A. J. Bailey

Collagen

Whatever the aetiology, the cellular biology and the overall biochemical changes, the proliferation of fibrous collagen is the major biochemical characteristic of Dupuytren's disease (DD). The excess collagen impairs normal function and also provides the disease with its clinical feature, the contracture of the fingers.

Considerable progress has been made in detailing the biochemical changes of collagen in DD but we still are not certain of the stimuli which actuate the process, or the mechanism by which the tissue contracts. In contrast to most fibrotic situations, in DD it is possible to distinguish the early and late stages of the disease. It is therefore possible to follow, at least in part, its course; from the early stages it should be possible to distinguish the stimulating factors, and hence the means to devise rational treatment. This chapter will seek to describe briefly the currently perceived role of collagen in DD.

DEPOSITION OF COLLAGEN

The lesion primarily involves the palmar fascia. In the early stages of DD discrete highly cellular nodules form, but in the later stages the characteristic feature is of dense fibrotic bands or cords in the palmar fascia. Biochemical investigations have therefore concentrated on comparing normal fascia with nodules and bands.

The nodules of DD are highly cellular, comparable to granulation tissue formed during wound healing. Amongst the collections of cell types present in the nodules are the fibroblasts, the major synthesizer of collagen. The excess collagen produced could result from an increasing fibroblast population, increased synthesis or by transformation of the fibroblasts. In the majority of fibrotic situations, it appears that a select number of fibroblasts synthesize more collagen per cell than the other fibroblasts in the tissue. Whether this is through stimulation via mediators or there is an inherent defect through transformation in these fibroblasts has not yet been established, and indeed the situation may be different in different fibrotic situations.

The disease often progresses to the irreversible deposition of thick fibrous collagen bands. Changes in the physical appearance of the fibres, their composition in terms of genetic type of collagen, changes in post-translational modification, extracellular cross-linking and changes in the organization of the tissues have been reported. An understanding of the mechanisms leading to these changes and their significance on the functional properties of the collagenous tissues requires some appreciation of the structure of collagen and details of its biosynthetic and degradative processes.

THE METABOLISM OF COLLAGEN

As a number of reviews adequately cover the genetics, structure, biosynthesis and degradation of collagen (see, for example Bailey & Etherington 1980; Piez & Reddi 1984; Fleischmajer et al 1985; Martin et al 1985 and its role in fibrosis (Evered & Whelan 1985), only the salient features of these properties in the metabolism of collagen need be outlined here.

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Structure

It is now apparent that there is a large family of collagens, which are for the most part tissue-specific (Martin et al 1985). At the time of writing 12 genetically distinct types of collagen have been identified. These molecules are basically similar in that they contain three helical polypeptide chains possessing the repeating sequence $(Gly\text{-}X\text{-}Y)_n$; wound into a stable triple helix to produce a thin rod-like molecule. The genetic differences lie in the small variations in primary structure, and in the size and composition of the globular domains at the N and C termini of the molecule. In addition, each of the molecular types undergoes different degrees of post-translational modification. These variations result in dramatic differences in the self-assembly of the molecules, thus providing a wide range of supramolecular structures. The identification of these distinct collagens over the last decade has provided some of the answers to the question of the biological diversity of the simple collagen molecule. This diversity is expressed in the rope-like fibres of skin and tendon, the transparent amorphous membrane of the lens capsule and glomeruli, and the filamentous collagen surrounding some cells.

Fibrous collagens

Collagen types I, II and III are the major fibrous collagens (Fig. 6.1). Self-assembly occurs through lateral association of the molecules in a quarter-stagger and end-overlap fashion (Hulmes et al 1973, Fraser et al 1987). This relative displacement of the molecules leads to the characteristic cross-striations observed in the electron microscope (Fig. 6.1).

Type I collagen is the most abundant structural component of skin, tendon and bone, generally comprising 90% of the collagen. Type II confers the structural framework on cartilage and the intervertebral disc. Type III occurs in many tissues,

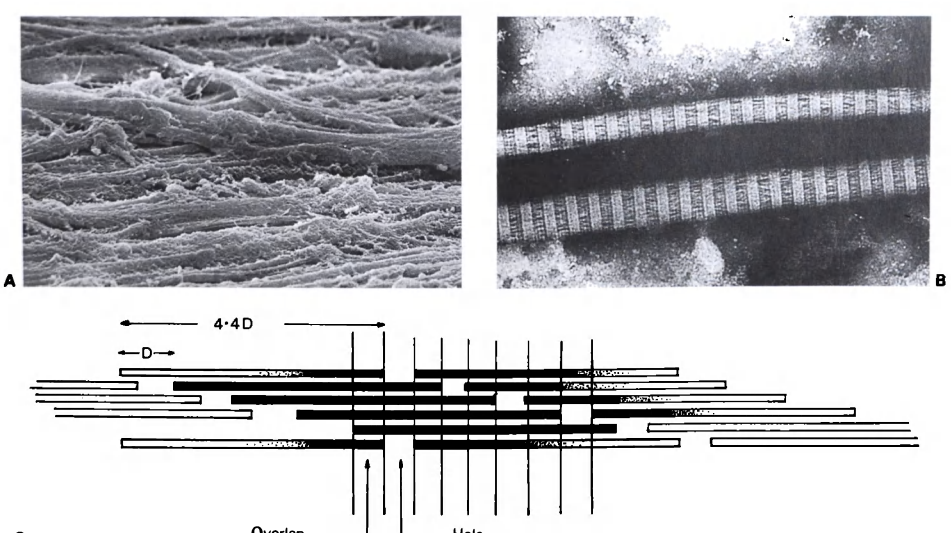

Fig. 6.1 **A** Scanning electron micrograph of fibrous collagen (magnification × 960); **B** Transmission electron micrograph of collagen fibres showing regular axial periodicity of 70 nm (magnification × 56 000); **C** Representation of the end-overlap and quarter-stagger alignment of molecules in collagen fibres responsible for the axial repeat observed in the electron microscope. The electron dense stain preferentially fills the whole region.

as a minor component in tendon (1–2%), a significant component in skin (10%) and a major component in the vascular system (40–50%). It forms fine fibres and occurs mainly in distensible tissues, although its function in relation to the other collagens has not been established. Similarly, type V collagen is a minor component (5–10%) and forms fine fibres in many tissues. The typical striations are not observed. The proportion of type V is generally higher in embryonic tissue. Like type III, the functional role of type V has not been identified.

