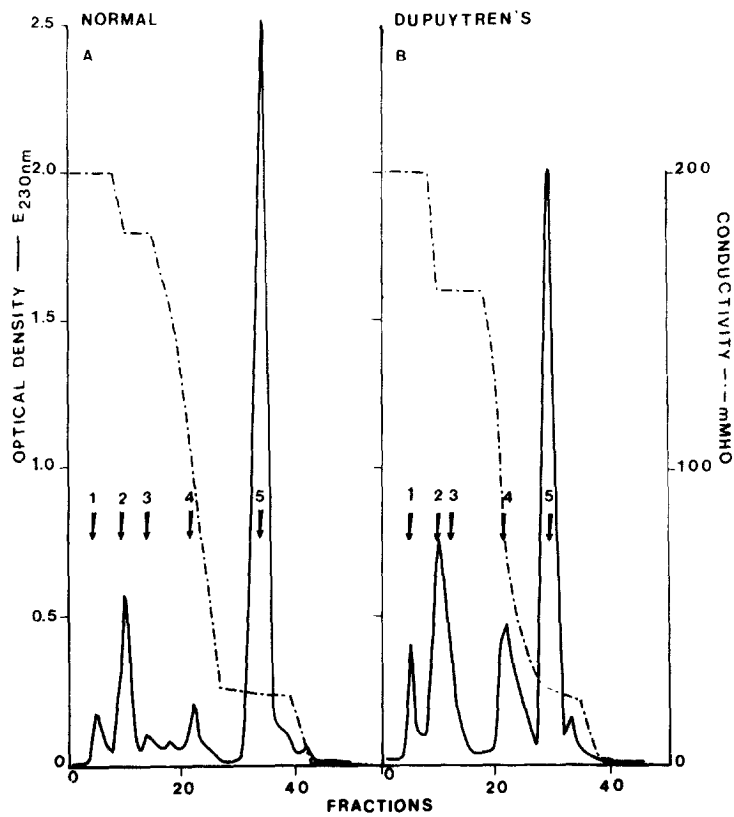
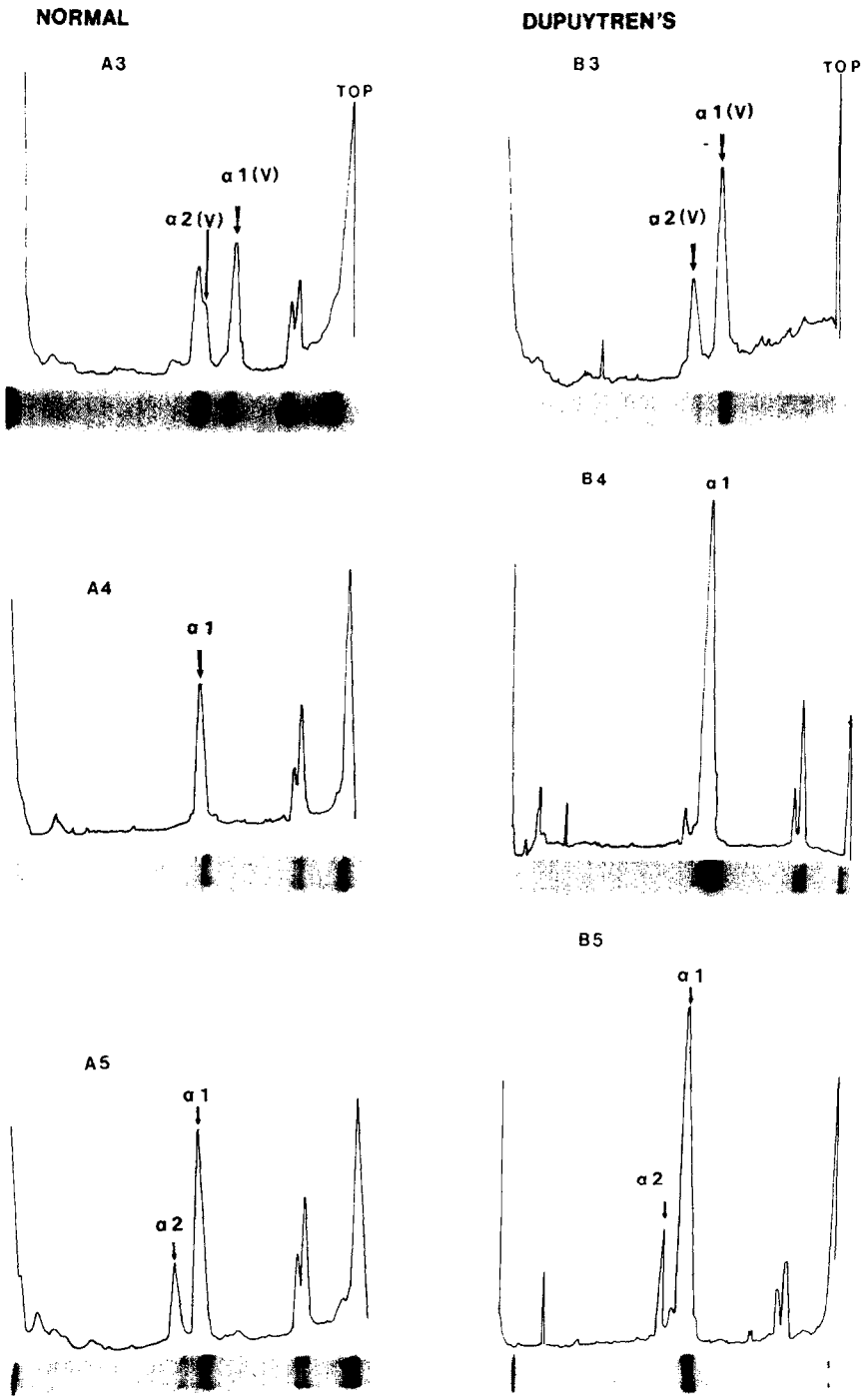


