Disease mechanisms

Diseases of the collagen molecule

C. I. LEVENE

From the Department of Pathology, University of Cambridge

The title of this paper has been chosen to contrast with the term 'collagen diseases' by which Klemperer and his colleagues (Klemperer et al., 1942) designated the group of diseases that included rheumatoid arthritis among others. In 1959 the basic lesion in osteoarthri-throm— an experimental condition produced in rats or chick embryos by treatment with the sweet pea seed 'lathyrus factor' (β-aminopropionitrile (BAPN)) and signs that included slipped epiphyses, tendon avulsion, kyphoscoliosis, hernias, and rupture of the aorta— was shown to be a defect in the cross-linking of collagen (Levene and Gross, 1959). The mechanism was believed to be the blocking of aldehyde groups in the collagen molecule (Levene, 1962) and essential for normal cross-linking (Tanzer, 1973; Bailey et al., 1974). Shortly afterwards, Pinnell and Martin (1968) isolated the enzyme responsible for the normal formation of these aldehydic precursors of the cross links. This enzyme, called lysyl oxidase, required copper as an essential cofactor and acted by oxidatively deaminating specific peptidyl lysine or hydroxylysine residues in the collagen molecule to produce aldehydes which subsequently formed either Schiff bases with amino groups in adjoining chains or condensed by the aldol reaction with adjoining aldehydic groups (Siegel et al., 1970; Siegel, 1974).

The effects of lathyrism were so striking that it seemed worthwhile using BAPN therapeutically in an experimental fibrotic condition to attempt to inhibit or to slow down the degree of fibrosis, a process which has generally been considered to be inexorable and irreversible. Pulmonary silicosis in the rat was selected as a model for a severe, progressive, and irreversible fibrosis. Treatment of silicotic rats with BAPN resulted in an approximately 50% inhibition of the degree of pulmonary fibrosis, despite the fact that the body weight was unaffected (Levene et al., 1968) (Table 1).

A consideration of the fibrotic process and the three major elements that lead to it after inflammation—that is, resolution, regeneration, and repair—indicates that while examples of the first two elements are sparse (for instance, lobar pneumonia as the classic example of resolution and epidermis and liver as two examples of regenerating tissues) examples of the third phase, repair, are numberless. The process—organisation—results in scar formation in such varied conditions as the healing of a surgical wound or fractured bone; mitral stenosis, where the 'healing' fibrosis causes the major pathology; cirrhosis of the liver; conical scarring resulting in blindness; rheumatoid arthritis, which may produce joint fusion; Crohn's disease of the small bowel; stenosis of the urethra after gonococcal infection; peritoneal adhesions; pulmonary silicosis; and coronary atherosclerosis, resulting in angina pectoris.

Clearly, while healing of a surgical wound, fracture or a myocardial infarction by fibrosis is a beneficial reaction, fibrosis elsewhere is often harmful. It may result in malfunction of an organ, as in liver cirrhosis; or in the narrowing of the lumen of a bile duct, urethra, or coronary artery; or in the contraction of scars such as result from skin burns. The word 'repair', with its beneficial connotations, seems less apt in these circumstances. It therefore seemed that an attempt to slow or inhibit fibrosis selectively might prove useful therapeutically. To achieve this, the pathway by which collagen is synthesised had to be studied to seek further potential sites for therapeutic attack on the fibrotic process. During the past 15 years or so several groups have between them elucidated the biosynthetic pathway of collagen and also the nature of the lesions in a number of experimental and naturally occurring diseases which illustrate errors in collagen biosynthesis (for reviews see: Grant and Prockop, 1972; Bornstein, 1974; Gross, 1974; Prockop et al., 1976; Uitto and

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of β-aminopropionitrile (BAPN) treatment on the lung collagen content of silicotic rats</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Total (±SD) collagen content (mg/lung)</td>
</tr>
<tr>
<td></td>
<td>Right</td>
</tr>
<tr>
<td>Normal controls</td>
<td>21.28 ± 1.4</td>
</tr>
<tr>
<td>Silicotic controls</td>
<td>76.10 ± 18.1</td>
</tr>
<tr>
<td>Silicotic treatment with BAPN</td>
<td>34.73 ± 1.8</td>
</tr>
</tbody>
</table>

