REVIEW ARTICLE

# COLLAGEN METABOLISM

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## *A Comparison of Diseases of Collagen and Diseases Affecting Collagen*

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COLLAGEN CONSTITUTES approximately one third of the body's total protein, and changes in synthesis and/or degradation of collagen occur in nearly every disease process. There are also a number of newly described specific diseases of collagen in both man and domestic animals. Thus, an understanding of the synthesis, deposition, and turnover of collagen is important for the pathologist, the clinician, and the basic scientist alike. Fortunately, during the last 10 years much progress has occurred in our understanding of the metabolism of collagen. Much of this progress is discussed in a number of recent books and reviews.[1–3,5–27] A significant part of this progress has been due to studies of the specific defects in the metabolism of collagen in disease of both man and domestic animals.[18,19,22–24,26,27] In fact, it appears likely that it was the discovery of the disease dermatosparaxis in cattle in Texas[133] and Belgium[121,126,131] that quickly led to the discovery of procollagen, the precursor of collagen. Veterinarians, therefore, played a key role in the initial steps leading to the recent major advances in our knowledge of the metabolism of collagen. By virtue of our constant exposure to the full range of diseases affecting a wide range of animals, veterinary pathologists are in an excellent position to identify and characterize the primary metabolic diseases of collagen, as well as the many diseases in which secondary changes in the metabolism of collagen are important. Consequently, in this review we will briefly discuss the current state of knowledge of the structure, synthesis, secretion, deposition, and turnover of collagen. This discussion will serve as a basis for a review of both the primary diseases of collagen discovered to date and the more prevalent disease processes in which changes in the metabolism of collagen are a prominent feature.

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### Types of Collagen

All collagen molecules are long, stiff rods consisting of a triple helix of three polypeptide chains called $\alpha$-chains. There are, however, at least seven and possibly ten genetically different types of collagen molecules in the body (Table 1). These different molecules may contain either one type of $\alpha$-chain or two genetically different $\alpha$-chains (Table 1).

The relative proportions of each of the different types of collagen are tissue-specific (Table 2).[1,5,12,16,58,61–65,68,69,71–73,75,76,81–83,85,86,87,99] For example, in the dermis Type I collagen predominates, but small to moderate amounts of three other types of collagen are also present in the dermis. Type III collagen is present in reticulin fibers and in the walls of muscular arteries, while small amounts of Type V collagen are associated with smooth muscle cells in skin. In addition, Type IV procollagen is present in both vascular and epidermal basement membranes in the skin.

The proportions of the different types of collagen change with time in the same tissue. For example, Type III collagen constitutes more than 60% of the collagen in fetal skin but makes up less than 20% of the collagen in adult skin.[63] This has caused Type III collagen to be called "fetal collagen," but this term is misleading, for Type III collagen constitutes a significant proportion of the collagen in the wall of the gastrointestinal tract, muscular arteries, lung, and uterus of the adult animal.[12,16,29,39,63,80,84,87]

Even though each tissue may contain from two to four different types of collagen, there is no evidence that different types of collagen molecules polymerize in the same fibril. For example, Type I collagen forms the thick banded collagen fibrils that predominate in skin, fascia, ligaments, tendon, and bone. The fibrils of Type I collagen range from 50 to 300 nm in diameter, and they have the 68-nm banded staining pattern that is characteristic of collagen.[10,66,150] Each collagen fibril is surrounded by a thin layer of ruthenium-red–positive noncollagenous glycoprotein or proteoglycan.[150] These fibrils are usually organized into fibers whose dimensions range from $<1\ \mu$ to $>50\ \mu$ in diameter, and each of these fibers is also surrounded by a thin layer of ruthenium-red–positive noncollagenous material.[150] Therefore, the boundary of both fibrils and fibers of collagen is demarcated by this ruthenium-red–positive noncollagenous material. Type II collagen, in contrast, tends to form thin, unbanded fibrils that form a meshwork with aggregates of proteoglycans in cartilage,[69] and Type III collagen is associated with argyrophilic glycoproteins in reticulin fibers.[10] To date, it is not known whether Type AB collagen makes up the thin fibers in the endomysium and perimysium or is a component of muscle basement membranes. It is generally assumed that Type AB colla-

Table 1—Types of Mammalian Collagens

| Polypeptide chains | Apparent molecular weight | Molecular formula | Collagen type designation | Tissue distribution | Selected references |
|---|---|---|---|---|---|
| A. Interstitial collagens | | | | | |
| $\alpha_1$(I)* | 95,000 | $[\alpha_1(I)]_2\alpha_2$ | Type I | All connective tissues | 8–10, 12, 16, 84, |
| $\alpha_2$ | 95,000 | | | | 87, 99, 119 |
| $\alpha_1$(I) | 95,000 | $[\alpha_1(I)]_3$ | Type I trimer | Skin, cartilage, aged or dedifferentiated chondrocytes and fibroblasts *in vitro* | 58, 68, 112, 113 |
| $\alpha_1$(II) | 95,000 | $[\alpha_1(II)]_3$ | Type II | All cartilages, nucleus pulposus, eye | 16–18, 64, 65, 75, 84, 85, 99, 112, 113, 119 |
| $\alpha_1$(III) | 95,000 | $[\alpha_1(III)]_3$ | Type III | Reticulin fibers, smooth muscle, fetal connective tissue | 10, 16, 63, 84, 87, 99, 119 |
| αA | 104,000 | $[\alpha A]_3$ and | Type AB | Placenta, lung, all | 61,62, |
| αB | 99,000 | $[\alpha B]_3$ or | or | muscle tissues, | 72, 77 |
| | | $\alpha A[\alpha B]_2$ | Type V | calvaria and cartilage Muscle and lung basement membrane? | |
| B. Basement membrane collagens | | | | | |
| [Pro-$\alpha_1$(IV)]$_3$ | 180,000 | [pro-$\alpha_1$(IV)]$_3$ | Type IV procollagen | Epithelial and endothelial basement membrane | 2, 14, 44, 83 |
| $\alpha_1$(IV) | 140,000 | $[\alpha_1(IV)]_3$ | Type IV | Derived by limited | 4, 14, 44, 62, |
| or | | or | collagen, | protease digestion | 79, 83, 85 |
| αC | | $[\alpha C]_3$ | basement membrane collagen | of Type IV procollagen | |
| $\alpha_1$(IV) | 115,000 | Peptide | Untyped | Proteolytic fragments | 2, 14, 62† |
| fragments | 95,000 | fragments | | of Type IV | |
| | 70,000 | | | collagen? | |
| | 55,000 | | | | |
| 80K | 80,000 | Presumptive fragments of arginine-rich αD chain | Untyped | Placenta and lens capsule basement membrane | † |

* The $\alpha_1$ and $\alpha_2$ chain designations correspond to the order of elution of these chains during ion exchange chromatography in carboxymethyl cellulose. Dimers of $\alpha$-chains are referred to as $\beta$-components and trimers or intact molecules are reffered to as $\gamma$-components.[76]

† Dr. E. J. Miller, personal communication.

Table 2—Compositional Characteristics of Different Types of Collagen

| | Partial amino acid composition of different α-chains (residues/1000 residues) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $\alpha_1$(I)* | $\alpha_2$(I)* | $\alpha_1$(II)† | $\alpha_1$(III)‡ | αA§ | αB§ | $\alpha_1$(IV) ‖ | 80K¶ |
| Amino acid | | | | | | | | |
| Hyl | 4 | 8 | 23 | 5 | 22 | 39 | 57 | 36 |
| Lys | 30 | 22 | 13 | 30 | 12 | 13 | 10 | 6 |
| Arg | 41 | 51 | 51 | 46 | 48 | 40 | 27 | 42 |
| 3-Hyp | 1 | 0 | 1 | 0 | 7 | 10 | 12 | 1 |
| 4-Hyp | 96 | 86 | 100 | 121 | 113 | 105 | 120 | 110 |
| Pro | 129 | 113 | 122 | 102 | 98 | 120 | 67 | 73 |
| Gly | 330 | 336 | 332 | 355 | 346 | 334 | 330 | 330 |
| Ala | 112 | 102 | 104 | 92 | 52 | 46 | 32 | 47 |
| Glycosylated hydroxylysine | | | | | | | | |
| Gal-Hyl | 0† | 1† | 4† | 1† | 3# | 5# | 3 | 2 |
| Glc-Gal-Hyl | 1 | 1 | 5 | 1 | 5 | 29 | 48 | 29 |

* Data from Piez et al.[76]
† Data from Miller.[16]
‡ Data from Epstein.[63]
§ Data from Burgeson et al.[61]
‖ Data from Kefalides.[14]
¶ Data from Dr. E. J. Miller, personal communication.
# Data from Chung et al.[62]

gen is an interstitial collagen, but this collagen may be a constituent of basement membrane in placenta, lung, and muscle tissue.[61,72]

**Structure of Collagen Molecules**

As shown in Table 1, each different type of collagen is a unique combination of three α-chains, and each of the different α-chains is a unique gene product with a unique amino acid sequence.[7-10,16] There are many similarities in these chains, however. All of the α-chains consist of a repeating triplet of glycine and two other amino acids. These polypeptide chains may be represented by the formula $(Gly\text{-}X\text{-}Y)_n$. In connective tissue collagens $n$ equals ~334, but in basement membrane procollagen $n$ may equal ~490. In both connective tissue and basement membrane collagens, the Y position is often occupied by 4-hydroxyproline, and the imino-acids proline and hydroxyproline constitute 20–25% of all residues (Table 2).[7-10,14,16] Similarly, in all chains many lysyl residues are hydroxylated, and some of these hydroxylysine residues are glycosylated with galactose or glucosylgalactose (Table 2).[7-10,14,16] These amino acids all have a major role in determining the structure and function of collagen molecules.[2,7-10,14-16,20] Furthermore, the unique distribution of charged amino acids in each different type of collagen molecule permits the electron microscopist to fingerprint the different types of collagen when they are precipitated from solution as segment-long-spacing crystallites.[10,60,66,79,87]

The repeating triplet Gly-X-Y is an absolute requirement for the formation of the triple helix.[10] Glycine is the smallest amino acid, and its small size permits the α-chains to form a tight triple helix.[10] This helix is then stabilized by hydroproline, which helps to form water bridges that stabilize the triple helix at body temperature.[59,78] This tight stable helicity is necessary for the normal secretion of collagen,[9,59,78] and it makes the native collagen molecule resistant to cleavage by all of the tissue proteases except collagenase.[10,21] This tight helix also makes the collagen molecule a relatively stiff, unbending rod, and this configuration is essential for the organization of collagen molecules into fibrils.[5,66] Similarly, the deposition of collagen in fibrils and the presence of hydroxylysine are both essential for the formation of intermolecular cross-links, and cross-links are essential for the development of the high tensile strength of collagen fibrils.[6,7,11,24,66,67] Thus, defects or changes in any of these structural components of collagen molecules will result in defects or changes in the structure and function of connective tissues.

An unwinding of the triple helix represents "denaturation" of collagen. This unwinding may occur at small foci within the molecule, or it may involve the entire molecule. In either case, sites of denaturation are susceptible to digestion with both acid hydrolases and neutral proteases.[11,21,23] Thus, when the local temperature of tissues is increased above ~40 C, hydrogen bonds and water bridges that hold the helix together are broken, and that portion of the helix melts, unwinds, and becomes susceptible to enzymatic digestion.[59,78] Similarly, when one or more of the α-chains is enzymatically cleaved with collagenase or when a chain is physically broken, the helix begins to unwind and become susceptible to further enzymatic digestion.[11,21] Consequently, those structural characteristics that contribute to the formation and stabilization of the triple helix of α-chains are not only required for the synthesis, secretion, and deposition of collagen; they are also essential for the normal maintenance and slow turnover of this protein in connective tissues.

The primary and tertiary structure of collagen also both play a critical role in the interaction of cells with collagen in their environment and in the interaction of collagen with other extracellular matrix proteins.[3,5,88–97,99] For example, myogenic differentiation of mesenchymal cells is promoted by all types of collagen, while chondrogenic differentiation of somite mesoderm is preferentially promoted by Type II collagen. In addition, the cell attachment glycoprotein fibronectin binds with most avidity to Type III collagen, with slightly less avidity to Type I and Type II collagens, and with progressively decreasing avidity to Type AB collagen, fibrinogen, elastin, and keratin. Furthermore, fibronectin binding is greater to denatured collagens than to native molecules, and this binding is great-

est to cyanogen bromide peptide 7 of the Type I collagen $\alpha_1$-chain and to cyanogen bromide peptides 8 and 12 of the Type II collagen $\alpha_1$-chain. This binding site of fibronectin to collagen corresponds to the binding site of mammalian collagenase to native collagen molecules, and the specificity of this binding suggests that this cell attachment glycoprotein may also play an important role in regulating the interaction of collagenase and its substrate. In addition, fibronectin on the surface of platelets has recently been shown to mediate the binding of platelets to collagen fibrils, and platelet aggregation is stimulated most by Type III collagen and least by basement membrane collagen.