FIG. 4. Elution of collagen extracted from palmar fascia by Zone Precipitation Chromatography. Two chromatographs of pepsin-solubilized collagen extracted from (A) normal human and (B) Dupuytren's diseased palmar fascia. Conductivity profiles show the descending salt gradient of this chromatographic system. Optical density profiles (——) represent native collagens eluted from both tissues. Peaks 1 and 2 are unresolved by gel electrophoresis and are proteins, peptides, and small molecular components. Peak 3 is type V collagen. Peak 4 is type I trimer collagen. Peak 5 is a mixture of type I and III collagens.

FIG. 5. Electrophoretic gel patterns and densitometry scans of the elution peaks from Zone Precipitation Chromatography. The A panels are gel patterns of three pooled fractions eluted from the Zone Precipitation Chromatography column of normal palmar fascia. In A3 type V collagen is characterized by α1(V) and α2(V) chains. In addition to the α2(V) chain there is also an α1(I) chain present. The α2(V) chain is the shoulder with less electrophoretic mobility. The peak height of α1(I) is twice that of the shoulder height referred to for α2(V). In A4, type I trimer collagen is characterized by a single α1 chain. In A5 there are two α chains and the densitometry ratio of α1 to α2 is 2.6. This is a mixture of type I and III collagens. The 5B panels are gel patterns of pooled fractions of Zone Precipitation Chromatography chromatograph of Dupuytren's diseased palmar fascia. In B3 there is type V collagen with α1(V) and α2(V) chains. In B4 there is a single α1 chain characteristic of type I trimer collagen. In B5 the α1 to α2 ratio is 3.0. This is a mixture of type I and III collagens; type III is estimated to be 33% by densitometry.


NORMAL
DUPUYTREN'S
A3
TOP
α1(V)
α2(V)
B3
TOP
α1(V)
α2(V)
A4
α1
B4
α1
A5
α1
α2
B5
α1
α2

collagen (see Fig. 5 (B4)). There is more type I trimer collagen in Dupuytren's fascia extracts as compared to normal fascia extracts. The major fraction from this collagen was fraction 5. The electrophoretic gel profile of fraction 5 (B5) has an $\alpha 1$ to $\alpha 2$ densitometry ratio of 3.0. This represents a mixture of type I and III collagens. The estimated amount of type III collagens is 33%. This agrees with the amounts of type III collagen reported by Bailey and co-workers (4).

Type V, I, and III collagens from Dupuytren's extract eluted earlier than collagens extracted from normal fascia (compare Figs. 4A and B). The reasons for these differences in collagen salt solubilities are not known. Salt solubility differences are noted between normal dermis and scar collagen extracts (19).