1Member of the external staff of the Medical Research Council.
Diseases of the collagen molecule

**STAGES**

1. Polysomal synthesis of unhydroxylated collagen

2. Hydroxylation of certain proline and lysine residues

3. Glycosylation of certain hydroxylysine residues

4. Assembly of 3α-chains → triple helix

5. Secretion of triple helical precursor to outside of cell

6. Excision of registration peptide → tropocollagen molecule

7. Assembly of fibril by 1/4 stagger alignment

8. Cross-linking of molecules in fibril

**ENZYMES**

- Prolyl and lysyl hydroxylase
- Glycosylation enzymes
- Procollagen peptidase
- Lysyl oxidase

**Figure** Diagram of collagen biosynthesis.
Collagen biosynthesis

Collagen is unique because of the various post-translational modifications involved in its biosynthesis (Figure). The precursor which is synthesised on the ribosomes is larger than the product, as in the case of fibrin, and has large N- and C-terminal extension peptides. It is important to note that although collagen contains about 12% hydroxyproline and 0-5% hydroxlysine, both of which are unique to collagen, neither are incorporated as such during synthesis. They are incorporated as proline or lysine and are therefore absent in the precursor. They appear only during the next stage, when selective hydroxylation of specific peptidyl proline and lysine precursor residues occurs via their respective enzymes prolyl and lysyl hydroxylase. These two enzymes both require α-ketoglutarate, molecular oxygen, ferrous iron, and ascorbic acid as essential cofactors. After that, galactose or galactosyl plus glucose are added to particular hydroxlysine residues by the two glycosylating enzymes, galactosyl or glucosyl transferase, both of which require Mn++ as an essential cofactor. Although some collagens—particularly kidney basement membrane—are highly glycosylated the actual role of glycosylation remains unknown. After glycosylation assembly of the three precursor α chains takes place to form the precursor procollagen molecule. Clearly, from the work of Prockop’s group (Berg and Prockop, 1973), hydroxyproline plays a vital role in the stabilisation of the collagen molecule. In the absence of hydroxyproline the collagen molecule is rapidly degraded.

The triple helix, still carrying the precursor peptides, is then secreted and the two extension peptides cleaved extracellularly by an enzyme called procollagen peptidase, so that the resultant polar tropocollagen molecules may align end to end and side to side but staggered at one-quarter of their length to form a fibril exhibiting a 640 Å axial periodicity. These fibrils, however, possess no tensile strength so cannot fulfil their major tensile function until they are cross-linked to each other. This takes place via the enzyme lysyl oxidase (Pinnell and Martin, 1968), which requires copper and, as has recently been shown, pyridoxal phosphate (Murray, 1976; Murray and Levene, 1977). The process is by an oxidative deamination of specific lysine or hydroxlysine residues to form aldehydic cross-link precursors which then either form Schiff bases with neighbouring amino groups on nearby chains or aldol condensation groups with neighbouring aldehydes. Little is known of the mechanisms of collagen fibril weave. In tendon the fibrils run parallel to each other. In cornea sheets of fibrils are superimposed at right angles to each other in contrast to neighbouring sclera where the collagen bundles form a three-dimensional network similar to that found in dermis.

It is relevant here to mention collagen polymorphism. At least four types of collagen are known. There is probably a fifth—membrane, or M, collagen. Polymorphism means that a particular tissue contains more than one type of collagen in health or, as we now suspect, in certain diseases (Nimni and Deshmukh, 1973). Meigel et al. (1977) illustrated this in human dermis using highly specific antisera to various collagen types labelled by an immunofluorescent technique. They showed that the thick collagen bundles that constitute most of the dermis and which stain red with the van Gieson stain consist of type I collagen, while the subepidermal fibres, which are much thinner and surround the pilosebaceous units, the eccrine and apocrine glands, and probably the blood vessels and which stain argyrophilically—that is, reticulin fibres—consist of type III collagen.

Diseases of collagen molecule of known aetiology

I think the time is now ripe to use the information on the collagen biosynthetic pathway as a basis for a logical classification of ‘diseases of the collagen molecule’. In going through the various stages of biosynthesis (Table 2) it may firstly be said that no disease involving an error of translation has so far been found.