#### Synthesis and Processing of Procollagen Polypeptides

Nearly 10 years ago biochemical studies of the skin of dermatosparactic cattle [131] led to the discovery of a precursor form of the $\alpha$-chains of Type I collagen, and this discovery was soon followed by the discovery that all collagen polypeptide chains are synthesized as larger procollagen chains, called pro-$\alpha$-chains.[8–10,16,20,31–40,42–44,47–57] These pro-$\alpha$-chains are synthesized on membrane-bound polysomes on the rough endoplasmic reticulum (RER) (Text-figure 1).[48,50,57] These pro-$\alpha$-chains consist of a central collagenous region, $(Gly\text{-}X\text{-}Y)_{334}$, and both amino (N) and carboxy (C) terminal noncollagenous propeptide regions (Text-figure 1). A short collagenous segment, $(Gly\text{-}X\text{-}Y)_{10}$, is present in the N-terminal propeptide of Type I collagen, but most of this propeptide and all of the C-terminal propeptide is noncollagenous.[9,10,20] Since these propeptides have apparent molecular weights of 20,000 (N-terminal) and 34,000 (C-terminal), the apparent molecular weight of pro-$\alpha$-chains of interstitial procollagen is ~154,000, and the apparent molecular weight of a procollagen molecule in connective tissues is ~450,000.[8–10,20]

Recent studies of collagen synthesis in cell-free systems have shown that procollagen chains are actually synthesized as preprocollagen chains with approximately 100 additional residues on the beginning of the N-terminal propeptide.[20] These residues are thought to represent a signal peptide or leader peptide for the initiation of synthesis and extension of nascent peptide chains into the RER.[20] This prepropeptide is then quickly removed from the pro-$\alpha$-chains in the RER, and the remainder of the N-terminal propeptide remains intact until procollagen is secreted into the extracellular space (Text-figure 1).[8–10,20] As noted below, this N-terminal propeptide has been shown to play a critical role in both the normal metabolism of collagen and in the disease dermatosparaxis in cattle, sheep, and man.

### Transcription and Translation

As with all proteins, transcription of the genetic message from DNA to messenger RNA (mRNA) is the first step in the synthesis of each preprocollagen chain (Text-figure 1A). Each different mRNA is then translated on the polysomes on the RER, and each nascent polypeptide chain extends through the membrane into the lumen of this organelle (Text-figure 1B). Since these processes determine the primary structure of proteins, we assume that defects in transcription or translation will be responsible for defects in the primary sequence of amino acids in collagen polypeptide chains. Furthermore, since most dominantly inherited diseases are thought to involve structural protein defects, we assume that the autosomal dominant defects in collagen are due to primary structural changes in the molecules. The existence of these defects has yet to be demonstrated, but a number of animal models with autosomal dominant defects in collagen are now available, and these models are valuable tools for studies of the role of transcriptional and translational errors in the pathogenesis of diseases of collagen. Furthermore, there are a number of acquired diseases in which there are significant changes in the rates or types of collagen synthesis, and these changes will result from regulatory changes in transcription and translation.

### Posttranslational Modifications

While the prepro-$\alpha$-chains are still attached to ribosomes, the first two of eight posttranslational modifications of these chains is begun. The hydroxylation of proline and lysine residues begins on nascent chains and is completed on free pro-$\alpha$-chains before the helix is formed in the lumen of the RER (Test-figure 1C).[8–10,15,20,41] In fact, these hydroxylations essentially stop when the polypeptide chains form a triple helix, and conditions that speed or slow triple helix formation, such as changes in temperature or changes in the rates of formation of disulfide bonds, may alter the amounts of hydroxyproline and hydroxylysine in each type of polypeptide chain.[8–10,15,16,20,55] Similarly, deficiences or defects in the hydroxylating enzymes or in their cofactors or cosubstrates will result in deficiencies of hydroxyproline or hydroxylysine, and this process will result in defects in the secretion, fibrillogenesis, cross-linking, or degradation of collagen.[15,20,22,26,27,36,40,41,49] It is not surprising, then, that defects in these hydroxylation reactions are the basis for a number of heritable and acquired diseases of collagen.

Smaller amounts of hydroxyproline occur in elastin, the Clq component of complement, and in the collagenous tail of the enzyme acetylcholinesterase. With these exceptions, hydroxyproline in vertebrate tissues

TEXT-FIGURE 1A–E—Synthesis, processing, secretion, deposition, and stabilization of collagen.

A—Transcription of the genes for each different procollagen chain.

B—Translation of the mRNA for each procollagen chain.

C—Posttranslational modifications of procollagen.

1. Hydroxylation of proline and lysine residues in nascent prepro-α-chains and in free pro-α-chains in the lumen of the RER.
   Requirements:

| Enzymes | Cofactors or cosubstrates |
|---|---|
| prolyl 4-hydroxylase | free oxygen |
| prolyl 3-hydroxylase | α-ketoglutarate |
| lysyl hydroxylase | ascorbate |
| | ferrous iron |
| | peptidyl proline and lysine |

2. Glycosylation of hydroxylysine residues in free pro-α-chains in the RER.
   Requirements:

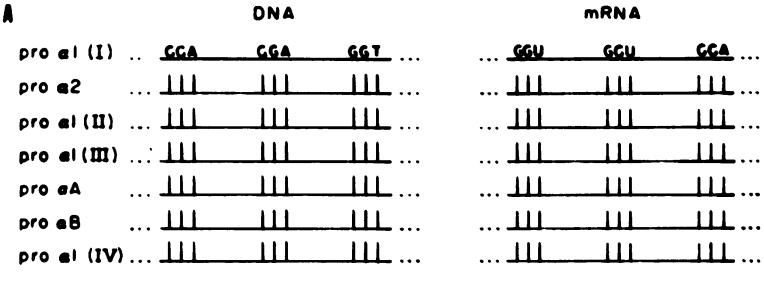
| Enzymes | Cofactors or cosubstrates |
|---|---|
| galactosyl transferase | $Mn^{++}$ |
| glucosyl transferase | UDP-sugars |
| | peptidyl hydroxylysine |

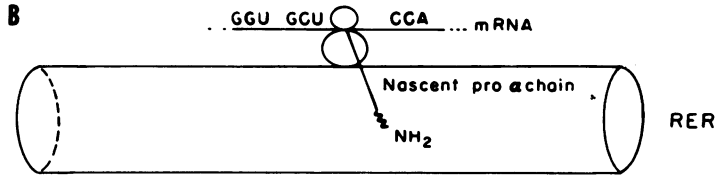
3. Glycosylation of propeptides.
   Requirements:

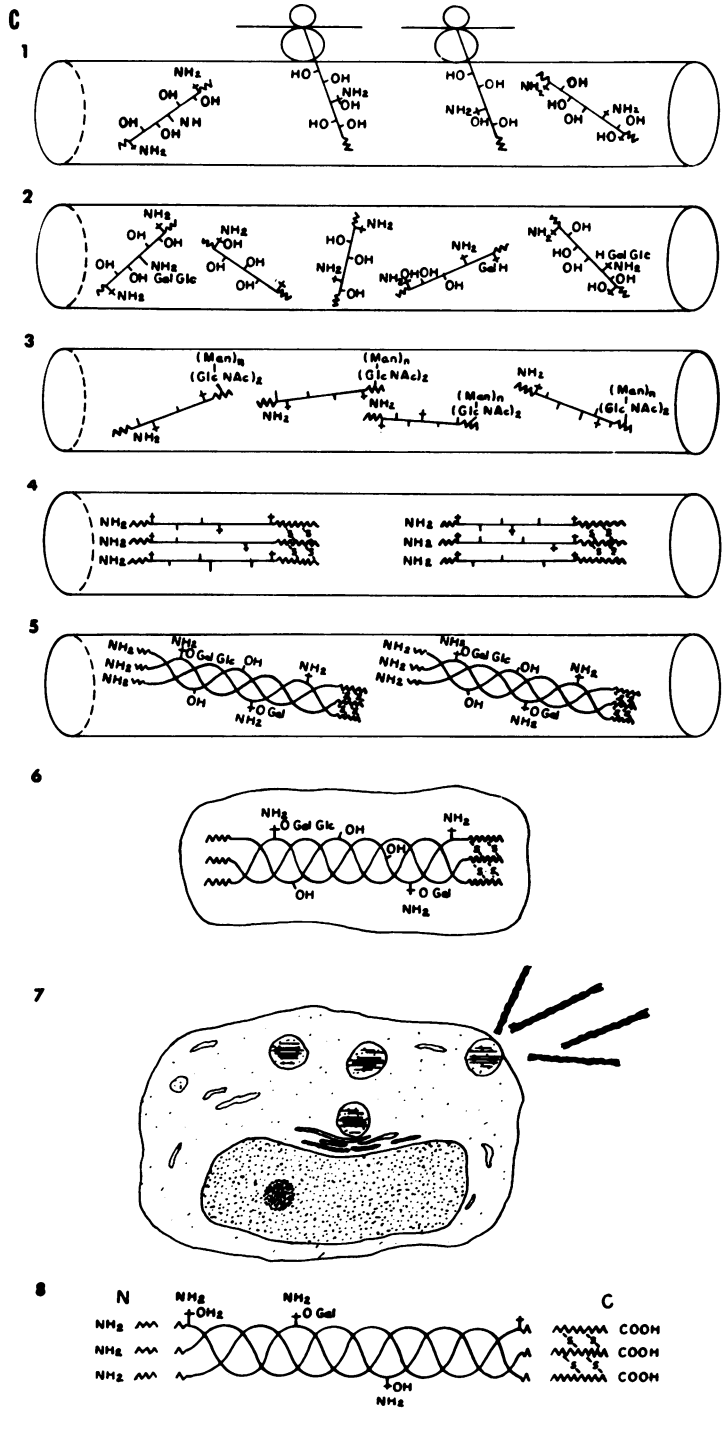
| Enzymes | Cofactors or cosubstrates |
|---|---|
| glucosyl transferase | GDP-mannose |
| mannosyl transferase | UPD-glucosamine |
| | peptidyl asparagine |

4. Interchain disulfide bond formation between C-terminal propeptide.
5. Triple helix formation.
6. Triple helical procollagen molecules are transported in coated vesicles from the RER to the Golgi complex.
   Requirements: energy
7. Secretion: procollagen molecules are aligned in condensed secretion granules on the maturing face of the Golgi complex, and these granules are transported to the cell surface, where procollagen is released into the extracellular space.
   Requirements: energy
   intact microtubules
8. Procollagen molecules are converted to collagen in the extracellular space.
   Requirements:

| Enzymes | Cofactors or cosubstrates |
|---|---|
| procollagen N-peptidase | $Ca^{++}$ |
| procollagen C-peptidase | $Zn^{++}$? |




C
1
2
3
4
5
6
7
8
N
C
COOH
COOH
COOH

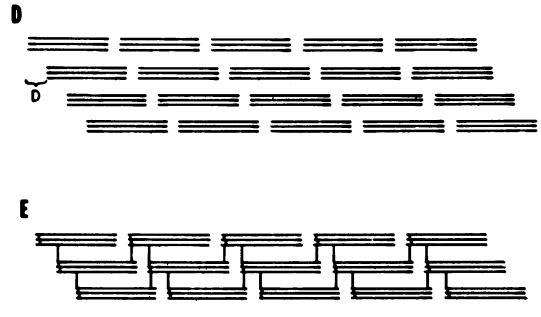
D—Triple-helical collagen molecules spontaneously undergo fibrillogenesis. Each diagonally associated molecule is staggered by one fourth of the molecular length (*D*). A hole (0.6D) exists between each linearly associated molecule.

Requirements: conversion of procollagen to collagen
physiologic salt and ion concentrations

E—Intermolecular lysyl derived cross-link formation.

Requirements:

| Enzymes | Cofactors or cosubstrates |
|---|---|
| lysyl oxidase | copper |
| | free oxygen |
| | hydroxylysine |
| | collagen fibrils |



is unique to collagen. Furthermore, since eukaryotic cells contain no transfer RNA for this amino acid, eukaryotic cells cannot incorporate free hydroxyproline into newly synthesized polypeptides. Thus, all hydroxyproline in the body is derived from the hydroxylation of proline residues in the Gly-X-Y triplet in newly synthesized peptides. Furthermore, since hydroxyproline cannot be reutilized, the excretion of hydroxyproline is a measure of the catabolism of collagen.[11,21,27]

The hydroxylation of proline and lysine are catalyzed by the enzymes prolyl 3-hydroxylase, prolyl 4-hydroxylase, and lysyl hydroxylase, and these reactions all require free $O_2$, ferrous iron, $\alpha$-ketoglutarate, and ascorbic acid (Text-figure 1C).[5,8,9,15,20] As indicated by their names, prolyl 3-hydroxylase hydroxylates the third carbon, and prolyl 4-hydroxylase hydroxylates the fourth carbon in the proline ring. In addition, prolyl 3-hydroxylase only recognizes proline in the X position, and prolyl 4-hydroxylase only recognizes proline in the Y position of the Gly-X-Y triplet. Since 4-hydroxyproline is predominant in all collagens, 4-hydroxyproline is the isomer that is usually referred to with the family name "hydroxyproline." However, 3-hydroxyproline constitutes ~10% of the total hydroxyproline in basement membrane procollagen (Table 1),[2,14,44,46] and it is possible that this amino acid contributes to basement membrane procollagen's higher thermal stability or its resistance to digestion with mammalian collagenase.