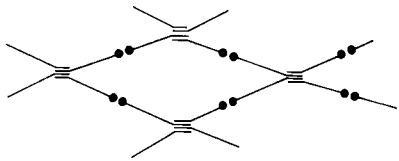

Fig. 6.2 Proposed 'chicken wire' net for the organization of type IV collagen molecules in basement membrane. The N-terminal regions are aligned in an anti-parallel fashion and the 'tetramers' so formed are organized into a network by the linking of the globular C-terminal regions.

Non-fibrous collagens

Type IV collagen provides the structural framework of the non-fibrous basement membranes which act as an underlying support for epithelial and endothelial cells, a protective sheath for myofibrils and the filtration membrane of the glomeruli. The type IV molecule possesses a longer triple helix than types I–III and, following secretion, the terminal globular domains are retained. This, together with differences in the primary sequence of the helix, causes a unique charge profile and a more flexible rod-like molecule which results in the self-assembly of an open network rather than the laterally associated fibril (Timpl et al 1981). The type IV molecules aggregate via their N-terminal ends in an anti-parallel fashion, and the tetramer formed acts as the unit 'monomer' to produce an open network through interaction of the C-terminal globular domains (Fig. 6.2). Our own recent evidence suggests that there may be some lateral aggregation of the type IV molecules, at least in lens capsule basement membrane (Barnard et al 1987a).

Filamentous collagens

Types VI and IX form loose assemblies of microfibrils rather than a tightly packed striated fibre (Fig. 6.3). Type IX appears to be associated with cartilagenous tissues (Duance et al 1982), whilst type VI seems to have a wide distribution (Hessle & Engvall 1984). It has been identified in cornea, skin and tendon, but its functional role has not been established. The type VI molecule is small — 100 nm compared to the fibrous (300 nm) and non-fibrous collagen (400 nm) — but

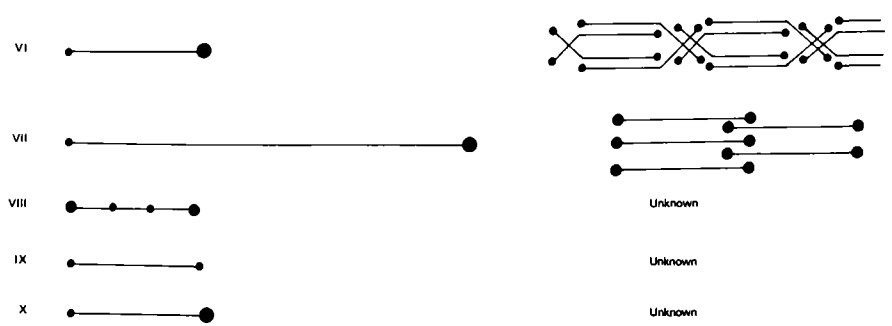

Fig. 6.3 Left: Diagrammatic comparison of the molecular length of the minor collagens types VI–X. Circles represent the globular regions at the ends of the triple helice. **Right:** The proposed macromolecular structures for types VI and VII.

self-assembles to a fibril by a complex series of anti-parallel and parallel interactions (Engel et al 1985 see; Fig. 6.3).

Biosynthesis

Collagen is synthesized as polypeptide chains by the same mechanism of mRNA and ribosomes as other proteins. The major difference is the number of post-translational modifications that take place in the nascent polypeptide chains (for reviews, see Bailey & Etherington 1980, Kivirikko & Myllylä 1984). These modifications require a complex series of enzymes and cofactors (Fig. 6.4). The prolyl residues in the Y position of the repeating sequence $(Gly\text{-}X\text{-}Y)_n$ are converted to hydroxyproline by prolyl hydroxylase; some of the lysine residues in the Y position are converted to hydroxylysine by lysyl hydroxylase; the hydroxylysine residues are 0-glycosylated with galactose or glucosyl-galactose by the relevant hexosyl transferases; a mannose-rich oligosaccharide is added to the carboxy terminal globular region by other transferases. Assembly of the triple helix is initiated by registration of the C-terminal globular region. The helical procollagen molecule is secreted from the cell and then undergoes further processing. The N- and C-terminal propeptides are, when necessary, selectively removed by N- and C-peptidases. In the case of the fibrous collagens this then permits self-assembly of the molecules in fibres. In contrast, the propeptides are retained in type IV and the molecules self-assemble in the extracellular milieu to form the network structure. These supramolecular structures are then stabilized by modification of specific lysine residues by lysyl oxidase which then spontaneously form intermolecular cross-links. Without the formation of covalent intermolecular cross-links, none of the collagenous assemblies described can function as a structural framework for body tissues.

Cross-linking

The cross-linking enzyme acts on the fibrillar form of collagen binding to a specific region of the triple helix, the sequence Hyl-Gly-His-Arg, and oxidizes a lysine or hydroxylysine in the vestigial propeptide region at the N and C termini. The lysyl-aldehyde produced then reacts with the Hyl in the enzyme-binding site sequence (Bailey et al 1974; Eyre et al 1984). This reaction results in a covalent intermolecular cross-link of the aldimine bond type (Fig. 6.5). If the residue in the N- or C-terminal telopeptide region is hydroxylysine then hydroxylysine aldehyde reacts with the hydroxylysine to form an oxo-imine bond (Fig. 6.5). The relative proportion of these two cross-links varies with the tissue and with age. The aldimine is the predominant cross-link in skin collagen, and the oxo-imine in cartilage and bone, whilst Achilles tendon contains equal proportions of both cross-links.