Faults in hydroxylation

Two diseases associated with faulty hydroxylation of proline and of lysine have been elucidated. Firstly, scurvy—a disease where proline fails to become hydroxylated (Gould, 1968; Barnes and Kodicek, 1972; Barnes, 1975; Levene and Bates, 1975). Ascorbic acid is essential for the maintenance of life. Man cannot synthesise his own ascorbate since he is deficient in one of the essential enzymes in his liver—gulonolactone oxidase. Like the guinea-pig, monkey, and a number of other species he is obliged to take ascorbic acid in his diet. Failure to do so results in scurvy, which eventually kills. Why it kills is not understood, but the cause of the failure of wound healing in scurvy is understood. Ascorbate is an essential cofactor in the hydroxylation of collagenous proline and lysine. Since hydroxysine is essential for the stability of the collagen molecule (Levene et al., 1972) underhydroxylation results in a lack of synthesis of normal collagen in the healing wound.
The lesion at the molecular level consists in a failure of hydroxylation of peptidyl proline in collagen and of certain peptidyl lysine residues, leading to the early degradation of the underhydroxylated collagen and consequently failure of the wound to heal.

Secondly, there has recently been described in two families a genetic disease characterised by hydroxylsine-deficient collagen due to defective lysyl hydroxylase activity (Pinnell et al., 1972; Eyre and Glimcher, 1972; Krane et al., 1972; Sussman et al., 1974; Quinn and Krane, 1976). The symptoms included skeletal deformities, stretchable skin, microcornea requiring enucleation after an accident, the 'floppy mitral valve' syndrome, and in one case death resulting from a dissecting aortic aneurysm. These cases have been classified as being of the type VI Ehlers-Danlos syndrome and the lesions are mostly explicable by faulty intermolecular cross-linking of collagen consequent on a lack of sufficient hydroxylsine residues.

**FAULTS IN GLYCOSYLATION**

Kidney glomerular basement membrane which contains type IV collagen is known to be highly glycosylated (Grant and Prockop, 1972; Kefalides, 1973). Spiro reported that the basement membrane in diabetic glomerulosclerosis was characterised by a collagen in which the hydroxylysine content and the number of glucosyl-galactose disaccharide units were much increased (Beisswenger and Spiro, 1970; Spiro and Spiro, 1971). Kefalides (1974) could not find this change in the diabetic glomerular basement membrane, but others have found chemical changes in the sugars (Kawamura, 1974). Consequently we must consider the possibility that change in the sugar composition of basement membrane collagen may represent the molecular defect in diabetic glomerulosclerosis. But obviously more evidence will be required before a firm conclusion can be drawn.

### FAULTS IN PROCOLLAGEN PEPTIDASE

An error in the procollagen peptidase cleavage step has been found in animals and in man. This enzyme normally excises the precursor peptides once the precursor triple helix is secreted by the cell. A condition named dermatosparaxis ('torn skin') has been described in calves (Ansay et al., 1968; O'Hara et al., 1970; Simar and Betz, 1971) and in sheep (Helle and Nes, 1972) and has been studied by Lapière’s group. They and others (Lenaers et al., 1972; Bailey and Lapière, 1973; Prockop et al., 1973; Hansel and Lapière, 1974; Fjølstad and Helle, 1974) showed that the skin fragility resulted from a genetic deficiency of procollagen peptidase, leading to a failure of complete excision of the precursor peptides, so that the resultant collagen molecules were unable to assemble and cross-link in the normal manner. In man there is an analogous condition, one of the Ehlers-Danlos types of genetic disease called the 'floppy mitral valve syndrome', in which myxomatous change of the mitral valve is accompanied by a systolic click. This too is thought to be due to a genetic deficiency of procollagen peptidase (Lichtenstein et al., 1973).

### FAULTS IN CROSS-LINKING

In a number of conditions, as follow, the basic lesion is a defect in collagen cross-linking.