It has been clearly demonstrated that adequate quantities of 4-hydroxyproline are necessary to stabilize the triple helix of collagen at body temperature.[59,78] Consequently, when the 4-hydroxyproline content is significantly reduced by conditions such as hypovitaminosis C or a local tissue hypoxia, newly synthesized collagen is denatured (non-triple-helical) at body temperature. This results in a marked reduction or failure in the secretion and deposition of collagen, as well as a marked increase in the rate of degradation of the underhydroxylated collagen that is secreted and incorporated into fibrils. To date, no heritable defect in prolyl hydroxylase has been identified; but scurvy is a prominent example of an acquired disease resulting from the underhydroxylation of proline and lysine residues.

It has also been clearly established that hydroxylysine residues are required for the formation of the intermolecular cross-links that stabilize collagen molecules within fibrils.[5,6,24,67,74] Like prolyl 4-hydroxylase, lysyl hydroxylase only recognizes lysine residues in the Y position of the Gly-X-Y triplet.[37] Thus, hydroxylysine, like hydroxyproline, is almost unique to collagen in vertebrate tissues. There is considerable variation, however, in the amounts of hydroxylysine in different types of collagen (Table 2).[2,5,10,14–20,22,24,26,27,61–63,77] In fact, there is even a significant difference in

the amounts of hydroxylysine in the same type of collagen in different tissues and at different ages in the same tissue.[28] There are also differences in the amounts of glycosylation of these hydroxylysine residues in different types of collagen (Table 2).[2,5,8–10,14,16,20] These modifications of collagen chains undoubtedly serve to increase the functional specificity of collagen molecules, but the significance of each of these variables has yet to be determined.

It is clear that defects in the hydroxylation of lysine must occur before procollagen molecules are organized into a triple helix in the RER, but a deficiency of hydroxylysine is only expressed when procollagen molecules are secreted, converted to collagen, and organized into fibrils. A deficiency of hydroxylysine results in a deficiency of intermolecular cross-links in collagen, and this deficiency results in a loss of tensile strength of collagen fibrils.[5,6,15,22,24,26] However, this defect is not as severe as that seen with defects in another enzyme, lysyl oxidase, that is required for the formation of all lysyl-derived cross-links in both collagen and elastin.[5,6,15,24,26] Nevertheless, a heritable deficiency of lysyl hydroxylase has been shown to have rather profound effects in man,[22,130,135,137,139,140] and it is likely that a similar defect will soon be found in other animals.

Glycosylation of hydroxylysine residues is the third posttranslational modification of the pro-$\alpha$-chains in the RER (Text-figure 1C). This process involves the enzymatic transfer of galactose to specific hydroxylysine residues in free pro-$\alpha$-chains.[8,9,12,14–17,20] Some of these galactosylhydroxylysine residues are then glycosylated further by the addition of a glucose residue.[2,9,14,15] These reactions are catalyzed by the enzymes glucosyl- and galactosyl-transferase, and these enzymes require $Mn^{++}$ as a cofactor for this transfer of the sugars from UDP-hexose to hydroxylysine or galactosylhydroxylysine. Since hydroxylysine is required as an acceptor for the sugars, this glycosylation cannot occur when the hydroxylation of lysine residues is blocked. Since the triple helical conformation also blocks this reaction, the amount of glycosylation may be affected by changes in the rates of triple helix formation. In fact, the time between synthesis and triple helix formation and the amount of glycosylation vary with the different types of collagen. Furthermore, a delay in triple helix formation is reflected by an increase in the lag time between synthesis and secretion of procollagen, and this is associated with an increase in the glycosylation of pro-$\alpha$-chains.[14,49,56] For example, the synthesis of a pro-$\alpha$-chain requires $<8$ minutes, but the time for secretion is 20 minutes for Type I procollagen, 33 minutes for Type II procollagen, and 60 minutes for Type IV procollagen.[15,26] These lag times are proportional to the increasing amounts of glycosylation of hydroxylysine in these different types of colla-

gen (Table 2). Nevertheless, the sugar residues do not control the lag time for secretion. They are, however, thought to affect the packing of collagen into fibrils and to have a role in the interaction of collagen with other extracellular matrix components and with cell surfaces.

The noncollagenous C-terminal peptide on each pro-α-chain is also glycosylated in the RER (Text-figure 1C).[33,34,37,51] This glycosylation is quite different from that in the collagenous regions of pro-α-chains. For example, the sugars on the propeptide include glucosamine and mannose that are linked to asparagine through an N-glycosidic linkage.[33] The addition of these sugars is blocked by tunicamycin [37] but is not affected by defects in the hydroxylation of lysine.[34] This suggests that the glycosylation of the propeptides involve dolichol phosphate intermediates, like those described for other glycoproteins.[37] To date, however, the function of these sugars on procollagen is not known.

Disulfide bonding and triple helix formation are the fifth and sixth post-transitional modifications of collagen (Text-figure 1C). Triple helix formation occurs in the lumen of the RER, and this process is initiated when disulfide bonds form between the C-terminal peptides of three pro-α-chains.[8,26,55] It is thought that these interchain disulfide bonds bring three pro-α-chains into register so that the collagenous regions can then spontaneously wind into a tight triple helix (Text-figure 1C).[8,26,55] Consequently, these C-terminal propeptides are referred to as "registration peptides." Presumably, these peptides also serve to bring the correct types of pro-α-chains together to make each specific type of collagen. This has not been proven, however, and it is possible that different collagens are made in separate compartments of RER. Since one cell may be simultaneously synthesizing up to three different types of collagen, plus elastin, proteoglycans, fibronectin, or other extracellular matrix glycoproteins, there must be some method of specific recognition or compartmentalization of the correct polypeptides that are necessary to make up each specific protein. Thus, it seems likely that the C-terminal propeptide may help with both recognition and registration in the association of three correct pro-α-chains.

Initially, it was thought that the N-terminal propeptides were registration peptides; but they have now been shown to contain intrachain, but not interchain, disulfide bonds.[9,32,35,38] This finding does not preclude the possibility that the N-terminal peptides may also have a role in recognition or registration of pro-α-chains, but the likelihood of such a role is reduced by the absence of interchain disulfide bonds in the N-terminal propeptides.

Once the triple helix is formed, procollagen is transported from the

RER to the Golgi complex, where it is packaged for secretion (Text-figure 1C).[48,50,57] During secretion procollagen molecules become aligned in condensed packets in condensed Golgi vacuoles.[57] These secretion vacuoles are then transported to the cell surface, where the procollagen is secreted by the usual process of exocytosis (Text-figure 1C).[57] Since this process of transport and secretion is blocked when microtubules are depolymerized with colchicine or vinblastine, these organelles are thought to play a role in the secretion of procollagen.[7,8,9] It is incorrect, however, to conclude that colchicine or vinblastine block either the conversion of procollagen to collagen, the process of collagen fibrillogenesis, or collagen cross-linking, simply because these events are extracellular, and the drugs that depolymerize microtubules block the secretion of procollagen into the extracellular space.

Recent studies have shown that during the lag before secretion relatively large amounts of newly synthesized collagen undergo intracellular degradation.[30] In fact, as much as one third of the collagen that is synthesized may actually be degraded before secretion occurs.[30] The function of this destructive process is not known, but it may serve to prevent abnormal molecules from being secreted. Such abnormal molecules may include those with defects in their primary sequences or those with abnormal combinations of different types of pro-$\alpha$-chains. Furthermore, intracellular degradation may be a mechanism for regulating the rate of secretion, which is independent of the rate of synthesis of procollagen (R. Crystal, personal communication).

#### Extracellular Processing of Procollagen and Collagen Fibrillogenesis

Since collagen molecules spontaneously precipitate as fibrils at physiologic salt and ion concentrations, it is fortunate that collagen is synthesized and secreted as a procollagen molecule that is soluble under physiologic conditions.[5,8–10,16,26] Otherwise, it would be impossible for collagen molecules to diffuse through extracellular fluids to sites of fibrillogenesis. Since procollagen is soluble, however, the sites of fibrillogenesis may be determined by the sites of localization of the enzymes that cleave the C- and N-terminal propeptides and convert procollagen to collagen. These enzymes are referred to as procollagen N-peptidase and procollagen C-peptidase, and it is a defect in the procollagen N-peptidase that results in dermatosparaxis.[42,43,131] These peptidase enzymes have now been partially purified, but their location in tissues has not yet been determined. However, the sites of fibrillogenesis of different types of collagen serve to imply the location of the sites of conversion of the different types of procollagen. For example, Type I collagen is deposited near the surface of fibroblasts,

while Type II collagen is deposited in the interterritorial matrix of cartilage at points that are roughly equidistant from surrounding chondroblasts. This implies that the procollagen peptidase enzymes in cartilage are localized in the extracellular matrix at some distance from the surface of chrondroblasts, while in fibrous connective tissue these enzymes may be located on the cell surface or in the extracellular matrix, near the surface of cells. In contrast, Type IV procollagen is not converted to collagen before it is deposited in basement membranes.[44-46] Instead, it is linked by disulfide bonds to noncollagen basement membrane glycoproteins,[2,14] and the new basement membrane is deposited between the secretory cell surface and the existing basement membrane.[45] This deposition of Type IV procollagen in basement membranes is thought to explain the absence of banded collagen fibrils in most basement membranes.[44,45] Thus, the sites and mechanisms of deposition of different types of collagen in the extracellular matrix are known to be important characteristics of each different type of collagen, but we do not know what mechanisms serve to control fibrillogenesis. Recent studies have shown, however, that some of the primary diseases of collagen involve defects in the control of both the linear and lateral growth of collagen fibrils, and animals with such defects should also serve as valuable models for basic studies of the mechanisms of control of collagen fibrillogenesis.[149,150]

One of the more prominent characteristics of collagen fibrils is a 68-nm periodic staining pattern. This periodicity may vary from 60 to 70 nm in different preparations, but it is a constant characteristic that serves to distinguish collagen fibrils from all other fibrillar structures in the extracellular matrix. It also serves to identify fragments of collagen fibrils that have been phagocytosed. This periodicity or banding results from the alignment of polar groups in staggered collagen molecules in fibrils of connective tissue collagens.[66] In these fibrils, adjacent collagen molecules are staggered by one fourth of their molecular length (Text-figure 1D).[66] This overlap distance is referred to as "D."[66] There is not, however, a D overlap of diagonally associated molecules (Text-figure 1D). Instead, the C-terminus of one molecule overlaps the N-terminus of diagonally associated molecules by 0.4 D, and there is a 0.6 D space or hole between each succeeding linearly associated molecule (Text-figure 1D).[66] This organization has important structural and functional ramifications, for intermolecular cross-links must develop in the region of the 0.4 D overlap in order to link collagen molecules into a cohesive fibril (Text-figure 1E).[5,6,24,67] In fact, the only sites of intermolecular cross-linking are between lysine and/or hydroxylysine residues in both the C- and the N-terminus of one molecule and reactive residues in adjacent collagen molecules (Text-figure

1E).[5,6,24,67] Thus, if there is a deficiency of lysine and/or hydroxylysine residues in either the N- or C-terminus, collagen cross-linking will be affected, and the tensile strength of collagen fibrils will be lost.

The lysyl-derived cross-links form during the first few hours or days after collagen is deposited in a fibril, and they are an absolute requirement for the development of a cohesive collagen fibril. However, after a short time the reducible lysyl-derived cross-links disappear from collagen, while the collagen continues to become more insoluble and the tensile strength of fibrils continues to increase.[5,6,24,67] This process is thought to be due to the conversion of the reducible lysyl-derived cross-links to a more stable, "mature," nonreducible cross-link. Nevertheless, the chemical nature of these mature cross-links has not yet been identified. These mature cross-links do not form in the absence of lysyl-derived cross-links, however; and both reducible and nonreducible cross-links must form to contribute to the development of the insolubility, inextensibility, slow turnover, and high tensile strength that characterize collagen fibrils.[5,6,24,67] Intramolecular lysyl-derived cross-links also form between the N-terminal regions of $\alpha$-chains, and these contribute to the stability of collagen molecules within fibrils. They may not, however, contribute directly to the development of the tensile strength of collagen fibrils.