During maturation of the tissue these cross-links are believed to be transformed into stable multivalent cross-links (Bailey et al 1980; Light & Bailey 1980). The stabilization of collagen through the formation of intermolecular cross-links appears to be a two-stage process. Initially cross-linking occurs through head-to-tail cross-linking of the end-overlap region, i.e. at 4D stagger, to form longitudinal cross-linked filaments (see Fig. 6.5). In the second stage these intermediate divalent cross-links react with cross-links in other filaments, i.e. with molecules in register at 0D stagger, to form transverse multivalent cross-links (Light & Bailey 1980). These longitudinal and transverse cross-links build up a three-dimensional network of cross-links (Fig. 6.6), and can therefore account for the increasing tensile strength of collagen as the tissue ages.

The nature of these multivalent cross-links has not been completely established. One trivalent compound, 3-hydroxy-pyridinoline (Fujimoto et al 1977), has been identified and a derivation from two oxo-imine cross-links has been proposed (Eyre & Oguchi 1980). The location of this cross-link has not been confirmed. An alternative cross-link, hydroxyaldolhistidine (Housley et al 1975) has been proposed as the mature cross-link of tissues that do not possess the precursor oxo-imine cross-link, for example, skin. More recently, the structure of this cross-link has been reassessed (Yamauchi et al 1987). On the other hand, one as yet uncharacterized mature cross-link has been identified in all mature tissues (Barnard et al 1987b). Confirmation of this compound as a cross-

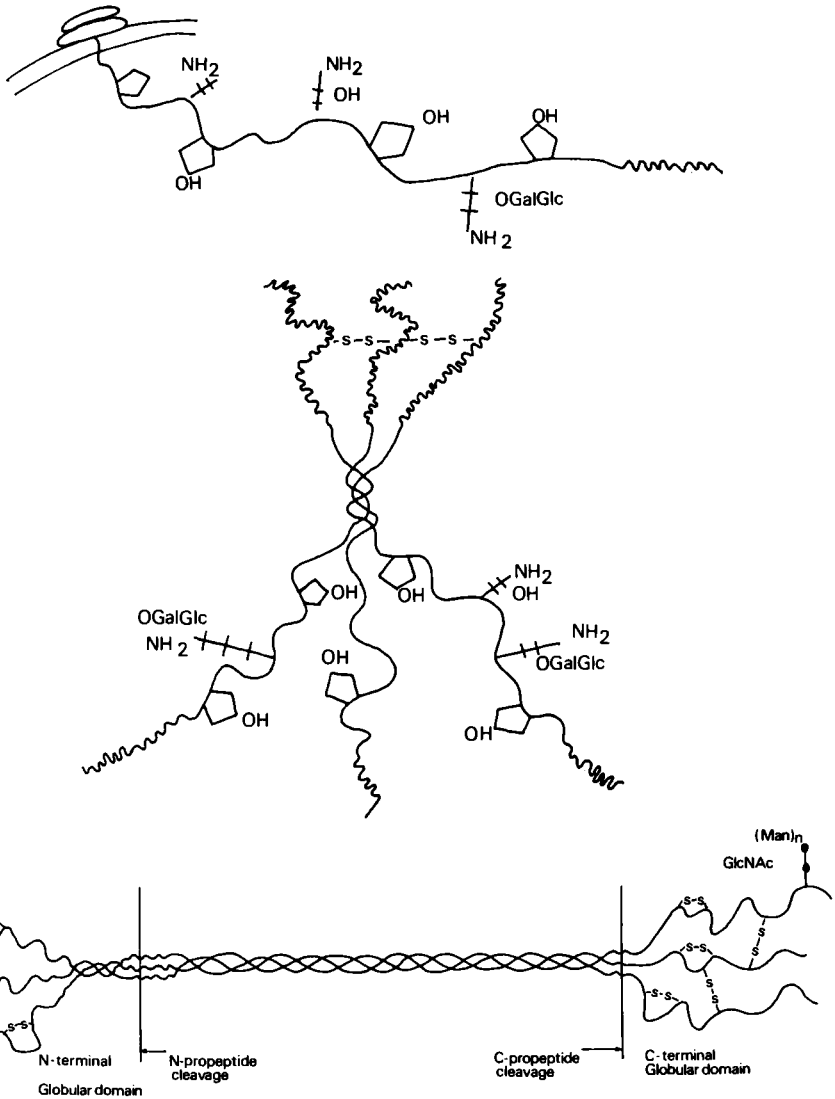

Fig. 6.4 Diagrammatic representation of the synthesis of procollagen (**top**) on the ribosome; (**middle**) during triple helix formation following aggregation of the C-terminal propeptides and (**bottom**) the procollagen molecule possessing a stable triple helix and globular domains at the N- and C-termini.

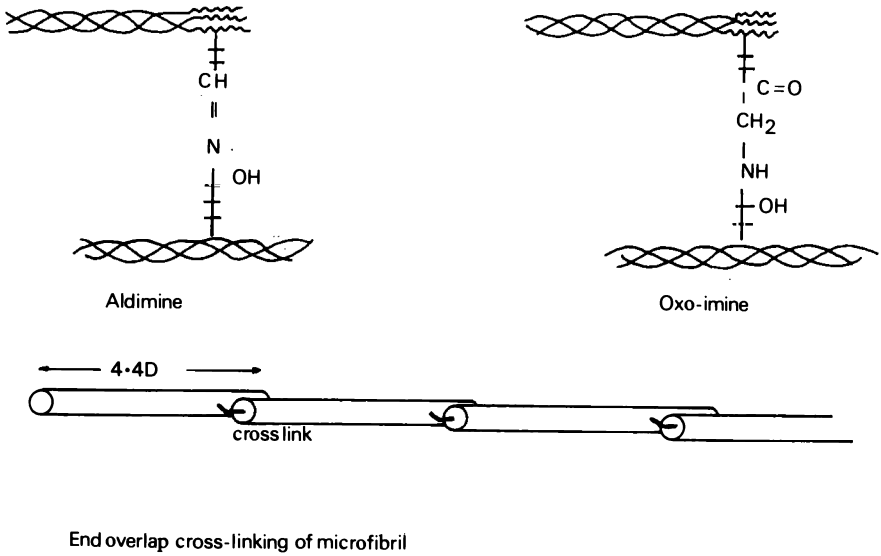

Fig. 6.5 Initial cross-linking of collagen molecules. The structure of the two major cross-links, the labile aldimine and the stable oxo-imine, is shown. The head-to-tail location, including the end-overlap of these cross-links in the microfibril, is also depicted.