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**Table 2 Diseases of the collagen molecule**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cofactors</th>
<th>Function</th>
<th>Examples of disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolyl hydroxylase</td>
<td>α-Ketoglutarate, Molecular oxygen, Ferrous iron, Ascorbic acid</td>
<td>Hydroxylation of specific proline residues</td>
<td>Scurvy</td>
</tr>
<tr>
<td>Lysyl hydroxylase</td>
<td>As for prolyl hydroxylase, Manganese</td>
<td>Hydroxylation of specific lysine residues</td>
<td>Ehlers-Danlos type VI, Alkaptonuria, ?Diabetic glomerulosclerosis</td>
</tr>
<tr>
<td>Galactosyl and glucosyl transferases</td>
<td>Unknown</td>
<td>Addition of galactose or glucosyl + glucose to specific hydroxylysine residues</td>
<td>Dermatosparaxis, Ehlers-Danlos types I and VII</td>
</tr>
<tr>
<td>Procollagen peptidase</td>
<td>Copper</td>
<td>Excision of N- and C-terminal precursor peptides to allow collagen fibril formation</td>
<td>Osteolathyrism, Copper deficiency, Vitamin B6 deficiency, Menkes's kinky-hair syndrome, d-Penicillamine, Mottled mouse defect, Ehlers-Danlos type V, Homocysteinuria, Cutis laxa</td>
</tr>
<tr>
<td>Lysyl oxidase</td>
<td>Copper, Pyridoxal phosphate</td>
<td>Intra- and intermolecular cross-linking of collagen to give fibrils tensile strength</td>
<td></td>
</tr>
</tbody>
</table>
(1) Osteolathyris (Levene, 1973) is characterised by skeletal deformity, increased solubility of collagen, and extreme fragility of the connective tissues. The last two give rise to a multiplicity of effects. The lesion is believed to be an inhibition of the cross-linking enzyme lysyl oxidase by the lathyrus factor BAPN resulting in a failure of the formation of the aldehydes essential for cross-link formation (Levene and Gross, 1959; Levene, 1962).

(2) Lysyl oxidase requires copper as a cofactor, which explains why aortic rupture occurs in copper-deficient swine, sheep, or chicks (O'Dell et al., 1961; Shields et al., 1962; Hill and Mann, 1962; Buffoni and Blaschko, 1964).

(3) It is now clear that pyridoxal phosphate constitutes a second essential cofactor for lysyl oxidase (Murray, 1976; Murray and Levene, 1977). Chicks deficient in vitamin B6 suffer severe deformity of the epiphyseal cartilages of the long bones associated with a reduction in the lysyl oxidase content of the cartilage. The aorta, which shows vague though distinct morphological changes, also has a reduced lysyl oxidase content. This may be restored to normal by treatment with vitamin B6 (Murray et al., 1978). Similar findings have been found in B6-deficient rats (Tane et al., 1976).

(4) A fourth example of a cross-link defect is the genetically-transmitted disease of children, Menkes's kinky-hair syndrome. The signs—cerebral degeneration and kinky, discoloured hair—are mostly explicable by a failure of absorption of copper in the small bowel. The neurological symptoms are thought to be due to cytochrome oxidase malfunction, the twisted hair to a failure of S-S bonding in keratin, and the hair discolouration to malfunction of tyrosinase since the three enzymes involved require copper (Menkes et al., 1962; Danks et al., 1972a; Danks et al., 1972b; Oakes et al., 1976). Oddly enough no anaemia occurs because the red cells are selectively spared.

(5) A fifth cross-link defect has emerged during the use of D-penicillamine as a chelating agent for copper in Wilson's disease (Walsh, 1977). Such treatment occasionally produces spontaneous rupture of tendons due, it is believed, to the formation of a thiazolidine complex between penicillamine and the aldehyde cross-link precursors which are normally formed, rendering them unavailable for the stabilisation of collagen (Nimni, 1977). Notably, the chemical structure and behaviour of penicillamine and homocysteine in relation to collagen cross-linking is similar.

(6) Finally, in a genetic condition in the mottled mouse a sex-linked defect in the cross-linking of collagen and elastin is associated with aortic aneurysm formation (Rowe et al., 1974).