The formation of lysyl-derived cross-links requires the enzyme lysyl oxidase, which catalyzes the oxidative deamination of the $\epsilon$-amino groups of selected lysyl and hydroxylysyl residues in both collagen and elastin.[5,6,24,25] This is an extracellular enzyme, and it has an absolute requirement for copper as a cofactor. As a result of this oxidative deamination, lysine and hydroxylysine are converted to reactive aldehyde (eg, allysine or hydroxyallysine) residues, and these residues react with each other or with other active sites to form aldol, hydroxyaldol, or ketoimine (Schiff base) cross-links in collagen or demosine and isodesmosine cross-links in elastin.[5,6,24,25] Since this process involves the formation of reactive aldehydes and requires free oxygen and copper, cross-linking of both collagen and elastin is blocked by 1) a deficiency of lysyl oxidase, 2) a deficiency of copper, 3) substances such as $\beta$-aminopropionitrile that irreversibly bind to the enzyme lysyl oxidase, 4) tissue hypoxia, or 5) drugs such as penicillamine that bind to reactive aldehydes.[5,6,24] Consequently, there are a number of both heritable and acquired diseases that result from defects in the cross-linking of collagen and elastin.

During the process of growth and maturation of connective tissues there is an increase in the mean and maximum diameter of collagen fibrils. These diameters are probably both tissue-specific and collagen-type–specific, but nothing is known about the mechanisms of control of ei-

ther the linear or lateral growth of collagen fibrils. In fact, it is possible that noncollagenous matrix components control the growth of collagen fibrils. For example, each collagen fibril is completely surrounded by a layer of ruthenium-red–positive noncollagenous material, and this material may affect fibril growth.[150] This layer may contain proteoglycans, but it also contains significant amounts of the cell attachment glycoprotein fibronectin. Since a similar layer of polyanionic material surrounds each collagen fiber, it is even possible that this material may play a role in the packing of collagen fibrils into fibers and in the interaction of cell surfaces with collagen fibers.[150] Since this layer is much thicker than the maximum length of any intermolecular cross-link, there can be no cross-links between molecules in adjacent collagen fibrils.[150] This suggests that frictional forces and/or collagen–noncollagen interactions must also play critical roles in determining the tensile strength of collagen fibers in connective tissues.[98,134,150]

#### Functions of Collagen in Connective Tissue

The most obvious role of collagen in the body is to provide the tensile strength that is necessary to hold all tissues together as functional units. This role is very apparent in the dense connective tissues of ligaments and tendons, but it is equally important in parenchymatous organs such as the liver, kidney, and lung. This tensile strength is provided by the net effects of 1) intermolecular cross-links, 2) frictional forces between fibrils and fibers of collagen, and 3) physical and/or chemical interactions of collagen with other structured extracellular matrix components. In tissues that contain elastin, this protein and the microfibrils associated with it in elastic fibers also contribute to the tensile strength of the tissue, but the tensile strength resulting from cell proteins is quite small. Unfortunately, good basic information on factors that affect tissue tensile strength is only available from studies of factors that affect the formation of the lysyl-derived cross-links, and these cross-links are only present in newly synthesized collagens and in elastin.[5,6,24,25] This should make it rewarding, however, for those who will study the functional properties of connective tissues in diseases in which there is a loss of tensile strength in the presence of normal or increased amounts of lysyl-derived cross-links between collagen molecules.

The second functional characteristic of collagen in connective tissues is that the fibrils and fibers must be organized so as to permit tissues to be pliable and/or extensible. This organization is critical, since individual molecules, fibrils, and fibers of collagen are all essentially completely inextensible. Thus, the pliability and extensibility of tissues must result from

a straightening of curved fibrils and fibers and/or from a sliding of fibrils and fibers past one another.[98,150] If a tissue is to return to its original shape, however, there can be no net movement of molecules within fibrils, no net movement of fibrils within fibers, and no net movement of the ends of fibrils and fibers within tissues. Thus, a tissue can only be extended until all associated collagen fibrils and fibers are fully extended in the direction of the lines of stress. Beyond this point no extension is possible until the stress exceeds the tensile strength, and this is the point at which tearing begins.

Another major function of collagen fibers is to limit the movement of other tissue components. For example, in cartilage, the collagen fibers form a meshwork that entraps aggregates of proteoglycans and large amounts of tissue fluids. This entrapment limits the net movement of proteoglycan complexes, which in turn limits the net movement of tissue fluids in all cartilages. Consequently, when collagen fibrils are degraded or broken in cartilage, the proteoglycans and tissue fluids are mobilized and the tissue structure is lost.

As noted above, collagen also serves to induce platelet aggregation and clot formation. At the same time collagen fibrils and fibers serve to immobilize the clot and limit the movement of substances from an inflammatory focus. However, the role of collagen in limiting the net movement of nonaggregating proteoglycans in fibrous tissue is probably an important part of this function, for the degradation of either collagen or proteoglycans will facilitate the movement of either microorganisms or toxic substances from an inflammatory focus. Consequently, it is important that we learn how to regulate pharmacologically the deposition and degradation of collagen in a limited area of tissue around inflammatory foci or at sites of wound repair in each type of tissue.

It is likely that basement membrane procollagen also serves as a stable backbone, or supporting meshwork, and that it limits the mobility of the more labile noncollagen glycoproteins in basement membranes.[2,14,44–46] Unlike the connective tissue collagens, basement membrane procollagen is linked by disulfide bonds to the noncollagen glycoprotein components of these extracellular matrices.[4,14] The organization of these constituents undoubtedly determines the function of basement membranes as both mechanical and ionic filters, and as substrates for cell support and separation. Even though almost nothing is known about the structural organization of the constituents of basement membranes, it is known that the procollagen component turns over much more slowly than the noncollagen glycoproteins,[46] and it was recently discovered that basement membrane collagen is resistant to digestion with mammalian collagenase.[120] This finding may

help to explain why basement membranes accumulate around regenerating capillaries and in diseases such as diabetes mellitus.[4] This makes it important that we learn how these collagen and noncollagen glycoproteins are organized in functionally different basement membranes, so that we can begin to understand their role in disease processes.

Another obvious function of collagen occurs in bone, where collagen fibrils serve as a substrate for the deposition of crystals of hydroxyapatite. Therefore, the deposition and removal of collagen regulates the growth, maintenance, remodeling, and repair of bone. These processes have been studied for years, but we still do not know how the degradation of collagen is regulated when calcium is mobilized from bone.

During embryonic development, collagen even plays a key role in the regulation of cell differentiation.[3,93] For example, vertebral development is initiated by cartilage-type collagen and proteoglycans, which are synthesized by the notochord.[3,96-97] These matrix components promote chondrogenic differentiation of the mesenchymal cells of the sclerotome and serve as a substrate for the medial migration and organization of these cells into a vertebral anlage.[96] Similarly, collagen that is synthesized by the corneal epithelial cells forms the initial orthogonal array of corneal collagen fibrils, and these fibrils serve as a substrate for the invasion of the definitive corneal fibroblasts.[93] Collagen also has a role in regulating the differentiation of myoblasts in developing muscles. In fact, collagen has even been shown to play an important role in regulating the pattern of branching of the developing bronchial tree in the developing lung, and it is well known that it affects the pattern of regeneration of the injured liver. Since veterinarians have ready access to all of the models that are necessary to study these phenomena, it is important that we take advantage of the opportunity to understand the role of extracellular matrix components in the regulation of cell proliferation, migration, and differentiation, both in the developing embryo and in the wide variety of disease processes in which collagen has important functional consequences.

#### Collagen Degradation

An extensive review of collagen degradation has recently appeared.[21] Therefore, we will refer the reader to this review and include only the highlights of this process at this time.

The complete breakdown of collagen requires a number of enzymatic steps, but mammalian collagenase enzymes make only one break or cut through each $\alpha$-chain in the triple helical molecule. This single break results in an unwinding or denaturation of the collagen molecule at body temperature. This denatured collagen is then susceptible to degradation

with other proteolytic enzymes. This two-step degradative process occurs with all known mammalian collagenases, but there are differences in the susceptibility of different types of collagen to different collagenase enzymes.[108,114] For example, granulocyte collagenase in the lung cleaved Type I faster than Type III collagen [108]; yet there was no difference in the rate of cleavage of different types of connective tissue collagens by other preparations of mammalian collagenase.[115] Current research has only begun to elucidate the importance of these differences in collagen degradation in many disease processes.[21] Experimental studies should also soon elucidate the pathway for the degradation of the collagenous component of different basement membranes.

The cleavage site for mammalian collagenase is at one specific residue in each $\alpha$-chain, and this is 75% of the distance from the N-terminal end of the molecule.[10,11,21,66] Thus, when a molecule is cleaved with collagenase, a three-quarter N-terminal fragment and a one-quarter C-terminal fragment result. These fragments unwind at body temperature and are then cleaved to small peptides or to free amino acids by less specific tissue peptidases. In contrast, bacterial collagenase enzymes act on the entire length of the Gly-X-Y triplet and reduce the entire collagen molecule to dialyzable peptides.

#### Regulation of the Metabolism of Collagen

Unfortunately, we still know very little about the regulation of the synthesis, secretion, deposition, or turnover of collagen in connective tissues. It is known that changes in the environment of cells may result in a change in the types of collagen being synthesized, and it is known that such simple changes as an increase in $K^+$ ions can result in an increase in both cell proliferation and extracellular matrix synthesis.[111] For example, an environment containing cartilage-type matrix components serves to induce somite mesenchyme to undergo chondrogenic differentiation and synthesize Type II collagen.[96,99,110] An organic matrix prepared from bone will also serve to induce mesenchymal cells to form cartilage and then bone,[3,5,119] and an injection of epithelial tumor cells into a mouse thigh muscle has been shown to induce muscle fibroblasts to form cartilage and then bone.[101] This process undoubtedly involves a switch from Type I to Type II collagen synthesis in muscle fibroblasts. On the other hand, a switch from Type II to Type I collagen synthesis is seen when cultured chondrocytes are exposed to increasing levels of cAMP or $CaCl_2$, to 5-bromo-2′-deoxyuridine,[106,113] or to adverse culture conditions or a prolonged time *in vitro*.[112] These processes all involve regulatory changes in the types of collagen synthesis, and these changes are induced or initiated

by changes in the environment of mesenchymal cells or immature fibroblasts. Similar changes undoubtedly occur in the development of a cartilaginous fracture callus, in ectopic bone formation, and in many repair processes.

Numerous factors have also been shown to change the amount of collagen synthesized.[100,102,103,107,109,112,113,116–118] For example, ascorbate has been shown to stimulate collagen synthesis, with or without an increase in prolyl hydroxylase activity.[103,109] Activated macrophages have also been shown to release a soluble factor that stimulates the synthesis of collagen and other proteins in granulation tissues,[100] and prostaglandins $E_1$ and $F_{1\alpha}$ stimulate collagen synthesis in skin and bone of chick embryos.[104] Since these prostaglandins increase in inflammatory lesions, these data may help to explain the stimulation of collagen synthesis associated with inflammation. On the other hand, various studies have shown that prostaglandin $E_2$ is a relatively specific inhibitor of collagen synthesis by osteoblasts,[118] and that other connective tissues contain one or more small basic proteins that simulate hyaluronate production but inhibit the production of collagen.[105] Nevertheless, these observations are all still somewhat preliminary, and there is an urgent need for many good basic studies of the control of transcription, translation, and posttranslational modifications of both collagen and the extracellular enzymes that are required for the deposition, stabilization, and degradation of collagen in all connective tissues.

#### Heritable Diseases of Collagen

Since the primary functions of collagen are to determine the tensile strength and extensibility of tissues, it is not surprising that tissue fragility and/or hyperextensibility are features of the many heritable disorders of collagen.[4,5,13,15,19,26] These disorders include the Ehlers–Danlos syndromes (EDS), which are a collection of eight to ten disease entities involving different defects in the metabolism of collagen in the human skin, gingiva, skeletal fibrous tissues, gastrointestinal tract, cardiovascular system, placenta, and eye.[4,13,26,151] There are, however, a number of primary heritable defects in the metabolism of collagen that are not appropriately included under the eponym of EDS, even though they present with fragility and hyperextensibility of a variety of tissues. These include 1) the collagen packing defects in the dog, mink, and cat, 2) dermatosparaxis or procollagen N-peptidase deficiency in cattle and sheep, 3) the X-linked, aneurysm-prone mottled-locus mouse defect, 4) Menke's kinky hair syndrome in man, 5) cutis laxa in man, 6) the Marfan syndrome in man, and 7) three or more specific entities in man that are included under the name "osteogenesis imperfecta." There is also at least one heritable disease, homo-

cystinuria, in which the metabolism of collagen is secondarily affected by accumulations of metabolites resulting from an inherited enzyme deficiency in an unrelated metabolic pathway in liver and kidney.

The Ehlers–Danlos syndromes are considered as the primary examples of diseases of collagen in man.[1,13] The different forms of EDS are classified on the basis of their heritability and clinical presentation (Table 3).[1,13,26] At least three of these entities are inherited as autosomal recessive defects; one is an X-linked recessive, and four have an autosomal dominant pattern of inheritance.[1,13,26] These diseases all present with fragility and/or hyperextensibility of one or more tissues, and they are all assumed to be due to a defect in the metabolism of collagen. Similar diseases occur in animals, but in essentially every case, the presentation of the disease in other animals is significantly different from the different forms of EDS, cutis laxa, or the other heritable diseases of collagen in man.[1,13,19,26,121,126,127,133,134,147,150] These differences in the clinical presentation must be due to either differences in the underlying defect in the metabolism of collagen or differences in the organization and function of collagen in different species. To distinguish between these alternatives, we must characterize the morphologic and biochemical lesions in affected tissues with each disease entity. This should permit us to identify differences in the organization and function of collagen in different tissues and in different species. In the absence of an identification of the same basic underlying defect in man and animals, however, it is wrong to use the eponyms of specific diseases of man to name those diseases of animals that have both similarities and differences in their presentation. In fact, since eponyms fail to tell anything about the clinical, morphologic, or biochemical characteristics of a disease process, the use of eponyms to identify a disease in any species may actually slow the spread of understanding.