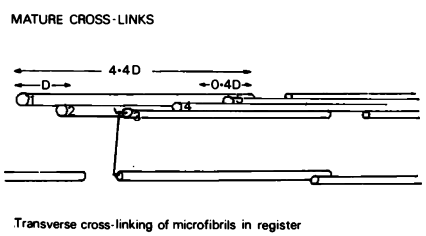

Fig. 6.6 The mature cross-linking of collagen fibres. The diagram illustrates the further reaction of the head-to-tail lateral cross-links within a microfibril to form interfibrillar transverse cross-links. These mature cross-links would form a three-dimensional network, thereby stabilizing the collagen fibre and providing the structure with a high mechanical strength.

link would suggest that there is a common mechanism for the maturation of collagen rather than different mature cross-links for different tissues.

Despite the unique network organization of type IV collagen in basement membranes, the molecules are intermolecularly cross-linked by the same mechanism (Bailey et al 1984). The divalent oxo-imine cross-link has been identified and localized in the N-terminal assembly of the tetramers. Although this cross-link must be present in cross-linking of the tetramers to form the larger network structure, its location has not been established but clearly must involve interaction of the C-terminal region. Basement membrane collagen also matures in the same way as the fibrillar collagen. However, analysis failed to reveal the presence of pyridinoline or hydroxyaldolhistidine. The unknown compound M was, however, found to be present (Barnard et al 1987b). To achieve the same maturation reaction through reaction of the divalent cross-links or their precursors necessitates the molecules of the network structure to be in register. This can be achieved if the type IV networks are overlaid in sheets.

The nature of the cross-links affects the properties of the collagen fibre by providing it with

tensile strength. Although the two intermediate divalent cross-links present in mature tissue are chemically different, the physiological difference in terms of function, if any, has not been established. Certainly the oxo-imine bond is chemically more stable than the aldimine bond and this may be reflected in mechanical properties. For example, tissues under stress may contain a higher proportion of the stable oxo-imine cross-link. In this context we have noted that the extensor tendon and the flexor tendon of the rabbit foot are cross-linked by the oxo-imine and the aldimine bonds respectively (Bailey, unpublished observations).

The presence of these cross-links affects the extractability of the collagen. Tissues containing the aldimine, e.g. skin, are readily extracted in dilute acidic solution, whilst those containing the oxo-imine, e.g. bone, are insoluble. As the tissue matures all the cross-links are stable and the collagen is virtually insoluble under these conditions, and can only be solubilized by degradative methods. These age-related changes in the collagen ensure that collagen is resistant to endogenous proteolytic enzymes, less susceptible to changes in pH and ionic concentrations, and that through the three-dimensional network of intermolecular cross-links it acquires its optimal functioning capacity.

Degradation

The degradation of collagen involves a complex and highly orchestrated series of enzymes (Murphy & Reynolds 1985). The connective tissue cells secrete a group of proteinases that are capable of acting on collagen under physiological conditions. A specific metallo-endopeptidase, generally referred to as mammalian collagenase, has been shown to cleave the three polypeptide chains of collagen at a single site along the helix (Woolley & Evanson 1980). These fragments of the helix are unstable at body temperature, hence are rapidly denatured, in which state they are then digested by many proteolytic enzymes (Fig. 6.7). The enzyme is synthesized as an active zymogen, and activation of the procollagenase is achieved by enzymes such as trypsin and plasmin.

The mammalian collagenase acts on mature fibrous collagen but release of the fragments is inhibited by the intermolecular cross-links. Release of these fragments can be achieved by the neutral cysteine or serine proteases (Burleigh 1977), by acting on the non-triple helical telopeptide ends of the molecule. Cleavage of peptide bonds on the helix side of the cross-links effectively depolymerizes the fibre (see Fig. 6.7).

Both types of enzyme are necessary for degradation of the fibre but the relative importance of each has not yet been established. The fragments are phagocytosed and degraded intracellularly by the lysosomal system of enzymes, primarily the cathepsins (Etherington 1980).

Metabolism

The biosynthesis and degradation has been shown to be a complex series of enzymic and non-enzymic reactions, the basic essentials of which are now understood. The rates of metabolism of collagens are less well known and collagen is generally thought to be metabolically inert. As a generalization, there is a high rate of turnover during early growth but this falls to a low level at maturity (Kivirikko 1970). The level of turnover in mature animals varies considerably with the particular tissue. In the majority of tissues — for instance, skin and tendon — the turnover is slow to non-existent whilst there is a slow but significant turnover in bone. In contrast some tissue, e.g. periodontal ligament, turns over in 2–3 days and therefore never matures (Sodek 1976).

STRUCTURAL CHANGES IN THE COLLAGENOUS TISSUE

Histological changes in DD have been well described in the literature (Larson et al 1960; Hueston 1963; Millesi 1966, 1974; Tubiana 1967), revealing two components — initially a highly cellular nodule and finally a virtually acellular fibrous cord. The nodules are characterized by a network of thin collagen fibres (reticulin) and proteoglycans staining metachromatically with toluidine blue. The collagen of the fibrous bands takes up the typical collagen stains, but the silver staining for reticulin is confined to fine fibres in the centre of

