Disease mechanisms

Diseases of the collagen molecule

C. I. LEVENE¹

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 osteolathyrism—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

¹Member of the external staff of the Medical Research Council.

Table 1 Effect of β -aminopropionitrile (BAPN) treatment on the lung collagen content of silicotic rats

	Total ($\pm$ SD) collagen content (mg/lung)	
	Right	Left
Normal controls	21.28 $\pm$ 1.4	11.95 $\pm$ 1.13
Silicotic controls	76.10 $\pm$ 18.1	21.86 $\pm$ 3.15
Silicotic treatment with BAPN	34.73 $\pm$ 1.8	16.75 $\pm$ 1.60

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; corneal 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

Figure Diagram of collagen biosynthesis.

Lichtenstein, 1976; Grant and Jackson, 1976; Kivirikko and Risteli, 1976).

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% hydroxylysine, 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 galactose plus glucose are added to particular hydroxylysine 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 hydroxylysine 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 hydroxylysine 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.

Diseases of the collagen molecule

Table 2 Diseases of the collagen molecule

Enzyme	Cofactors	Function	Examples of disease
Prolyl hydroxylase	α -Ketoglutarate Molecular oxygen Ferrous iron Ascorbic acid	Hydroxylation of specific proline residues	Scurvy
Lysyl hydroxylase	As for prolyl hydroxylase	Hydroxylation of specific lysine residues	Ehlers-Danlos type VI ?Alkaptonuria
Galactosyl and glucosyl transferases	Manganese	Addition of galactose or galactose + glucose to specific hydroxylysine residues	??? Diabetic glomerulosclerosis
Procollagen peptidase	Unknown	Excision of N- and C-terminal precursor peptides to allow collagen fibril formation	Dermatosparaxis ?Ehlers-Danlos types I and VII
Lysyl oxidase	Copper Pyridoxal phosphate	Intra- and intermolecular cross-linking of collagen to give fibrils tensile strength	Osteolathyrism Copper deficiency Vitamin B6 deficiency Menkes's kinky-hair syndrome D-Penicillamine Mottled mouse defect Ehlers-Danlos type V ?Homocysteinuria ?Cutis laxa

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 hydroxylysine-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 hydroxylysine 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 (Ansary *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; Hanset 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.

(1) Osteolathyrism (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 (Walshe, 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 osteolathyrism 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

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

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

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 hydroxylysine-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 hydroxylysine 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 α_2 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 (Fujii *et al.*, 1977; Fujii 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 hydroxylysine 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. Murray *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 Klemperer *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). Teloptides 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 Fleishmajer, 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 $(\alpha 1)_3$ 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 paraquat 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 C1q moiety of complement (Al 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