### Recessive Dermatosparaxis

"Dermatosparaxis," meaning "torn skin," was the first "true" collagen disease to be identified and fully characterized.[42,43,121,125,126,131,133,134,138] It was discovered and named by veterinarians in Belgium.[121,126] At approximately the same time, veterinarians in Texas studied a "connective tissue dysplasia" in cattle that presented with fragile, hyperextensible skin.[133] Electron-microscopic studies of the cattle in Texas showed that there was an extreme defect in the packing of collagen into fibrils and fibers in the skin of these cattle.[133] Instead of having cylindrical fibrils packed into parallel fiber bundles, the collagen fibrils in affected cattle were twisted ribbons. These ribbons failed to form thick fibers like those in normal skin.

Table 3—The Ehlers–Danlos Syndrome in Man

1. Dominant Ehlers–Danlos syndromes (EDS I, II, III, and VIII)

| Syndrome | Synonym |
|---|---|
| EDS I | gravis-type EDS |
| EDS II | mitis-type EDS |
| EDS III | benign hypermobile-type EDS |
| EDS VIII | periodontitis-type EDS |

a. Clinical features

| | |
|---|---|
| EDS I | Skin: very hyperextensible, fragile, bruisable, and scarred<br>Joints: extremely hyperextensible<br>Placenta: pregnancy often terminates prematurely due to ruptured membranes |
| EDS II | Skin and Joints: extensibility is only slightly increased and may be localized to hands or feet<br>Tissues are not friable |
| EDS III | Skin: few if any abnormalities<br>Joints: severe generalized hyperextensibility<br>Tissues are not friable |
| EDS VIII | Skin: moderate fragility with scarring, mild hyperextensibility and bruisability<br>Joints: mild hypermobility<br>Teeth: severe generalized periodontitis, alveolar bone lysis, premature loss of teeth |

b. Pathogenesis

| | |
|---|---|
| EDS I | A defect in collagen fibrillogenesis has been identified in two laboratories, but the presumptive defect in a structural protein has not been identified. |
| EDS, II, III, VIII | No information is available on the basic defect in these forms of EDS. |

2. Autosomal recessive Ehlers–Danlos syndromes (EDS, IV, VI, VII)

| Syndrome | Synonym |
|---|---|
| EDS IV | ecchymotic, arterial, or Sacks EDS or Type III collagen-deficient EDS |
| EDS VI | ocular-type EDS or hydroxylysine-deficient EDS |
| EDS VII | dermatosparaxis, procollagen N-peptidase-deficient EDS, or arthrocalasis multiplex congenita |

a. Clinical features

| | |
|---|---|
| EDS IV | Skin: thin, pale, easily bruisable, and scarred over bony prominences<br>Joints: normal<br>Bowel and Muscular Arteries: frequently distended and ruptured |
| EDS VI | Skin: thin, pale, hyperextensible, and fragile<br>Joints: moderately hyperextensible<br>Sclera: thin, blue, and ruptures easily |
| EDS VII | Skin: slightly to moderately hyperextensible and bruisable<br>Joints: severely hyperextensible |

b. Pathogenesis

| | |
|---|---|
| EDS IV | A decrease in the proportion of Type III collagen has been reported, especially in tissues containing smooth muscle. This corresponds to the clinical involvement of tissues containing smooth muscle and Type III collagen. |
| EDS VI | A deficiency of the enzyme lysyl hydroxylase has been clearly demonstrated. This enzyme is necessary for the hydroxylation of lysine, and hydroxylysine is involved in the formation of the most important forms of lysyl-derived cross-links in collagen. Affected tissues represent those tissues in which hydroxylysine-derived cross-links in collagen play the most important role in determining the tensile strength of the tissues. |

Table 3—Continued

| | |
|---|---|
| EDS VII | A deficiency of procollagen N-peptidase that cleaves the N-terminus of procollagen has been clearly demonstrated in cattle and sheep and has been reported in a group of human patients with a distinct form of EDS. The retention of the N-terminal propeptide on Type I procollagen interferes with the packing of collagen into fibrils and fibers, and this results in the poor tensile strength in dermal and ligamentous connective tissues. A deficiency of procollagen C-peptidase that cleaves the C-terminal end of procollagen may occur in some forms of this syndrome, but this deficiency has not been clearly demonstrated. |
| 3. X-Linked Ehlers–Danlos syndrome (EDS V) | |
| Syndrome | Synonym |
| EDS V | lysyl oxidase deficiency EDS |
| a. Clincial features | |
| | Skin: hyperextensible, fragile and easily bruisable |
| | Joints: moderately hyperextensible |
| | Heart: congenital defects, floppy valve syndrome |
| | Supporting Connective Tissues: short stature, inguinal hernias |
| | Urine: increased excretion of hydroxylysine glycosides and Val-Pro dimers |
| b. Pathogenesis | |
| | A deficiency of lysyl oxidase has been reported in two maternal cousins with this syndrome. This enzyme is required for the oxidative deamination of lysine and hydroxylysine for the formation of lysyl-derived cross-links in both collagen and elastin. |
| | A decrease in the cross-linking of collagen and elastin may explain the poor tensile strength and more rapid turnover of collagen and elastin in these patients. |

The explanation for these morphologic abnormalities was found in biochemical studies of the cattle in Belgium.[43,125,131] These studies showed that there was a deficiency of procollagen N-peptidase that resulted in an accumulation of a partially converted procollagen in the skin. The N-terminal propeptide was retained on the $\alpha_1$(I) chains in skin collagen, and these propeptides interfered with the packing of the pN-collagen molecules into fibrils and fibers. This packing defect also resulted in a decrease in the rate of formation of intermolecular cross-links in dermal collagen. Thus, the studies of the cattle showed that there was a remarkable correlation among the molecular defect in procollagen N-peptidase, the morphologic defect in collagen fibrils, the loss of tensile strength in the skin, and the clinical presentation of this disease of cattle.[42,43,121,125,126,131,133,134,138]

Dermatosparaxis was actually discovered in sheep in Norway before it was discovered in cattle. Affected lambs were found to tear their skin during nursing, and they often died from secondary bacterial infections during the first few weeks of life. The skins of these lambs were reportedly stored frozen but were not studied for at least 10 years. During this time dermatosparaxis was discovered and characterized in cattle. When the sheep were finally studied, electron microscopy and biochemical analyses

showed that there were similar morphologic abnormalities in collagen fibrils [124,127] and an accumulation of pN-collagen in the skin of affected sheep.[122,123] There has been no explanation, however, for the fact that the disease in sheep is much more severe than that in cattle. Consequently, additional studies of the defect in sheep are needed to determine whether there is an additional or different enzyme defect that accounts for the increased severity of dermatosparaxis in sheep.

### Recessive Forms of EDS

The recessive forms of EDS in man, like most other recessive diseases, involve specific enzyme defects. Thus, each of the enzymes that are required for the synthesis and posttranslational modification of collagen is a potential site for the development of a specific heritable disease. Conversely, each recessive defect in the metabolism of collagen must involve a specific enzyme defect.

### EDS VI

The so-called ocular form of EDS, or, more simply, EDS VI, in man has been shown to be due to a deficiency of lysyl hydroxylase.[128,130,135,137,140] This defect was the first true collagen disease identified in man, and the specific enzyme deficiency is the most firmly established.[26,130,135,137] This enzyme deficiency results in a deficiency of hydroxylysine in newly synthesized collagen molecules, and this results in a deficiency in the hydroxylysine-derived cross-links and subsequently in a deficiency of mature cross-link formation in collagen fibrils. Consequently, the extractability and turnover of collagen are both increased, and the tensile strength of all affected tissues is greatly reduced in this disease.

Even though the deficiency of lysyl hydroxylase in EDS VI is firmly established, at least three studies of other cases of EDS VI have shown that there are significant differences in the amount and distribution of the deficiency of enzymatic activity and in the amounts of hydroxylysine deficiency in different tissues in different cases.[128,139,140] These biochemical differences also correlated somewhat with differences in the clinical presentation of each case of this disease. For example, two affected sisters with EDS VI were floppy babies with retarded motor development, hyperextensible skin and joints, ocular fragility, and a number of skeletal deformities.[135] The hydroxylysine deficiency and decrease in lysyl hydroxylase activity in these sisters was quite severe.[135] However, in another case with fragile hyperextensible skin, joint laxity, and both an ocular deformity and fragility, there was only a mild decrease in hydroxylysine in the skin, even though assays of skin fibroblasts showed that the lysyl hydroxy-

lase activity was greatly reduced.[139] The difference in these results is as yet unexplained, but they could be explained by the presence of two different lysyl hydroxylase enzymes in connective tissues or by differences in the nature of the defect in a single enzyme. There is no firm evidence to distinguish between these alternatives, but a comparison of the hydroxylation of lysine in Type I collagen in embryonic and adult tissues suggests that there may indeed be two different lysyl hydroxylase enzymes.[15,28] If so, there may be two distinct diseases that present as EDS VI. It is hoped that a defect in lysyl hydroxylase will soon be identified in a domestic animal so that we can carry out more extensive studies to help resolve this question.

### EDS VII

The second true collagen disease identified in man was a presumptive autosomal recessive form of EDS that presented with extreme hypermobility of joints, multiple dislocations, scoliosis, microcornea and myopia, and hyperextensible fragile, scarred, easily bruisable skin.[132] In all three patients studied there was an accumulation of pN-collagen in which a portion of the N-terminal propeptide remained attached to the $\alpha$-chains of collagen in the skin. There was also an increase in the extractability of skin collagen, and this suggested that there was a decrease in cross-linking of collagen in the skin of these patients.[132] It was assumed that this disease was due to a deficiency of procollagen N-peptidase like that in dermatosparaxis in cattle and sheep, but the morphologic defect in collagen fibrillogenesis found in cattle skin was not demonstrated in the human disease, and there was no explanation as to why the defect in cattle and sheep was confined to the skin while the defect in man included an extensive involvement of ocular and skeletal connective tissues.[132] Therefore, additional studies of the human syndrome are necessary to determine the exact cause for the failure in the change of the N-terminal propeptides of Type I procollagen. In the meantime this defect should be referred to as EDS VII or arthrocalasis mulitplex congenita, for the procollagen chains that accumulate in EDS VII in man may be different from those that accumulate in dermatosparactic cattle and sheep.

### EDS V

The third true collagen disease reported in man was a deficiency of the cross-linking enzyme lysyl oxidase in an X-linked recessive defect that presented with fragile hyperextensible skin, a mild hypermobility of joints, a floppy mitral valve syndrome, and an increase in the proportion of skin collagen that was extractable with neutral salt solutions.[152,154] This

disease is classified as EDS V. However, the results of one biochemical study showed that the acid solubility of skin collagen was not increased in either of the affected cousins,[154] and a marked increase in acid-extractable collagen would be expected with a deficiency of lysyl oxidase. Consequently, additional studies of EDS V are needed.

### Lysyl Oxidase Deficiency in the Mouse

The mottled-locus, aneurysm-prone mouse is an excellent model for studies of an X-linked deficiency of lysyl oxidase.[155,156] With some alleles of the mottled locus there is a loss of tensile strength in the skin and a number of skeletal malformations, and massive aneurysms develop along the full length of the aorta.[155] This clinical syndrome correlates well with a deficiency of lysyl-derived cross-links in collagen and elastin, and these abnormalities in cross-linking have been shown to result from defects in lysyl oxidase in all connective tissues in the mouse.[155,156] The studies of this mouse model showed that the gene for lysyl oxidase was on the X chromosome and served as a basis for the hypothesis that the X-linkage of EDS V was indicative of a deficiency of lysyl oxidase.

### X-Linked Cutis Laxa

There is also an X-linked form of cutis laxa in man that is thought to be due to a lysyl oxidase deficiency.[26,158] This disease presents with thick, sagging, pendulous, nonresilient, relatively inextensible, and prematurely aged skin. Histologically this condition primarily shows a fragmentation of elastic fibers and shows little or no change in collagen.[1,26] Thus, even though this is thought to be a cross-linking defect, there are many discrepancies that still require explanation. For example, there is no explanation as to 1) why the presentation of this condition is so different from that of EDS V or the lysyl-oxidase–deficient mouse model, 2) why elastic fibers appear to be more affected than collagen fibers in the dermis, or 3) why fibrous connective tissues are more affected than elastic arteries. Studies of these differences may help us to learn whether there are different isoenzymes of lysyl oxidase for collagen and elastin, different isoenzymes of lysyl oxidase in different tissues, or different isoenzymes for different types of collagen in the same tissue.