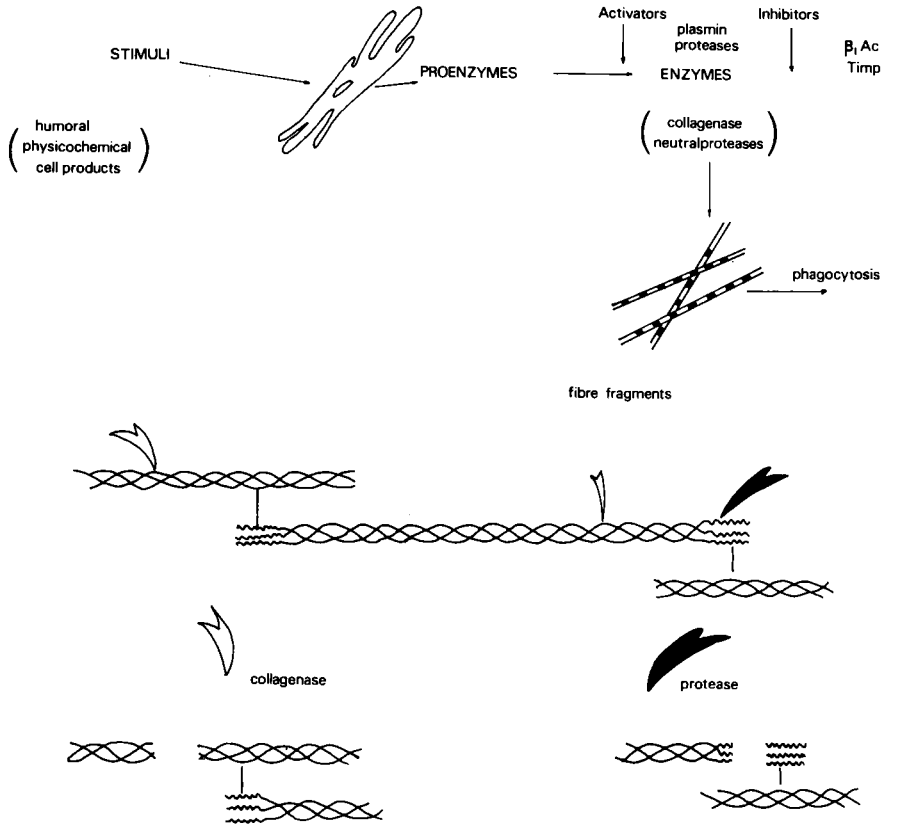

Fig. 6.7 Proposed degradative pathway of collagen fibres. **Top:** Cells are stimulated to synthesize proenzymes (procollagenase and other proteinases) which are then activated to digest intact collagen fibres. Control is believed to be exercised by the presence in the tissue of inhibitors (Timp). **Bottom:** The mechanism of two types of proteinase is illustrated. Collagenase acts at a highly specific location within the triple helix, whilst the other neutral proteinases act on the non-triple helical N- and C-terminal domains.

the band. Like the normal aponeurosis, metachromatic staining of the bands is minimal. The fibres are more highly oriented in a preferred direction in the band compared to the normal aponeurosis. At intermediate stages the collagen fibres appear to be degraded whilst others fuse together to form thicker fibres, and it would appear that the bundle structure breaks down. The elastic fibres are no longer evenly distributed but are reduced in number and located in the periphery of the fibre bundles. The subaponeurotic fat usually remains free of fibrotic infiltration.

Examination of the organization of the fibre bundles by the scanning electron microscope has revealed significant changes (Hunter & Ogdon 1975; Legge et al 1981). The normal parts of the palmar fascia consist of flat interweaving fibres.

Nodules are characterized by a meshwork of fine fibrils which appeared to be incompletely formed. In the bands the fibres are more compact and tended to be oriented in a specific direction. The fibres possess a wavy appearance indistinguishable from normal tendon. Legge et al (1981) also observed tighter bundles which had a shorter wave pattern than the normal fibres. The waves were frequently seen in the form of a helix that was never observed in normal tissue.

In cells in the proliferative stage, Gokel & Hübner (1977) observed intracellular structures surrounded by trilaminar membranes continuous with the endoplasmic reticulum containing banded structures with a periodicity of about 100 nm. These workers concluded the material was intracellular 'fibrous long-spacing' (FLS) collagen, based on comparison with in vitro reprecipitated FLS collagen fibres, but subsequent studies indicate that these FLS fibres were probably type VI collagen.

At high magnification using transmission electron microscopy the individual collagen fibres were seen to possess the normal structure and typical axial banding pattern of 67 nm. Similarly, analysis of the fibres by X-ray diffraction, both wide and low angle, showed no detectable difference between the normal tissue and that from DD patients (Brickley-Parsons et al 1981). It appears from these results that the structure of the individual collagen fibres in DD is indistinguishable from normal collagen fibres. However, at a higher level of order the histological evidence suggests that the structure of the fibre bundles appears to have broken down.

BIOCHEMICAL CHANGES IN THE COLLAGENOUS TISSUE

Recent studies in DD have attempted to identify and separate for analysis at least three regions of the aponeurosis:

1. The highly cellular nodules.
2. The fibrous bands.
3. The apparently unaffected regions, in terms of macroscopic appearance.

There is a progressive increase in the proportion of collagen in the aponeurosis from the control at about 60%, to the bands at about 90% and even higher in the nodules (Bazin et al 1980; Brinkley-Parsons et al 1981; Hamamoto et al 1982). The water content of this collagen increased from 55% in the controls to 62% in the nodules. The proportion of neutral salt-soluble and acid-soluble collagen was very small, about 0.2%, from the diseased tissue compared to virtually nothing from the control. Similarly, the amount of collagen digestible by pepsin treatment increased from 80% in the controls to almost complete solubilization for the diseased tissue. The latter tissues were also solubilized more rapidly by the pepsin than were the controls, as would be expected for immature collagen.

Amino acid compositional analysis of the collagen extracted revealed a higher level of hydroxylation, increasing from 5 to 13 residues of hydroxylysine per 1000 residues (Brickley-Parsons et al 1981). This increase was accompanied by a parallel increase in the number of glycosylated hydroxylysines so that the relative proportion of glycosylated hydroxylysine remained constant. However, it has not been established whether the increased glycosylation occurs predominantly in the type I or type III collagen.

All these changes in chemical and physical properties are consistent with the presence of increased quantities of newly synthesized collagen in the diseased tissue. Similar changes occur in the granulation tissue of healing wounds. Unfortunately no studies have been carried out on the time-related changes analogous to those in the healing wound.