Diseases of collagen molecule of uncertain aetiology

Heritable disorders of connective tissue

A number of diseases are probably due to genetic errors in collagen biosynthesis but their aetiologies are as yet incompletely understood, although they are being gradually unravelled. These are among the 'heritable disorders of connective tissues' so admirably described by McKusick (1972). During the last decade or so they have been investigated in the few laboratories equipped to do this work by culturing the patients' fibroblasts and examining the various steps in their biosynthesis of collagen. Table 3 lists most of these diseases, the major signs, and what is thought to be the basic defect. Clearly, in no case is the lesion plainly elucidated but there are, nevertheless, some clues as to the nature of the particular lesion.

Marfan's syndrome

The three major sites affected in this disease are the skeletal system, eyes, and aorta (McKusick, 1972). The results of studies on the collagen in such patients have been conflicting. Some workers have found an increase in the amount of soluble collagen produced by cultured fibroblasts (Laitinen et al., 1968; Priest et al., 1973). Francis et al. (1976a) also found increased collagen solubility but interpreted the data cautiously, since the cross-linking of collagen occurred at a later stage than expected. Other workers, however, found no change in collagen solubility (Martin et al., 1971) or in the production of lysyl oxidase (Layman et al., 1972) or of specific cross-links (Bailey et al., 1974). Moreover, the measurement of polymeric collagen in the skin, believed to reflect the degree of collagen cross-linking, was not greatly abnormal (Francis et al., 1976b). Consequently, it seems discreet to keep an open mind on the lesion in this disease, of which osteolathyris appears to be such a striking phenocopy.

Ehlers-Danlos syndrome

This complicated syndrome consists of at least seven types and is well documented by McKusick (1972). It is clear from the literature that in these various types malfunction is at different points in collagen biosynthesis.

Firstly, under-hydroxylation of collagenous lysine was the first sign of a genetic fault in lysine hydroxylation found in two siblings who suffered severe scoliosis and lesions in the eyes, some of which eventually required enucleation (Pinnell et al., 1972). The collagen in these girls was deficient in hydroxylysine due to a deficiency in the enzyme lysyl
Diseases of the collagen molecule

Table 3  Heritable disorders of connective tissues (adapted from McKusick, 1972)

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Skin</th>
<th>Joints</th>
<th>Bone</th>
<th>Eye</th>
<th>CVS</th>
<th>Fundamental defect suspected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marfan syndrome</td>
<td>Fragility,</td>
<td>Hyperextensibility</td>
<td>Long, thin extremities</td>
<td>Ectopia of lens</td>
<td>Aortic aneurysm</td>
<td>? Collagen defect</td>
</tr>
<tr>
<td></td>
<td>hyperelasticity, bruisability</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Conflicting data; lesion still obscure I, VII.</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>II, III. Unknown lesion</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>IV. Probably lack of type III collagen</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>VI. Lysyl hydroxylase deficiency</td>
</tr>
<tr>
<td>Osteogenesis</td>
<td>Brittle bones, deafness</td>
<td>Blue sclera</td>
<td></td>
<td></td>
<td>Arterial and venous thromboses</td>
<td>Cystathionine synthetase deficiency producing homocysteine excess and cystathione deficiency; homocysteine probably causes collagen cross-link defect</td>
</tr>
<tr>
<td>imperfecta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Homogentisic acid oxidase deficiency; produces ? in-vivo collagen tanning; ? lysyl hydroxylase deficiency</td>
</tr>
<tr>
<td>Homocysteinuria</td>
<td>Long, thin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>? Lysyl oxidase deficiency collagen and elastin cross-link defect</td>
</tr>
<tr>
<td></td>
<td>extremities</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaptonuria</td>
<td>Ochronotic</td>
<td>Arthritis</td>
<td></td>
<td></td>
<td>Ochronotic pigmentation</td>
<td></td>
</tr>
<tr>
<td>(Garrod's disease)</td>
<td>pigmentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutis laxa</td>
<td>Pendulous, stretchable,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>prematurely aged look</td>
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</table>