### Menke's Kinky Hair Syndrome

Menke's kinky hair syndrome is another disease in man that is inherited as an X-linked recessive defect in the cross-linking of collagen and elastin.[153] This is not due to a defect in lysyl oxidase, however. Instead, it is due to a defect in the intestinal absorption of copper,[153] and lysyl oxidase

is a copper-dependent enzyme. This suggests that the gene(s) for an enzyme that regulates copper absorption is also located on the X chromosome, and it is an excellent example of how a defect in an enzyme that regulates the metabolism of a cofactor may mimic a defect in an enzyme that is required for the modification and functional organization of a structural protein.

### Homocystinuria

Homocystinuria is an autosomal recessive enzyme deficiency that results in a defect in the cross-linking of collagen and elastin in man.[129] In this disease, however, the enzyme deficiency is in a metabolic pathway in the liver and kidney; and it results in an accumulation of a metabolite that in turn blocks the stabilization of aldehyde cross-links in collagen and elastin. In homocystinuria a deficiency of cystathionine synthetase in liver and kidney results in an accumulation of methionine, serine, and homocystine in tissue fluids and the excretion of homocystine in the urine.[129] It appears that one of these metabolites blocks the conversion of aldehyde cross-links to the more stable mature cross-links, and it is this failure of maturation of cross-links in collagen and elastin that results in the high incidence of aortic aneurysms and thrombosis in homocystinuric patients.[26,129]

### EDS IV

EDS IV is an autosomal recessive defect that presents with a loss of tensile strength in the wall of large arteries, the gastrointestinal tract, and the skin.[4,26] It is known as the arterial, ecchymotic, or Sack-type EDS, and patients with this defect usually die before the age of 20 as a result of a rupture of the aorta or colon.[4] All of the studies of this form of EDS to date have found that there is a deficiency of Type III collagen,[26,136] and Type III collagen is most prevalent in those tissues that are clinically affected. In none of these studies, however, has an enzyme defect been identified. The autosomal recessive pattern of inheritance certainly suggests that an enzyme defect should underlie the deficiency in Type III collagen in these cases, but nothing is known about the enzymatic control of the synthesis of different types of collagen. Consequently, it will be important to learn whether this defect is due to an enzyme regulating transcription or translation for Type III collagen or an enzyme that is specific for a posttranslational modification that is required for the synthesis and secretion of Type III collagen in all connective tissues.

### Dominant Forms of EDS

The autosomal dominant forms of EDS include only EDS I, II, III, and VIII in man (Table 3).[4,13,26] Type I, or gravis-type EDS, is the most severe

of the different forms of EDS, and it is predominantly fibrous connective tissues that are affected. This form is also considered to be typical of the entire syndrome. The skin is very friable, hyperextensible, and easily bruisable, and there is a severe generalized hypermobility of joints.[4] Premature termination of pregnancy frequently results from a tearing of placental membranes, and surgical suture lines often fail as a result of the tissue friability. There is no agreement, however, on the nature of the morphologic abnormalities associated with EDS I. Some authors have reported that there is a deficiency and fragmentation of collagen fibers; yet others have found either no abnormalities or an increase in collagen. Some have reported that there is an excess of elastic fibers, but others have found that the elastic fibers are also normal. Some have even reported that there is an excess of mucopolysaccharides. Recently, Holbrook et al [149] found that there were packing defects in the collagen in skin biopsies of patients with both EDS I and EDS IV, but previous electron-microscopic studies had detected no abnormalities in EDS I. Nevertheless, the presumptive structural protein defect underlying this disease has not been identified. When this is known, the reasons for the discrepancies in these reports may be readily evident.

EDS II is referred to as the mitis-type EDS, because the skin and joints are only mildly affected with hyperextensibility and hypermobility.[1] Only a limited number of joints may be affected, and none of the tissues are friable. In contrast, EDS III is referred to as benign hypermobile EDS, for there is a generalized and severe hypermobility of joints but no hyperextensibility of skin.[1] There is also no tissue friability in EDS III, but skeletal deformities may result from the severe abnormalities in the distribution of the stresses of muscle tension and weight bearing resulting from the hypermobility of all joints. Although precise incidence figures are not available for these forms of the EDS, it is believed that Type II may be relative common, while Type III is relatively uncommon. It is assumed that most people with familial double jointedness are affected with Type II EDS, but this condition is of little clinical significance, and little is known about either the morphologic or biochemical changes associated with it. Similarly, little is known about the mechanisms underlying the hypermobility of joints in Type III EDS. This lack of knowledge of underlying mechanisms is also true of Type VIII EDS, which is a newly described variant that presents with fragile hyperextensible skin and severe periodontal disease.[13,151]

#### Dominant Collagen Packing Defect I

We are currently using the name "dominant collagen packing defect I" for an autosomal dominant defect in collagen fibrillogenesis that results in

a severe fragility and hyperextensibility of the skin in dogs, mink, and cats. Arlein [141] and Hegreberg et al,[145-148] who first described this defect in dogs and mink, have referred to this disease as EDS I or "cutaneous asthenia," while others have called it "rubber puppy disease." Hegreberg, et al [148] showed that the tensile strength of the skin of the affected dogs and mink was <20% of normal, and found that this loss of tensile strength was associated with a fragmentation of collagen fibers in the dermis.[146] Patterson and Minor [150] recently described a similar defect in a cat and found that there were defects in both the lateral growth of collagen fibrils and in the parallel packing of collagen fibrils into fibers. These studies showed that in affected animals the diameter of more than half of the collagen fibrils in abnormal collagen fibers was larger than that of the largest collagen fibril in the unaffected skin.[150] This gave rise to two populations of diameters of collagen fibrils in the skin of affected cats. A comparable defect has now been identified in a dog with hyperextensible fragile skin (Figures 1 and 2). In addition, in both species, there was a defect in the normal packing of fibrils into parallel bundles in collagen fibers (Figure 1).[150] These studies have since been confirmed by Holbrook et al,[149] who found similar defects in collagen fibrillogenesis in skin biopsy specimens of affected dogs, mink, and man. Consequently, the morphologic defect in the packing of collagen into fibrils and fibers as well as the dominant pattern of inheritance have been combined in the name for this disease. Presumably a better name will be evident when the basic biochemical defect is identified. In the meantime we are avoiding the use of the terms "EDS I" and "cutaneous asthenia," due to the fact that "asthenia" refers to a general muscle weakness or malaise, and the skin is the only tissue that is clinically involved in affected animals.[150] Furthermore, all fibrous connective tissues are markedly involved in EDS I in man.[1] Since affected dogs and cats are lively and playful and have no muscle involvement or malaise, it may even be preferable to use one of the clinical terms "dominant dermatosparaxis" or "dominant dermatorrhexis" to indicate the fragile nature of the skin of affected animals. The single name "dermatosparaxis" has been preempted, however, for the recessive defect in cattle and sheep with a deficiency of procollagen N-peptidase.

Since the clinical presentation with fragile, hyperextensible skin and the morphologic characteristics of the collagen packing defect in the dermis of affected dogs and cats were identical, we have assumed that these diseases are due to the same underlying protein defect. It also appears that Hegreberg et al [143,145-148] have assumed that the defects in dog and mink are identical. It is possible, however, that these assumptions are not valid. These diseases could each be due to a similar but different substitution, insertion, or deletion of one or more amino acids either in a collagen poly-

peptide chain or in a protein that regulates the packing of collagen molecules into fibrils and fibers in connective tissues. Counts et al [143] have found that there is an increased rate of collagen synthesis in explants of skin biopsies from affected mink, but we have found either reduced or normal rates of synthesis and turnover of collagen in the whole skin of affected kittens (Minor and Patterson, unpublished data). The dominant pattern of inheritance suggests that these diseases will all involve structural protein defects, but there is no evidence to suggest that there will be a preferential selection for a specific point mutation in the same gene in different species. Such a selection of a specific point mutation must occur if these diseases are truly identical in the different species. In fact, a similar selection for a given point mutation must occur within each species if each new occurrence of these dominant defects is due to the same amino acid substitution. Alternatively, these defects may result from a combination of amino acid substitutions that result in a specific conformational and/or functional change in the same protein. To distinguish between these alternatives, it is important that we identify the basic biochemical defect in affected tissues from each species and from each new occurrence of this disease in unrelated animals. Consequently, the availability of heterozygous dogs, cats, and mink for breeding provides us with the valuable opportunity of producing matched pairs of affected and unaffected animals for basic studies of this disease process.[150]

Our microscopic studies of skin from kittens with this defect have shown that differences in the density and organization of collagen fibers and differences in the thickness of skin cannot be adequately identified in histologic sections of small biopsies of affected and unaffected skin. This problem with the histologic evaluation of fixed connective tissues is apparently due to differences in the shrinkage of small biopsy specimens during fixation and dehydration. This became apparent when our clinical evaluation suggested that the skin of affected kittens was thinner; yet histologic examination of biopsy specimens from these kittens showed no difference in the thickness of affected and unaffected dermis. To resolve this discrepancy, we fixed the skin of a pair of these kittens by perfusion with a buffered paraformaldehyde–glutaraldehyde fixative followed by immersion in this same fixative. The microscopic appearance of dermal collagen and the thickness of the skin that was fixed *in situ* was then compared to that of the small biopsy specimens. The results of these studies showed that the density of collagen fibers and skin thickness were both greatly reduced in the affected skin that was fixed *in situ*, even though these differences could not be detected in small biopsy specimens of skin from the same pair of kittens. Thus, differences in tissue shrinkage may help to explain some of the differences in the results of different studies of both the

various forms of EDS in man and the collagen packing defects in animals. These observations are important, for similar shrinkage differences may be affecting the density and orientation of collagen fibers in connective tissues in a wide variety of tissue biopsies. It is also important to compare the diameters and packing of collagen fibrils in matched biopsy specimens of affected and unaffected tissues.[150] Byers et al [157] have recently found collagen packing defects in a new variety of spondylo-epiphyseal dysplasia.

### Dominant and Recessive Forms of Osteogenesis Imperfecta

Osteogenesis imperfecta (OI) appears to be directly analogous to EDS in that OI, like EDS, occurs in both autosmoal dominant and recessive forms.[4,26] Both forms of OI are also further subdivided into a severe congenital form and a less severe tarda form.[4,26] All of these forms of OI are considered to be due to defects in the metabolism of collagen in both bone and fibrous tissues.[159–167] In those cases that were studied to date these defects have included 1) an increase in the proportion of Type III collagen synthesis in cultured fibroblasts, 2) a defect in which $\alpha_2$ chains are missing from Type I collagen, and 3) abnormal proportions of collagen cross-links in skin and bone. It is not known, however, whether the decrease in the Type I:Type III collagen ratio was due to an increase in Type III collagen synthesis, a decrease in Type I collagen synthesis, or an increase in the degradation of newly synthesized Type I collagen.

Since Type I collagen is essentially the only collagen in bone, it seems likely that a structural protein defect involving Type I collagen will underlie the dominant form of OI and that an enzyme defect in the pathway of synthesis, cross-linking, or degradation of Type I collagen will underlie the recessive forms of this disease. In both cases a failure to accumulate a matrix of Type I collagen fibrils in bone and fibrous connective tissues will account for the presentation of the different forms of OI. Further studies are necessary to prove these hypotheses, but to date no animal models have been identified to facilitate these studies in the whole animal. It is not clear whether this absence of animal models for studies of OI is due to our failure to recognize the defect in perinatal animals or whether it is lethal during the early embryonic period in animals other than man. Since it is likely that similar metabolic defects occur in different species, more detailed studies of collagens in animals are likely to identify defects in the metabolism of Type I collagen that are similar to those in OI in man.

### Dominant and Recessive Forms of Cutis Laxa

Pendulous sagging, redundant, hyperextensible nonresilient skin and pulmonary emphysema are the primary features of cutis laxa (CL) in

man.[4,26] The sagging of the skin and the absence of fragility distinguish CL clinically from EDS, and the absence of resiliency gives CL skin the appearance of premature aging. This defect, like EDS and OI, is inherited in both autosomal dominant and recessive forms and in an X-linked recessive pattern. In addition, there is an acquired form of CL. In all of these forms histologic examination of the skin, lung, and large arteries shows a deficiency and fragmentation of elastic fibers and an accumulation of glycosaminoglycans.[4,26] The collagen fibers appear to be histologically normal. However, as noted above, one recent study found that there was a deficiency of lysyl oxidase activity in cultured skin fibroblasts from two male cousins with an X-linked recessive form of CL.[158] In other cases there has been a suggestion of a copper deficiency, and this, too, would lead to a deficiency in cross-link formation in both elastin and collagen. In most cases of CL the basic defect is not known, but the tissue distribution suggests that it may involve both collagen and elastic fibers, histologic study suggests that it is primarily due to elastolysis, and the results of biochemical studies suggest that there may be a defect in the cross-linking of both collagen and elastin. Therefore, further studies are necessary to identify and characterize the underlying defect in both the dominant and recessive forms of this disease.