Collagen types

The major collagen of normal aponeurosis is type I, although using the sensitive immunofluorescence technique (Fig. 6.8) it is possible to detect a small amount of type III collagen (Bazin et al 1980). This is similar to the situation in normal tendon where the type III can be located in the endotendinium surrounding the bundles of collagen fibres within the tendon (Duance et al 1977). This hierarchy of fascicles bound together by a collagenous sheath is presumably a requirement for mechanical integrity and normal function of

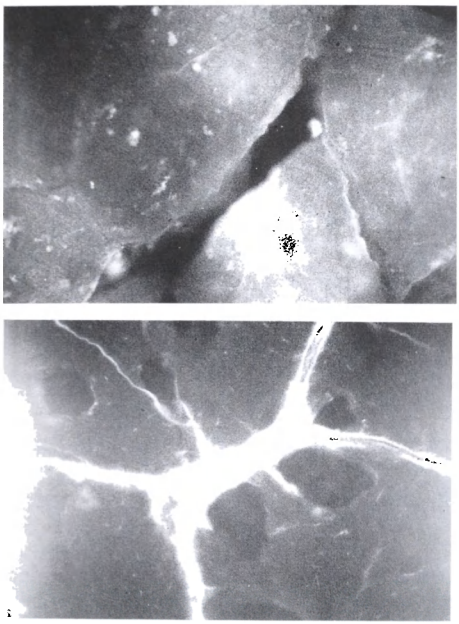

Fig. 6.8 Immunofluorescence location of type I and type III collagen in tendon fibres. **A** Overall staining of fascicles by type I collagen antibody; **B** Preferential staining of endotendinium by anti-type III collagen

tendons. Immunofluorescence studies also indicate the presence of types IV and V as part of the endotendinium sheath (Duance et al 1977) but these have not been confirmed biochemically. The presence of type IV and V in the interfascicular connective tissue has not been described, but is probably analogous to the endotendinium.

Using either pepsin digestion, which in this case solubilized over 90% of the collagen and therefore gave a representative sample (Bailey et al 1977; Bazin et al 1980; Gelberman et al 1980), or the complete dissolution of the sample by cyanogen bromide in formic acid (Brickley-Parsons et al 1981), similar results were obtained for ratios of types I to III (Table 6.1). Basically there was an increase from 1–2% type III in the normal aponeurosis to 10–15% in the apparently uninvolved, 10–20% in the nodules, and 30–40% in

Table 6.1 Analysis of normal and Dupuytren's aponeurosis

	Total Collagen (%)	Solubility (%)		Type III (%)	Type V (%)
		Acid	Pepsin		
Normal aponeurosis	65	0	80	<5	<2
Dupuytren nodules	100	0.2	97	30–40	10
Dupuytren bands	90	0.3	95	20–30	
Apparently unaffected Dupuytren	70	0	90	10–15	

the fibrous bands (Fig. 6.9). The more accurate technique of cyanogen bromide peptide mapping indicated the nodule possessed a higher (28%) type III content than the bands (25%). These small differences between the bands and nodules may be due to differences in experimental techniques or in the duration of the disease in the tissue from which samples were taken.

These changes in collagen type are analogous to those occurring in the granulation tissue of dermal wounds (Bailey et al 1975a) and in hypertrophic scars (Bailey et al 1975b). As in the case of physicochemical properties, the time-related changes in the ratio of types I:III have not been investigated. One would expect a greater amount of type III in the nodules where there is a rapid proliferation of collagen and decreasing amounts in the bands as they mature if the Dupuytren's contracture follows the pattern of normal wounds and fibrotic lesions. On the other hand, a high type III would be retained over a long time period if the bands followed a similar course to the hypertrophic scar.

Other collagen types have been detected, as in granulation tissue, but in much smaller amounts. Type V collagen has been reported to be increased (Ehrlich et al 1982) and this may be associated with increased vascularity since it is a component of basal lamina; type IV of the capillary basement membrane has not been studied. The relative proportions of both type I trimer and type V were found to double from 2 to 5% and from 5 to 9% respectively and these increases are again similar to those found in hypertrophic scars (Ehrlich et al 1982).

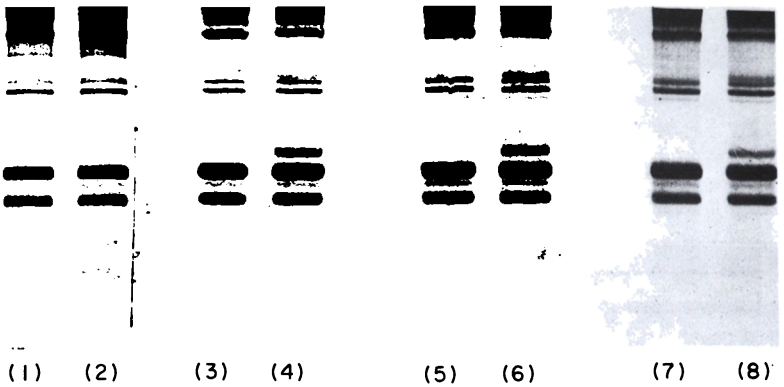

Fig. 6.9 SDS gel electrophoresis of pepsin-solubilized collagen from the aponeurosis of normal and Dupuytren's subjects. **1** and **2**: normal type I collagen without and with mercaptoethanol; **3** and **4**: collagen from the nodules; **5** and **6**: collagen from the bands; **7** and **8**: collagen from the apparently uninvolved aponeurosis. Tracks 3, 5 and 7 show the presence of type I collagen, and tracks 4, 5 and 8 type I and type III after treatment with mercaptoethanol.

Recently type VI collagen has been identified in a number of tissues (Hessle & Engvall 1984). This unusual collagen is not detected in normal tendon but can be readily observed in the electron microscope following in vitro incubation (Bruns et al 1986). Similarly, examination of the fibrotic bands in the aponeurosis of DD reveals the 100 nm banded filaments of type VI collagen (unpublished results). These fibres are probably the 'fibrous long spacing' with a banding periodicity of 100 nm, previously reported by Gokel & Hübner (1977). The suggestion is supported by the immunohistochemical studies on DD tissue using antibodies to type VI collagen (Bailey et al, unpublished results).