Normal fascia has 2% type V collagen, 5% type I trimer, and 93% type I and III collagens. Dupuytren's fascia extract has 3 to 5% type V, 9% type I trimer, and 85% type I and III collagens (see Table 1). These estimates are based upon collagens fractionated by Zone precipitation Chromatography.

DISCUSSION

Type I trimer and type V collagens have not been previously reported in Dupuytren's Contracture collagen extracts (4). Reasons for these omissions appear to be related to their solubility in 5% NaCl under acid conditions (9). In 20% NaCl under neutral conditions, these collagens are precipitable.

The collagen composition of connective tissue matrix from Dupuytren's fascia is different from that of normal palmar fascia. Dupuytren's collagen extracts show more type III, type I trimer, and type V collagens. Palmar fascia extracts from normal individuals also have these collagen types.

TABLE 1
COMPARISON OF COLLAGEN TYPES

	Palmar fascia[a]		Dermis[b]	
Collagen type	Normal	Dupuytren's	Normal	Hypertrophic scar
I and III	93	85	96	89
I trimer	5	9	2	6
V	2	5[c]	2	5

Note. Results are expressed as percentages.

[a] Results based upon Zone Precipitation Chromatography.

[b] Preliminary data from hypertrophic scar connective tissue studies using bulk salt fractionation techniques.

[c] Bulk salt fraction showed higher proportion.

Collagens extracted from Dupuytren's Contracture fascia appear to have salt solubility differences when compared to normal fascia collagens. In bulk salt preparations type V collagen appears in both the "2.2 M NaCl fraction" and the "1 M NaCl fraction." With Zone Precipitation chromatography, type V collagen from Dupuytren's fascia is more soluble in high salt and coelutes with the small peptides, salts, and amino acids. In contrast, normal fascia type V collagen elutes later and is free of these materials. We are unable to explain why there is a difference in the salt solubility of collagens from normal or Dupuytren's palmar fascia. Similar results were found between normal dermis and hypertrophic scar collagen (11). Further studies are in progress to examine these differences, and their possible relationship to glucosylgalactosylhydroxylysine and galactosylhydroxylysine amino sugar residues in α chains.

Type V collagen in palmar fascia is composed of two α1(V) chains and one α2(V) chain. Type V collagen was initially reported to be composed of two αB chains and one αA chain (8). To keep collagen nomenclature consistent, α1(V) and α2(V) are preferred for reporting the polypeptide chains of type V collagen (10).

Type V collagen is a component of the basal lamina of capillaries (19). Since cellular nodules, as opposed to fibrous cords of Dupuytren's Contracture fascia, have enriched vasculature, increased amounts of type V collagen may originate there.

Type I trimer collagen can appear when pathological alterations of connective tissue matrix caused by chronic inflammation occur (6).

Type I trimer may be a component of the fibrous tissue matrix which makes up the fibrous cords of Dupuytren's Contracture. More type I trimer may be deposited in fibrous cords because of chronic inflammation associated with this disease.

Increased amounts to type I trimer and type V collagens in Dupuytren's Contracture palmar fascia may be important for further understanding the pathology of the disease. These collagens are reported to be abundant in inflamed and embryonic tissues (8,20). Hypertrophic scar has similar collagen patterns. Hypertrophic scar is characterized by excess amounts of connective tissue.

Dupuytren's Contracture may be related to hypertrophic scarring. The mechanism(s) for both pathological conditions is not understood, but may be related to connective tissue matrix.

Young connective tissue is immature and undergoes rapid turnover and remodeling. Type I trimer and type V collagens in Dupuytren's tissues therefore may point to an immature and unstable tissue matrix. This condition could promote tissue movement or contracture. A similar movement, "wound contracture," is seen in hypertrophic scars of skin (21). Current findings in our laboratory show that hypertrophic scars also

have increased concentrations of type V and type I trimer collagens, suggesting that Dupuytren's palmar fascia and dermal hypertrophic scars have a common alteration in the structural components which make up their connective tissue matrix.

ACKNOWLEDGMENTS

These studies were supported by U.S. Public Health Service Grant GM 25561. This work was done during tenure as an Established Investigator of the American Heart Association, H.P.E.

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