### The Marfan Syndrome

The Marfan syndrome is a complex autosomal dominant defect in man that affects a wide variety of connective tissues. This syndrome presents with an excessive growth of the long bones, joint hypermobility, scoliosis, pectus excavatum, lens luxation, myopia, and a number of cardiovascular defects, including dissecting aneurysms of the aorta and valvular insufficiency.[4,26] Histologic examination shows predominantly a fragmentation of elastic fibers and an accumulation of metachromatic material in the aortic lesions.[4,26] Biochemical studies have shown that there is a fivefold increase in hyaluronate synthesis by skin fibroblasts, which accounts for the accumulation of metachromatic material. Biochemical studies have also shown that there is an increase in the extractability of collagen from the skin of patients with this syndrome. This finding suggests that both collagen and elastin may be affected, and this idea would be consistent with a defect in lysyl-derived cross-links. To date, however, biochemical studies have failed to identify a cross-linking defect in affected tissues.

## Acquired Diseases and Repair Processes Affecting Collagen

Most disease processes involve tissue injury, and the response to this injury very quickly results in a localized response of fibroblasts or mesenchymal cells. The localized nature of this response in most acquired diseases

and repair processes contrasts with the more generalized regulatory defects in heritable diseases of collagen. This localized response usually includes a stimulation of both cell proliferation and extracellular matrix synthesis. Furthermore, the response to nearly all forms of tissue injury involves regulatory changes in the rates and/or the types of collagen synthesis (Table 4). In spite of the ubiquitous nature of these changes, we know very little about the mechanisms regulating these cellular processes. We do not even know whether the primary site of control is at the level of transcription, translation, or one of the posttranslational modification steps in the pathway of collagen synthesis. Since we must know the sites and mechanisms of control if we are going to regulate the amounts and types of collagen synthesis in different disease processes, research in this area is very important.

### Acquired Changes in the Types of Collagen Synthesis

Recent studies have shown that the types of collagen synthesis change with differentiation and aging,[58,63,80,93,99,112] as well as in the different stages of the repair response to cell death and inflammation (Table 4). Numerous studies suggest that these changes in synthetic activity may result from changes in the environment of the responding mesenchymal cells.[3,93,96,97,119] These localized changes may involve either an increase or a decrease in the relative or absolute rates of synthesis. These changes may in turn lead to either an increase or a decrease in the Type I:Type III collagen ratio, an increase in the Type I: Type II collagen ratio, or an increase in the amounts of Type I trimer (Table 1, Row 2). For example, in the early stages of fibrous tissue repair there is an increase in the synthesis of Type III collagen.[169,176,182,202,203] As this repair process progresses, there is a return to a marked predominance of Type I collagen.[169,202] In fact, it seems likely that the transient increase in Type III collagen synthesis is a physiologic component of the repair process in fibrous tissues. This increase in Type III collagen synthesis may be related to the presence of myofibroblasts in the earlier phases of the repair process.[176] If the myofibroblasts fail to develop in either repair processes *in vivo* or cultures of cells from inflamed tissues, there may be no increase in the synthesis of Type III collagen.[175,189,190,195]

Connective tissue repair processes become pathologic when there is a failure in the normal regulatory changes in the types of collagen synthesis in either the acute or chronic stages of repair. For example, in the normal scar there may be a small to moderate increase in Type III collagen, but in the hypertrophic or abnormal scar there may be a marked increase in the amount of Type III collagen [202]; yet the rates of collagen synthesis in

Table 4—Collagen Metabolism in Acquired Diseases and Repair Processes*

I. Acquired changes in types of collagen synthesis (regulation of transcription and translation)
  A. Changes in response to cell death and inflammation
    1. Fibrous tissue repair
      a. granulation tissue
      b. scar formation
      c. hypertrophic scar formation
      d. Dupuytren's contracture of tendon
    2. Repair in bones and joints
      a. osteoarthropathies (osteoarthritis)
      b. rheumatoid arthritis
    3. Parenchymatous organ repair
      a. pulmonary fibrosis
      b. cirrhosis
    4. Atherosclerosis
  B. Age-related changes
  C. Changes with neoplasia
II. Acquired changes in amounts of collagen synthesized (regulation of transcription, translation, and/or posttranslational steps)
  A. Excess collagen synthesis
    1. Responses to cell injury
      a. fibrous tissue repair
      b. parenchymatous organ repair
        i. pulmonary fibrosis
        ii. cirrhosis
        iii. glomerulosclerosis or interstitial fibrosis
      c. diabetes mellitus
    2. Hypertrophic scar formation
    3. Neoplasia
    4. Osteopetrosis
    5. Scleroderma
  B. Deficient collagen synthesis
    1. Nutritional deficiencies
      a. hypovitaminosis C
      b. zinc deficiency
      c. starvation
    2. Glucocorticoid excess
    3. Prostaglandins
    4. Virus infection
III. Acquired changes in hydroxylation of proline and lysine
  A. Underhydroxylation
    1. Hypovitaminosis C
      a. scurvy
      b. deficient wound repair
    2. Hypoxia
      a. deficient wound repair (rabbit cornea)
    3. Increasing age
    4. Glucocorticoid excess
  B. Increased hydroxylation of lysine
    1. Dermal scar
    2. Hypertrophic scar or keloid
    3. Scleroderma
    4. Rachitic osteoid
IV. Acquired changes in collagen cross-links
  A. Increased reducible cross-links
    1. Rapidly growing tissues
    2. Dermal scar

Table 2—Continued

3. Hypertrophic scar or keloid
4. Scleroderma
5. Cirrhosis
6. Virus-induced osteopetrosis

B. Deficient or defective reducible cross-links
1. Lathyrism
2. Copper deficiency
3. Zinc deficiency
4. Hydroxylysine deficiency
5. Penicillamine toxicity

C. Defects in cross-link maturation
a. scleroderma

V. Acquired defects in collagen degradation
A. Excess degradation
1. Increased collagenase activity
a. acute inflammation
b. immune mediated cell injury
c. mast cell degranulation
d. bacterial infection
e. tumor invasion
2. Increased susceptibility of collagen
a. denaturation of collagen
i. tissue hyperthermia
ii. underhydroxylation of proline
b. deficient cross-links

B. Deficient degradation
1. Decreased collagenase activity
a. cirrhosis
b. scleroderma
c. osteopetrosis
2. Decreased susceptibility of collagen
a. diabetes mellitus
b. hypertrophic scar

* Each acquired disease or repair process may involve more than one site of regulation of the metabolism of collagen.

these scars may be the same or less than the rates of collagen synthesis in the normal dermis.[175] In contrast, in the interlobular spaces in the liver there is an increase in Type III collagen synthesis in the acute response to injury, but there is a marked increase in Type I collagen synthesis in these areas when the liver injury becomes irreversible.[204] Similar changes occur in osteoarthrotic cartilage where there is an increase in the synthesis of Type I collagen and Type I trimer instead of Type II collagen.[18] Similar changes also occur in atherosclerotic plaques, where there is a marked increase in Type I collagen instead of Type III collagen synthesis.[187] To date, however, essentially nothing is known about the mechanisms leading to the failure in the regulation of these transitions in the types of collagen synthesis; yet these mechanisms are obviously very important.

An increase in the relative amounts of Type III collagen synthesis in fibrous connective tissue may be indicative of the development of a popu-

lation of fibroblasts in the early stages of differentiation. The young fibroblasts or mesenchymal cells in adult tissues undergoing repair may be comparable to differentiating mesenchymal cells in the embryo. The less differentiated phenotype in these cells may also be comparable to the phenotype in fibroblasts that have undergone neoplastic transformation. At least one study has shown that Type III collagen synthesis predominated in a fibrosarcoma in fibrous connective tissues that would normally synthesize predominantly Type I collagen.[185] Presumably the neoplastic cells would differ, in that they could not switch to a more differentiated phenotype as they underwent cell replication. With normal fibroblasts in both embryonic and adult connective tissues, the portion of the genome that is expressed is obviously switched as these cells proliferate and differentiate in response to local environmental stimuli.

### Acquired Changes in Amounts of Collagen Synthesized

Changes in the amounts of collagen in acquired diseases and repair processes are more apparent than changes in the types of collagen (Table 4, Part II).[168,171-175,177-183,189-194,199-201] An excess of collagen synthesis is certainly a major problem in parenchymatous organs such as the lung, liver, kidney, and gastrointestinal tract. This excess is especially prominent in the fibrotic response to paraquat-induced alveolar epithelial cell injury in the lung, in the cirrhotic response to prolonged or extensive injury to hepatocytes, and in stenotic lesions of the alimentary tract resulting from a severe cell injury and/or prolonged inflammatory response.[10,15,18,21,173,174,177,193,194]

In the inflamed lesion, fibrin may serve to induce a localized fibroblastic response, and this may be important in the initiation of the increase in collagen synthesis following inflammation in soft tissues.[23] The amount of fibrin will be determined by the site and nature of the cell injury, and this fibrin will in turn stimulate the fibrotic response. Thus, fibroplasia may be reduced by decreasing fibrin deposition, by increasing fibrinolysis, by pharmacologically decreasing collagen synthesis and stabilization, or by increasing collagen degradation.[21,23,30,174,180]

In most repair processes the increase in the amount of collagen synthesis is reflected by an increase in the activity of prolyl and lysyl hydroxylase, an increase in the rate of accumulation of soluble collagens, and an increase in reducible cross-links in the tissues.[15,193,194,197,199] Nevertheless, an absolute increase in the amount of collagen in tissues may also result from a decrease in the rate of degradation in the presence of normal or decreased rates of synthesis.[21] In most repair processes with increased amounts of collagen, however, it is likely that there is an increased rate of

extracellular matrix synthesis in existing fibroblasts as well as a localized increase in the numbers of active fibroblasts that result from cell proliferation, migration, and differentiation.

### Acquired Changes in Hydroxylation of Proline and Lysine

As noted above, ascorbate is required as a cofactor for the enzymes prolyl and lysyl hydroxylase in the hydroxylation of proline and lysine residues in the free pro-α-chains of collagen. Consequently, scurvy in man and guinea pigs is a key example of an acquired disease that is due to a primary defect in the metabolism of collagen.[184] In the presence of a deficiency of ascorbate there is an underhydroxylation of proline and lysine in collagen. The deficiency of hydroxyproline results in a reduction in the melting temperature of the newly synthesized collagen molecules,[59,78,184] and the deficiency of hydroxylysine results in a deficiency of intermolecular cross-links.[15,184] For example, the melting temperature of fully hydroxylated Type I collagen is ~39 C, and that of unhydroxylated Type I collagen is ~23 C. Consequently, as the degree of underhydroxylation increases, the newly synthesized collagens melt at normal body temperatures. There is considerable variation, however, in the temperature of tissues throughout the body, and this variation may affect the rate of formation and stabilization of the triple helix in underhydroxylated collagen in different tissues, in different species, and in different environments. The same underhydroxylated collagen molecule may be denatured in a weight-bearing segment of long bone or native in the cooler dermis. In either tissue, however, a lowering of the melting temperatures of newly synthesized collagen below the local tissue temperature will have the same effect: the newly synthesized collagen will fail to form a stable triple helical molecule. This failure of underhydroxylated collagen to form a stable triple helix in the RER will result in an increase in intracellular degradation and a reduction in the rate of packaging and secretion of this procollagen. Since the underhydroxylated procollagen that is secreted will also be denatured, it will not be deposited into fibrils, or it will be degraded by the nonspecific tissue proteases or peptidases. In addition, the decrease in hydroxylysine may result in a decrease in the formation of intermolecular cross-links in collagen that is deposited in fibrils.

As noted above, an excess of corticosteroids will also result in a decrease in the hydroxylation of proline and lysine. Presumably the major effect of this block will be to decrease the stability of the triple helix of collagen at body temperature. It should be noted, however, that the concomitant decrease in hydroxylysine will result in a decrease in cross-linking in the collagen that is secreted and deposited in fibrils. This decrease, too, will result in a decrease in the stability of newly formed collagen fibrils and may

result in a decrease in the tensile strength in newly formed scars and defects in the growth and maintenance of bone in cases of both hypovitaminosis C and hypercorticosteroidism.

*In vitro* studies have shown that extreme hypoxia and the chelation of ferrous iron with $\alpha,\alpha'$-dipyridyl will both block the hydroxylation of prolyl and lysyl residues and mimic hypovitaminosis C.[15,55,59,78,116] *In vivo*, however, the levels of iron will never be sufficiently low to produce an underhydroxylation of collagen. It is possible that a localized tissue hypoxia may result in a transient underhydroxylation of collagen, but it does not seem likely that tissue oxygen levels will be sufficiently low for a sufficient length of time to cause a primary defect in the hydroxylation of collagen. It is possible, however, that a local tissue hypoxia will slow tissue repair processes. It is also possible that increasing age or tissue injury will result in changes in the organization and activation of the peptide components of the hydroxylating enzymes in the RER.[199]

Increased amounts of hydroxylysine and glycosylated hydroxylysine have also been identified in Type I collagen in embryonic tissues [15,28] and in repair processes in the adult.[15,193,194] This change is inconsistent, however, for increased levels of hydroxylysine were found in some dermal scars,[15,199] in some cases of scleroderma,[15,178,200] and in rachitic osteoid [198]; yet there were normal or decreased levels of hydroxylysine in corneal scar collagen,[172] in other dermal scars,[171] and in other cases of scleroderma.[182] However, an increase in hydroxylysine may lead to an increase in cross-linking and a decreased rate of degradation of newly synthesized collagen (15,21). This, in turn, may lead to an excessive accumulation of collagen in tissues undergoing fibroplasia.