Cross-linking

Distinct differences in the cross-link pattern were reported by Bailey and co-workers (Bailey et al 1977; Bazin et al 1980) and have been confirmed by others (Gelberman et al 1980; Brickley-Parsons et al 1981; Hanyu et al 1984). The control tissue, as expected from previous studies of mature tissue, revealed the hexosyl-lysines as the major reducible components; the divalent reducible cross-links were barely detectable. In contrast, the major reducible components of the nodules and the bands were the two reducible cross-links dehydro-dihydroxylysinonorleucine and dehydro-hydroxylysinonorleucine (Fig. 6.10). The reported increased levels of lysyl oxidase (Hamamoto et al 1982) is consistent with the high levels of these reducible cross-links. Surprisingly, the apparently unaffected parts of the aponeurosis also showed increased amounts of the reducible cross-links although a significant level of hexosyl-lysines was still present. It should be remembered that the hexosyl-lysines are not cross-links, but may be considered a good indicator of maturity (Robins & Bailey 1972).

As discussed earlier, the reducible cross-links are only present in immature, and the hexosyl-lysine in mature tissue. The gradual change of pattern shown by the different parts of the diseased aponeurosis indicates an increasing activity of the cells in synthesizing collagen. The apparently unaffected aponeurosis shows clear signs of some newly synthesized collagen, whilst the highly active nodules contain completely new collagen. The bands are mainly newly synthesized collagen, but contain some mature features similar to the control, clearly indicating that some maturation of the tissue has occurred. Surprisingly, Hanyu et al

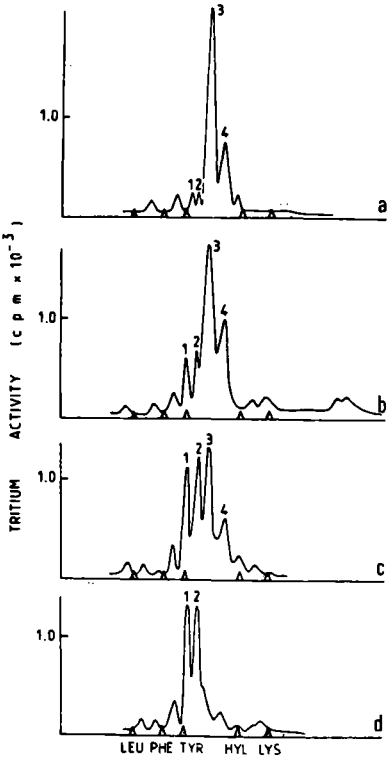

Fig. 6.10 Chromatographic profiles of the reducible cross-links present in control and Dupuytren aponeurosis. a nodules; b bands; c apparently uninvolved; d control. Peaks 1 and 2 represent hexosyl-lysines, 3 dihydroxylysinonorleucine, and 4 hydroxylysinonorleucine.

(1984) reported equal amounts of pyridinoline in the normal and affected aponeurosis and concluded the cross-link was not involved in the pathogenesis of the disease. The presence of the mature cross-link in the bands has not been reported. However, to be of value it would be crucial to know the physiological 'age' of the band in order to correlate this with the amount of pyridinoline and compound M.

The changes detailed above are characteristic of those occurring in hypertrophic scars (Bailey et al 1975b) which, in contrast to normal wounds, fail to mature and retain some of the characteristics of embryonic collagen. These studies indicated a continuing rapid turnover of the collagen even in hypertrophic scars that are many years old.

However, in contrast to this analogy with scar tissue, we observed that the disease is not strictly focal and limited to the nodules, but is clearly evident in the apparently unaffected parts of the aponeurosis (Bailey et al 1977; Brickley-Parsons et al 1981). This is consistent with the well accepted clinical observation that Dupuytren's disease can recur within the same aponeurosis, presumably due to the failure to eliminate the disease by excision only of the grossly affected tissue. We have suggested (Bazin et al 1980) that the disease is initiated and/or propagated by the cells migrating along the collagen bundles. The pathway would presumably be equivalent in the aponeurosis of the endotendinium. The presence of myofibroblasts in the apparently unaffected aponeurosis (Bazin et al 1980) supports the proposal, but this latter finding was not supported by Brickley-Parsons et al (1981). On the other hand, Gelberman et al (1980) have correlated recurrence of DD with those patients in whom myofibroblasts were detected.

Immunolocalization

Using the indirect immunofluorescence technique it is possible to determine the distribution of the various collagen types in tissues (von der Mark 1982). Few studies of the aponeurosis have been carried out. When stained with antibodies to type I collagen, uniform staining occurred as expected; with types III and V, however, the staining was limited to the periphery of the regularly arranged bundles (Bazin et al 1980). This can be compared to the staining of Achilles tendon where the fibre bundles of type I were surrounded by fibres of type III as a peritenon and, closer to the bundles, by a sheath of types IV and V collagen (Duance et al 1977; Fig. 6.11). A similar analysis of the diseased aponeurosis provided a different picture (Bazin et al 1980). The nodules were intensively stained with antibodies to type III and type V within the major bundles, and the bundles appeared to be dissociated into finer fibres (Fig. 6.11). Staining of the apparently unaffected areas revealed much the same picture as for the normal aponeurosis, but in some areas the staining was more intense and the distribution was similar