hydroxylase (Krane et al., 1972). Moreover, a reduction in reducible cross-links was found (Eyre and Glimcher, 1972). Such lysyl hydroxylase as was present had abnormal chemical, physical, and kinetic properties (Quinn and Krane, 1976). Since hydroxylsine-derived cross-links produce a more stable collagen than those which are lysine-derived (Miller and Robertson, 1973) a causal relationship between the signs and the hydroxylsine deficiency is likely in this condition, which has been classified as type VI Ehlers-Danlos. A clinical attempt has been made to correct the lesion by treatment with ascorbic acid (Elsas et al., 1974), which is essential for the hydroxylation of those residues participating in cross-link formation (Levene et al., 1972). Diagnosis requires culture of the patients' fibroblasts as well as analysis of their native collagen (Steinmann et al., 1975). Also there may be variants of the condition (Judisch et al., 1976). Finally, it seems that the collagen abnormality in this type may affect the collagen-platelet interaction, leading to a bleeding tendency (Karaca et al., 1972).

Secondly, a defect has been described in which, like dermatosparaxis, the conversion of the precursor procollagen molecule into the tropocollagen molecule fails owing probably to an insufficiency of the enzyme procollagen peptidase (Lichtenstein et al., 1973; Shinkai et al., 1976). Thirdly, Ehlers-Danlos type V is believed to be due to a deficiency in the cross-linking enzyme lysyl oxidase (Di Ferrante et al., 1975). Finally, Pope et al. (1975) showed that patients with Ehlers-Danlos type IV lack type III collagen.

Osteogenesis imperfecta
This disease seems to be due to a genetic fault in the mechanism that controls the types of collagen made and should perhaps be classified under the heading 'polymorphism'. Again, it is well-documented by McKusick (1972). Some types of osteogenesis imperfecta are evidently caused by failure of the fibroblasts to synthesise normal amounts of type I collagen (Meigel et al., 1974; Sykes et al., 1977). Control fibroblasts in culture were found to make only type I collagen whereas osteogenesis imperfecta fibroblasts synthesised type III as well as type I (Müller et al., 1975), suggesting that control of the type of collagen synthesised lay at the root of the condition. Other workers found that fibroblasts synthesise both types but that while the normal ratio of type I:type III is 5-6:1 it falls to 1:1 in the diseased fibroblasts (Penttinen et al., 1975). Moreover, the type I collagen produced in this disease had fewer α2 chains than normal (Lichtenstein et al., 1975). Some have also considered the lesion to be a failure of maturation of collagen due to the production of labile intermolecular cross-links (Fuji et al., 1977; Fuji and Tanzer, 1977; Müller et al., 1977), and Trelstad et al. (1977) found in at least one type of the condition an increase in the amount of hydroxylsine and in the degree of glycosylation, particularly in calcifying tissues.

Homocysteinuria
This interesting genetically-transmitted condition is well-documented by McKusick (1972). The signs comprise central nervous system manifestations,
including thromboses and mental retardation, and various eye and cardiovascular lesions. The most distinctive pathological feature is arterial obstruction by thrombosis and severe changes in the arterial wall. Fairly certainly the lesion consists of an inhibition of collagen cross-linking. Some years ago Harris and Sjoerdsma (1966) noted that the chemical structure of homocysteine greatly resembled that of D-penicillamine. They therefore suggested that homocysteine, like D-penicillamine, might also inhibit collagen cross-linking by complexing with the aldehydic cross-link precursor, thus inhibiting normal cross-linking. It was later shown that homocysteinuric skin collagen is deficient in cross-links and that homocysteine can inhibit cross-link formation in vitro (Kang and Trelstad, 1973).

Siegel (1975) has suggested that homocysteine may prevent the further stabilisation of aldehyde-derived cross-links and so prevent newly synthesised collagen fibres from acquiring their normal tensile strength. Uitto and Lichtenstein (1976) have reservations about the in-vitro studies of Kang and Trelstad because they used 10-times the concentration of homocysteine maximally found in the disease. This criticism may well be unfair. The basic lesion seems to be a deficiency of cystathione synthetase in homocysteinurics and in fibroblasts cultured from their skin (Uhlendorf and Mudd, 1968) leading to an accumulation of homocysteine and to a deficiency of cystathione (McKusick, 1972). Apparently care should be taken in using culture methods to make a diagnosis, since cystathione synthetase levels in such cultures seem to vary with the composition of the medium and the age of the cultured fibroblasts (Griffiths and Tudball, 1976).