### Acquired Changes in Collagen Cross-Links

As noted above, the formation of lysyl-derived reducible cross-links is essential for the stabilization of newly synthesized collagen. Consequently, there is nearly always a direct association between an increased rate of collagen synthesis and a quantative increase in reducible cross-links in tissues that are undergoing either growth or repair. This increase in cross-linking may contribute to a pathologic process by decreasing the rate of collagen degradation,[21] but in most cases an increase in reducible cross-links may simply reflect the increase in the deposition of newly synthesized collagen. It was recently shown, however, that in virus-induced osteopetrosis in chickens there is a specific increase in the dihydoxylysine-derived cross-links in the virus-infected bone.[170]

Acquired deficiencies or blocks in the formation of reducible cross-links are well known in veterinary medicine. In fact, studies of the disease lathyrism in turkeys, laboratory animals, and chick embryos were responsible

for much of the progress in our understanding of the stabilization of collagen fibrils and the development of tensile strength in connective tissues.[6,7,11,24,70,174] In lathyrism, the cross-linking of collagen and elastin is blocked by an amino nitrile such as β-aminopropionitrile, aminoacetonitrile, or methylene aminoacetonitrile that are derived from the seeds of a wild vetch or sweet pea of the genus Lathyrus.[7,70] One form of the disease involves the central nervous system, another affects bone, and a third affects elastic arteries. These forms are referred to as neuro-, osteo-, and angiolathyrism, and the different presentations of these forms are a reflection of differences in the toxic effects of the different aminonitriles.[7]

β-Aminopropionitrile (BAPN) binds irreversibly to lysyl oxidase and thus blocks the formation of lysyl-derived cross-links in newly synthesized collagen and elastin. Consequently, in lathyrism, the tensile strength fails to develop in newly synthesized collagen and elastin, and all growing connective tissues become fragile. This fragility results in aneurysms and rupture of elastic arteries or a breakdown in the growing ends of weight-bearing bones. Since existing collagen and elastin are not affected, this disease is essentially only seen in growing animals, and it is now of little clinical significance. However, the binding of BAPN to lysyl oxidase is so specific that this compound has proven to be an invaluable tool for experimental studies of the synthesis, deposition, and stabilization of collagen in essentially every connective tissue laboratory in the world.

Copper deficiency, especially in pigs, is another classic example of a disease in animals that has contributed greatly to progress in studies of connective tissue proteins, especially elastin.[25] Since copper is an essential cofactor for lysyl oxidase, a deficiency of copper will also block the formation of lysyl-derived cross-links in collagen and elastin. This results in a loss of tensile strength, especially in elastic tissues, and accounts for the frequent rupture of elastic arteries in the copper-deficient animal.

A recent study has shown that a deficiency of zinc may also lead to a decreased rate of collagen synthesis.[186] At the same time there is an increase in the formation of intramolecular cross-links and a decrease in the formation of intermolecular cross-links in the newly synthesized collagen.[186] These results suggested that a deficiency of zinc decreased collagen synthesis by decreasing the replication and transcription of DNA and RNA, but it was not clear why a deficiency of zinc affected the cross-linking of collagen. Perhaps zinc is required for the procollagen peptidase enzymes. Intramolecular cross-links may form in free procollagen molecules, but intermolecular cross-links will decrease if procollagen is not converted to collagen and packed into fibrils.

Penicillamine is a drug that has been used pharmacologically to decrease the stabilization of collagen in fibrotic processes by blocking the

formation of reducible cross-links in newly synthesized collagen.[174,180] The early studies suggested that this drug reacted with the aldehyde group that resulted from the oxidative deamination of lysine and hydroxylysine. More recent studies have shown, however, that penicillamine may only block the formation of polyfunctional intermolecular cross-links from the Schiff base cross-link precursors; and these results helped to explain the specificity of penicillamine for cross-links in fibrous tissue collagen and its ineffectiveness in bone collagen.[196]

Abnormalities in the cross-linking of collagen may also involve defects in the conversion of reducible cross-links to nonreducible cross-links. A decrease in the rate of conversion may result in an accumulation of reducible cross-links, while an increase in the rate of conversion may decrease the rate of degradation or turnover of collagen. Thus, an increase in the conversion of cross-links may contribute to the development of fibrotic processes. Neither the nature of the nonreducible cross-links nor the mechanism of this conversion has been identified, however.

#### Acquired Defects in Collagen Degradation

No heritable defects in collagen degradation have been indentified, but numerous acquired disease processes involve either an increase or a decrease in the rate of collagen degradation. An outline classifying these processes is presented in Table 4, Part V, and the reader is referred to the excellent comprehensive review of collagen degradation by Perez-Tamayo.[21]

## Conclusion

It should be readily apparent that this review has not begun to cover even a fraction of those diseases in which the metabolism of collagen is markedly changed. It is hoped, however, that it will have served to show how much progress has occurred during the last 10 years in our understanding of the metabolism of collagen in both health and disease. More importantly, it should have shown how much more there is to learn about the metabolism of collagen. At the same time, it will have illustrated the importance of animal models for the wide variety of studies that are necessary in our finding answers for the important questions about the regulation of the synthesis, deposition, and removal of collagen in essentially every disease process.

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[*Illustrations follow*]

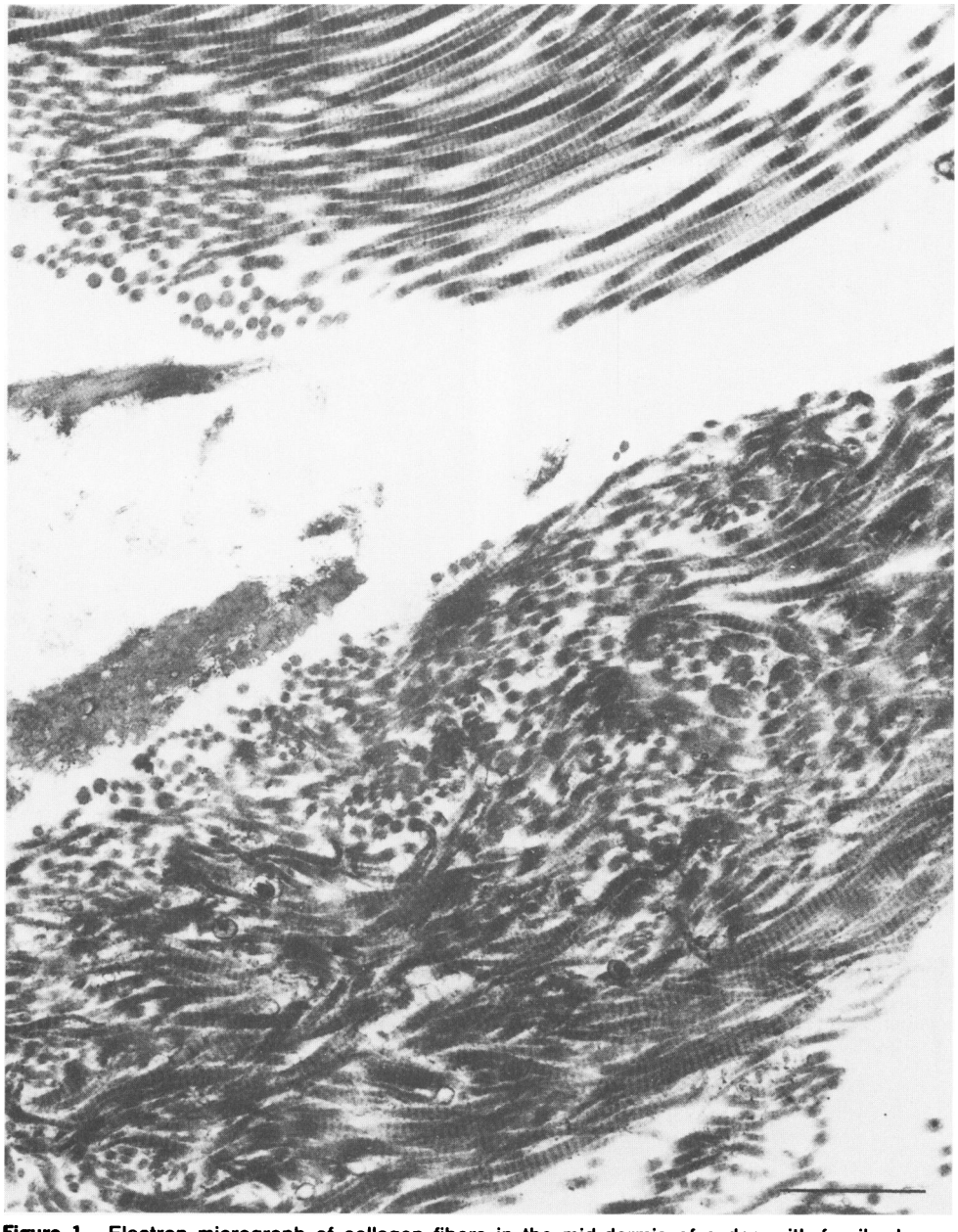

**Figure 1**—Electron micrograph of collagen fibers in the mid-dermis of a dog with fragile, hyperextensible skin. The fiber at the bottom is very abnormal, and the fiber at the top is morphologically normal. There is a wide variation in fibril diameter and a severe disorganization in the packing of fibrils in the abnormal fiber. The entire dermis of affected heterozygous dogs and cats consists of a mixture of morphologically normal and abnormal fibers. These defects may not be detectable, however, with light microscopy. Bar = 1 $\mu$. (×22,000)

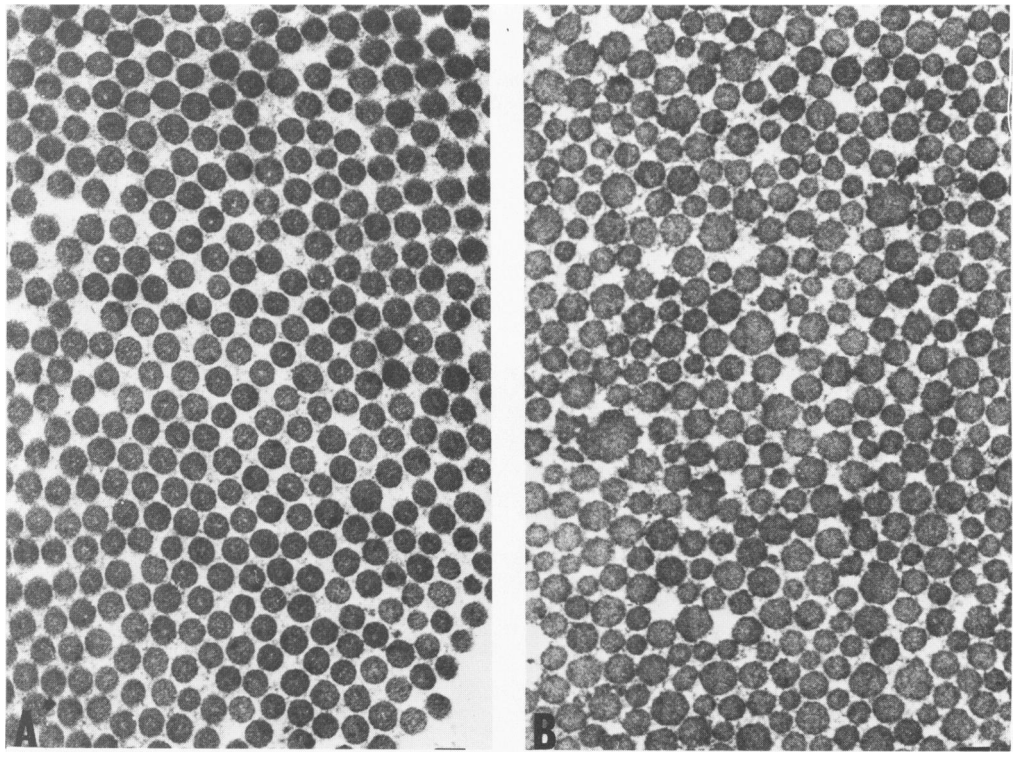


**Figure 2**—Electron micrographs of cross-sections of collagen fibrils in the mid-dermis of dorsal back skin of a normal dog (**A**), and a dog with fragile hyperextensible skin (**B**). Many of the collagen fibrils in affected dermis are larger than the largest fibril in normal dermis. This loss of control of the lateral growth of collagen fibrils is similar to that seen in cats with dominant collagen packing defect I.[150] Bar = 100 nm. (×40,000)