- Al Adnani, M. S., O'Reilly, K. M., and McGee, J. O. (1975). Clq production and secretion by fibroblasts. *Journal of Medical Microbiology*, **8**, xix.
- Allison, A. C., Clarke, I. A., and Davies, P. (1977). Cellular interactions in fibrogenesis. *Annals of the Rheumatic Diseases*, **36**, Supplement 2, 8-13.
- Andriopoulos, N. A., Mestecky, J., Miller, E. J., and Bradly, E. L. (1976). Antibodies to native and denatured collagens in sera of patients with rheumatoid arthritis. *Arthritis and Rheumatism*, **19**, 613-617.
- Ansay, M., Gillet, A., and Hanset, R. (1968). La dermatose héréditaire des bovines: biochimie descriptive de la peau. *Annales de médecine Vétérinaire*, **112**, 449-451.
- Bailey, A. J., and Lapière, C. M. (1973). Effect of an additional peptide extension of the N-terminus of collagen from dermosparactic calves on the cross-linking of collagen fibres. *European Journal of Biochemistry*, **34**, 91-96.
- Bailey, A. J., Robins, S. P., and Balian, G. (1974). Biological significance of the intermolecular cross-links of collagen. *Nature (London)*, **251**, 105-109.
- Barnes, M. J. (1975). Function of ascorbic acid in collagen metabolism. *Annals of the New York Academy of Sciences*, **258**, 264-277.
- Barnes, M. J., and Kodicek, E. (1972). Biological hydroxylations and ascorbic acid with special regard to collagen metabolism. *Vitamins and Hormones*, **30**, 1-43.
- Barnes, M. J., Morton, L. F., and Levene, C. I. (1976). Synthesis of collagens types I and III by pig medial smooth muscle cells in culture. *Biochemical and Biophysical Research Communications*, **70**, 339-347.
- Bashey, R. I., and Jimenez, S. A. (1977). Increased sensitivity of scleroderma fibroblasts in culture to stimulation of protein and collagen synthesis by serum. *Biochemical and Biophysical Research Communications*, **76**, 1214-1222.
- Bashey, R. I., Jimenez, S. A., and Perlish, J. S. (1977). Characterization of secreted collagen from normal and scleroderma fibroblasts in culture. *Journal of Molecular Medicine*, **2**, 153-161.
- Beisswenger, P. J., and Spiro, R. G. (1970). Human glomerular basement membrane chemical alteration in diabetes mellitus. *Science*, **168**, 596-598.
- Berg, R. A., and Prockop, D. J. (1973). The thermal transition of a non-hydroxylated form of collagen. Evidence for a role of hydroxyproline in stabilising the triple helix of collagen. *Biochemical and Biophysical Research Communications*, **52**, 115-120.
- Bornstein, P. (1974). The biosynthesis of collagen. *Annual Review of Biochemistry*, **43**, 567-603.
- Buffoni, F., and Blaschko, H. (1964). Benzylamine oxidase and histaminase: purification and crystallization of an enzyme from pig plasma. *Proceedings of the Royal Society. Series B. Biological Sciences*, **161**, 153-167.
- Byers, P. H., Narayanan, A. S., Bornstein, P., and Hall, J. G. (1975). An x-linked form of cutis laxa due to deficiency of lysyl oxidase; the collagen and elastin cross-linking enzyme. Abstract, *Birth Defect Conference*, p. 206.
- Calnan, J. (1977). Keloid and Dupuytren's contracture. *Annals of the Rheumatic Diseases*, **36**, Supplement 2, 18-22.
- Cheung, H. S., Harvey, W., Benya, P. D., and Nimni, M. E. (1976). New collagen markers of 'depression' synthesized by rabbit articular chondrocytes in culture. *Biochemical and Biophysical Research Communications*, **68**, 1371-1378.
- Danks, D. M., Campbell, P. E., Stevens, B. J., Mayne, V., and Cartwright, E. (1972b). Menkes' kinky-hair syndrome: An inherited defect in copper absorption with widespread effects. *Pediatrics*, **50**, 188-201.
- Danks, D. M., Campbell, P. E., Walker-Smith, J., Stevens, B. J., Gillespie, J. M., Blomfield, J., and Turner, B. (1972a). Menkes' kinky-hair syndrome. *Lancet*, **1**, 1100-1103.
- Di Ferrante, N., Leachman, R. D., Angelini, P., Donnelly, P. V., Francis, G., and Almazon, A. (1975). Lysyl oxidase deficiency in Ehlers-Danlos syndrome type V. *Connective Tissue Research*, **3**, 49-53.
- Elsas, L. J., Hollins, B., and Pinnell, S. R. (1974). Hydroxylysine deficient collagen disease: effect of ascorbic acid. (Abstract). *American Journal of Human Genetics*, **26**, 28a.
- Epstein, E. H., Jr., and Munderloh, N. H. (1975). Isolation and characterization of CN Br peptides of human [α 1(III)]₂ collagen and tissue distribution of [α 1(I)]₂ α ₂ and [α 1(III)]₂ collagens. *Journal of Biological Chemistry*, **250**, 9304-9312.
- Eyre, D. R., and Glimcher, M. J. (1972). Reducible cross-links in hydroxylysine-deficient collagens of a heritable disorder of connective tissue. *Proceedings of the National Academy of Sciences of the United States of America*, **69**, 2594-2598.
- Fjølstad, M., and Helle, O. (1974). A hereditary dysplasia of collagen tissues in sheep. *Journal of Pathology*, **112**, 183-188.
- Fleckman, P. H., Jeffrey, J. J., and Eisen, A. Z. (1973). A sensitive microassay for prolyl hydroxylase: activity in normal and psoriatic skin. *Journal of Investigative Dermatology*, **60**, 46-52.
- Francis, G., Donnelly, P. V., and Di Ferrante, N. (1976a). Abnormally soluble collagen produced in fibroblast cultures. *Experientia*, **32**, 691-693.
- Francis, M. J. O., Sanderson, M. C., and Smith, R. (1976b). Skin collagen in idiopathic adolescent scoliosis and Marfan's syndrome. *Clinical Science and Molecular Medicine*, **51**, 467-474.