Alkaptonuria
This inborn error of metabolism, termed ‘Garrod’s disease’ by McKusick (1972), is typified by dark urine; pigmentation of the connective tissues, especially of cartilage; progressive arthropathy; often cardiovascular disease; and urinary and prostatic calculi. The disease is due to a deficiency of homogentisic acid oxidase, normally present in the liver and kidney. As a result homogentisic acid, a normal metabolite, accumulates behind the enzyme block. Milch (1961) believes that homogentisic acid is then auto-oxidised and may act as a potent in-vivo tanning agent leading to the production of ochronosis and degenerative arthropathy. Murrays et al. (1977) believe from their in-vitro studies that homogentisic acid in vivo may inhibit lysyl hydroxylase, resulting in a hydroxylysine-deficient and structurally modified collagen, although the cross-links and the hydroxylysine content of ochronotic cartilage have not yet been examined. Exactly how homogentisic acid may inhibit lysyl hydroxylase remains unexplained. Unfortunately it is not feasible to study this disease by using cultured fibroblasts from cases of alkaptonuria in vitro (McKusick, 1972).

*Cutis laxa*
This genetically transmitted disease, also described by McKusick (1972) and by Uitto and Lichtenstein (1976), is characterised by pendulous stretchable skin which produces an appearance of premature ageing; the skin is not friable. These patients may suffer from hernias, prolapse of the rectum and uterus, and pulmonary emphysema. The lesion had been thought to be a defect in the elastic fibre (McKusick, 1972) but Byers et al. (1975) found in two male cousins suffering from the condition a deficiency of lysyl oxidase in their cultured fibroblasts leading to a cross-link defect in both collagen and elastin, since, so far as we know, the same enzyme is used for cross-linking both proteins.

*Tight-skin mutation of mouse*
This genetically transmitted disease was found in mice by Green et al. (1976). The gene, a dominant one, was located on chromosome 2. Heterozygotes had tight skins with much hyperplasia of the loose subcutaneous connective tissue, increased growth of cartilage and bone, and small tendons surrounded by hyperplastic tendon sheaths. The body weight was not increased. It was proposed that the condition was produced by a somatomedin-like factor that promoted the growth of cartilage, bone, and connective tissues.

The ‘Collagen Diseases’
This group of diseases, first described by Klemper et al. (1942), includes rheumatoid arthritis, scleroderma, disseminated lupus erythematosus, and polyarteritis nodosa. That collagen can stimulate antibody production is well established (Steffen, 1970; Furthmayr and Timpl, 1976; Holborow et al., 1977). Telopeptides make good antigens, and probably these diseases have auto-immune aspects. Antibodies to collagen have been found in rheumatoid arthritis (Michaeli and Fudenberg, 1974; Ziff, 1974; Menzel et al., 1976; Andriopoulos et al., 1976) and in progressive systemic sclerosis (Stuart et al., 1976). Antibodies to membrane (M) collagen of the fibroblasts may also exist and produce a cytotoxic effect (Lichtenstein et al., 1976).

This subject is too vast to do justice to here. Scleroderma deserves a particular mention since, although there is no absolute certainty about the basic lesion, most workers are agreed that excess collagen is synthesised in the skin. Keiser and Sjoerdsma (1969) and Keiser et al. (1971) showed
that prolyl hydroxylase concentrations are raised in sclerodermatous skin and that it synthesises more collagen than normal. This was confirmed by others (Uitto et al., 1969; Uitto et al., 1970a; Uitto et al., 1970b; Uitto, 1971; Leroy, 1972; Fleckman et al., 1973; Giro et al., 1974). Recently it has been suggested that fibroblasts cultured from patients with scleroderma may have more stringent biosynthetic requirements and need more serum than normal fibroblasts (Kovacs and Fleishmayer, 1974; Bashey et al., 1977; Bashey and Jimenez, 1977). The significance of this finding is difficult to interpret but it seems that sclerodermatous fibroblasts differ from the normal in some way.