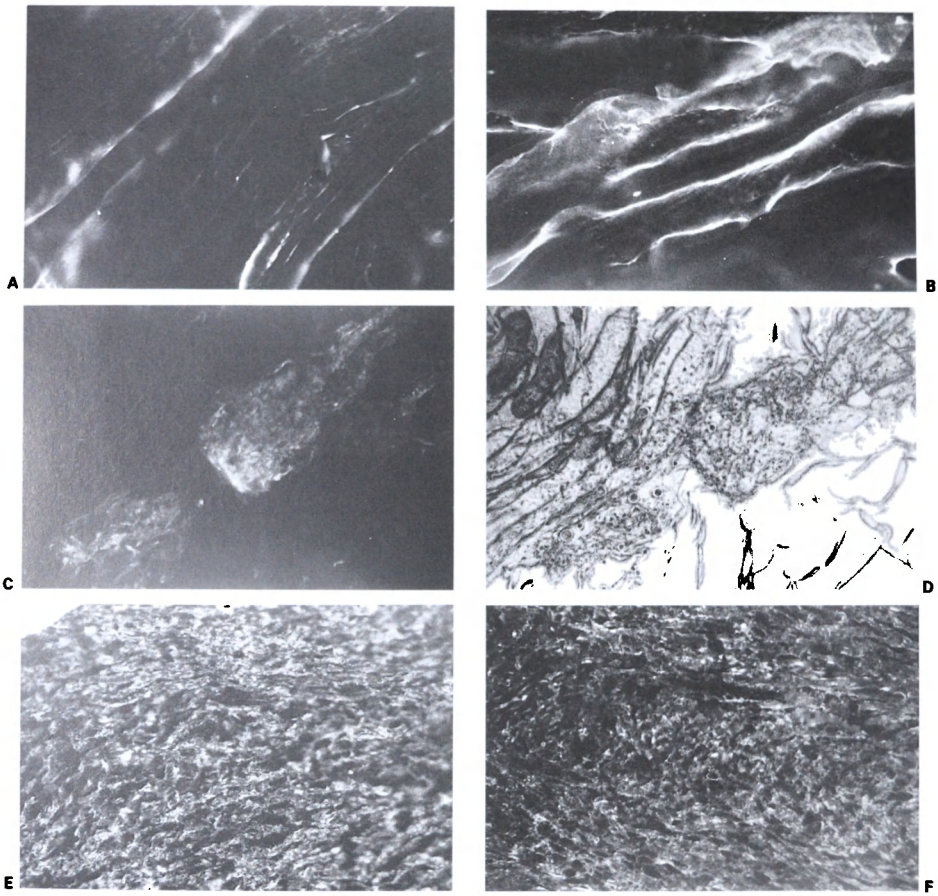

Fig. 6.11 **A**, Immunofluorescent staining of a longitudinal section from a normal human aponeurosis using anti-type III collagen, showing the type III collagen sheath surrounding the packed type I fibres (as in Fig. 6.8) (magnification × 160). **B**, Longitudinal sections from the apparently uninvolved region of the aponeurosis from a Dupuytren's subject demonstrating the large early increase in type III collagen (magnification × 160). **C**, Aponeurosis staining with anti-type III collagen showing the early stage of disorganization (magnification × 60). **D**, Phase contrast picture of similar field to Fig. 6.2c (magnification × 60). **E**, Late stage highly disorganized area of the aponeurosis from a Dupuytren's subject stained with anti-type I collagen (magnification × 160). **F**, Similar field to Fig. 6.2e but stained with anti-type III collagen, again demonstrating the random distribution and high proportion of type III collagen (magnification × 160).

to that in the nodules, although not as extensively dissociated into microfibrils. Unfortunately, no detailed immunohistochemical examination of the 'band' regions has been reported.

These observations certainly support the proposal that the initiating site is within the aponeurosis. However, more detailed studies on the changing location of the different collagen

types using these techniques and following a time sequence in the disease would provide valuable information on the development of DD. In addition, the thin argyrophilic fibrils reported to be present by a number of workers are almost certainly type III collagen but this needs confirming both in the nodules and the bands. The presence of type V indicates vascularization but this requires confirmation using type V antibodies, since Type V may also exist as fibrils in the extracellular matrix. Similarly, the 100 nm banded fibrils seen in the electron microscope need to be confirmed as type VI using antibodies.

CONCLUSIONS

Although much has been learnt about the changes of the collagen in DD this knowledge has not as yet helped our understanding of the nature of the stimulating factors or the mechanism of contraction. Whilst the disease is initiated in the palmar connective tissues, it seems likely that these changes are not unique, but follow the same pattern as those occurring in wound granulation tissue and hypertrophic scar. However, there appears to be a significant difference in that DD does not remain localized but progresses along the tissue.

Collagen does not always respond the same way even to the same stimulus, and the nature of the tissue could alter the response. It is therefore conceivable that the collagen of DD patients is defective and responds in an abnormal way to stimuli that do not affect the majority of individuals. If indeed the collagen of DD patients is genetically abnormal, the change must be subtle since no major biochemical difference has been reported to date. A more detailed analysis of the collagen should be undertaken. Furthermore, the aberration is likely to show up as connective tissue defects in other tissues. The genetic background to DD is well known and the recent typing of patients using collagen antibodies suggests that it may be an inherited collagen disorder.

The ultimate solution to the disease may be to search for and identify the stimulating factor: an exogenous mediator or transformed cell which, based on the recurrence — albeit slow — of the disease appears to be retained in the tissue. A genetically defective collagen could produce an abnormal response to the trauma. Unfortunately, in DD fibrosis usually develops before clinical presentation and knowledge of the stimulating factor is unlikely to help clinically at this stage. However, based on our understanding of the fundamentals of the synthesis and degradation of collagen, several approaches may result in regression of the fibrosis:

1. Control of the production of mRNA at the transcriptional level. New techniques in molecular biology are becoming available to provide an understanding of collagen synthesis at the gene level. For example, dexamethasone and gamma-interferon decrease levels of type I mRNA in chick fibroblasts, and in the future it may be possible to regulate gene expression through the promoter regions of the gene.
2. Control of the post-translational modification. Specific inhibitors of prolyl hydroxylase are known to be effective in reducing synthesis. These act by sufficiently reducing the level of hydroxyproline to destabilize the triple helix at body temperature, and consequently leading to rapid degradation. Inhibition of secretion by colchicine and the use of a feedback mechanism utilizing the C-propeptide are feasible alternatives.
3. Controlled degradation of the collagen. The selective use of collagenases and neutral proteases, or in the future a genetically engineered collagenase, could be effective in removing the excess collagen.

Unfortunately, at the present time inhibiting synthesis and removing excess collagen present formidable difficulties in targeting the therapeutic agents to the specific tissues involved.

The increased understanding of the nature of collagen, its complex biosynthetic and degradative pathways, together with a more detailed biochemical analysis of the progression of the disease will surely provide not only a better explanation of the mechanism of contracture but at the same time may be an alternative treatment to surgery.