**POLYMORPHISM**

Nimni and Deshmukh (1973) described how osteoarthritic human articular cartilage which normally contains only type II collagen also contains type I collagen. This was the first description of polymorphism in disease. Polymorphism in health was first described by Miller et al. (1971) and later by Epstein and Munderloh (1975). We now know that polymorphism occurs normally in tissues such as lung, which contains type I and III (Hance et al., 1976), and aortic smooth muscle, which contains types I and III (McCullagh and Balian, 1975; Barnes et al., 1976; Layman et al., 1977; Rauterberg et al., 1977; Scott et al., 1977) as well as segment-long-spacing type III (Rauterberg and von Bassewitz, 1975) and type IV collagen (Trelstad, 1974). The human fibroblast has been shown to contain not only type I collagen but also the M (membrane) collagen (Lichtenstein et al., 1976), and the human liver to contain collagens of types I and III (Seyer et al., 1977).

Examination of various diseased tissues has shown that changes occur in the proportion of the various types, and that this may prove to be significant. For example, the lungs in idiopathic pulmonary fibrosis contain types I and III, as in the normal, but their hydroxylysine content is diminished (Seyer et al., 1976). In the normal human liver 47% of the collagen is type III, the remainder being type I, but in the human cirrhotic liver there is a much smaller proportion of type III ranging from 18 to 34%, with a corresponding increase in type I (Seyer et al., 1977). Indeed, type III collagen, normally present in human gingival fibroblasts together with type I, was absent in diseased human gingival fibroblasts which contained a significant amount of the (α1S) type I trimer (Narayanan and Page, 1976).

Among the genetic conditions must be mentioned Ehlers-Danlos type IV, where the lesion probably consists of a lack of type III collagen (Pope et al., 1975), and osteogenesis imperfecta, where it seems that the proportion of type III increases as that of type I diminishes (Meigel et al., 1974; Müller et al., 1975; Penttinen et al., 1975; Lichtenstein et al., 1975; Sykes et al., 1977; Fujii et al., 1977; Müller et al., 1977).

Some light on the significance of these changes may be shed by the work on polymorphism in cultured chick cartilage cells. Normally these synthesise type II only, but after treatment with 5-bromo-2′-deoxyuridine two different types of collagen were detected—type I and type I trimers (Mayne et al., 1975). The identical change was observed in cultured chick chondrocytes that were allowed to age in culture without the addition of 5-bromo-2′-deoxyuridine (Mayne et al., 1976). Cheung and his colleagues noted that cultured rabbit chondrocytes underwent a progressive ‘depression’, finally synthesising collagens of types I, III, and another unknown type instead of the normal type II (Cheung et al., 1976). This instability in the phenotypic expression of these cells may eventually explain some of the changes described earlier and shed light on whether they are fundamental to the disease process or merely incidental.

**KELOID, DUPUYTREN’S CONTRACTURE, AND PEYRONIE’S DISEASE**

The aetiology of these conditions remains obscure (Calnan, 1977) In the last two a contractile aspect of the fibrotic process possibly plays a role in the pathogenesis, but their aetiology remains as obscure as ever.

**FIBROSIS**

Many more fibrotic conditions may be listed. The aetiology of some, such as practolol peritonitis (Read, 1977) or paracetamol poisoning leading to pulmonary fibrosis is known. The mechanism, however, remains obscure.

**CHRONIC INFLAMMATION**

Is it possible to comment usefully on a cause of chronic inflammation when there are so many disparate conditions leading to the same end product? Histologically, chronic inflammation features lymphocytes and plasma cells, generally taken to signify immunoglobulin production. Macrophages too are present, thought by Allison et al. (1977) to play a key role in the control of fibrogenesis. Lastly, fibroblasts abound. McGee has recently demonstrated the production by fibroblasts of the Clq moiety of complement (Adnani et al., 1975). Without wishing to cloud an already difficult issue any further by useless speculation, it does nevertheless seem possible to construe the foregoing facts as pointing to the activation of an immunological pro-
cess in the pathogenesis of chronic inflammation. It may perhaps eventually prove that chronic inflammation relates to acute inflammation in the way that hypersensitivity relates to immunity—one being harmful the other useful, but both being different sides of the same coin. If this be so it may one day prove feasible, once the mechanism has been clarified, to control the harmful aspects of chronic inflammation therapeutically as it has proved possible to do in the case of hypersensitivity.

References


Diseases of the collagen molecule


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