- Fujii, K., Kajiwar, T., Kurosu, H., and Tanzer, M. L. (1977). Osteogenesis imperfecta: altered content of type III collagen and proportion of the cross-links in skin. *FEBS Letters*, **82**, 251-254.
- Fujii, K., and Tanzer, M. L. (1977). Osteogenesis imperfecta; biochemical studies of bone collagen. *Clinical Orthopaedics and Related Research*, **124**, 271-277.
- Furthmayr, H., and Timpl, R. (1976). Immunochemistry of collagens and procollagens. *International Review of Connective Tissue*, **7**, 61-99.
- Giro, M. G., Peserico, A., and Volpin, D. (1974). Collagen and elastin in scleroderma. *Connective Tissue Research*, **2**, 309-313.
- Gould, B. S. (1968). The role of certain vitamins in collagen formation. In *Treatise on Collagen*, edited by G. N. Ramachandran, vol. 2A, pp. 323-365. Academic Press, London and New York.
- Grant, M. E., and Jackson, D. S. (1976). The biosynthesis of procollagen. *Essays in Biochemistry*, **12**, 77-113.
- Grant, M. E., and Prockop, D. J. (1972). The biosynthesis of collagen. *New England Journal of Medicine*, **286**, 194-199, 242-249, 291-300.
- Green, M. C., Sweet, H. O., and Bunker, L. E. (1976). Tight skin, a new mutation of the mouse causing excessive growth of connective tissue and skeleton. *American Journal of Pathology*, **82**, 493-512.
- Griffiths, R., and Tudball, N. (1976). Studies on the use of skin fibroblasts for the measurement of cystathione synthase activity with respect to homocysteinuria. *Clinica Chimica Acta*, **73**, 157-162.
- Gross, J. (1974). Collagen biology: structure degradation and disease. *Harvey Lecture*, **68**, 351-432.
- Hance, A. J., Bradley, K., and Crystal, R. G. (1976). Lung collagen heterogeneity. Synthesis of type I and type III collagen by rabbit and human lung cells in culture. *Journal of Clinical Investigation*, **57**, 102-111.
- Hanset, R., and Lapière, C. M. (1974). Inheritance of dermatosparaxis in the calf. A genetic defect of connective tissues. *Journal of Heredity*, **65**, 356-358.
- Harris, E. D., Jr., and Sjoerdsma, A. (1966). Collagen profile in various clinical conditions. *Lancet*, **2**, 707-711.
- Helle, O., and Nes, N. N. (1972). A hereditary skin defect in sheep. *Acta Veterinaria Scandinavica*, **13**, 443-445.
- Hill, J. M., and Mann, P. J. G. (1962). The inhibition of pea-seedling diamine oxidase by chelating agents. *Biochemical Journal*, **85**, 198-207.
- Holborow, E. J., Page-Faulk, W., Beard, H. K., and Conochie, L. B. (1977). Antibodies against reticulin and collagen. *Annals of the Rheumatic Diseases*, **36**, Supplement 2, 51-56.
- Judisch, G. F., Waziri, M., and Krachmer, J. H. (1976). Ocular Ehlers-Danlos syndrome with normal lysyl hydroxylase activity. *Archives of Ophthalmology*, **94**, 1489-1491.
- Kang, A. H., and Trelstad, R. L. (1973). A collagen defect in homocysteinuria. *Journal of Clinical Investigation*, **52**, 2571-2578.
- Karaca, M., Cronberg, L., and Nilsson, I. M. (1972). Abnormal platelet-collagen reaction in Ehlers-Danlos syndrome. *Scandinavian Journal of Haematology*, **9**, 465-469.
- Kawamura, M. (1974). Chemical structure of glomerular basement membrane in diabetic glomerulosclerosis. *Jikeikai Medical Journal*, **21**, Supplement, 35-42.
- Kefalides, N. A. (1973). Structure and biosynthesis of basement membranes. *International Review of Connective Tissue Research*, **6**, 63-104.
- Kefalides, N. A. (1974). Biochemical properties of human glomerular basement membrane in normal and diabetic kidneys. *Journal of Clinical Investigation*, **53**, 403-407.
- Keiser, H. R., and Sjoerdsma, A. (1969). Direct measurement of the rate of collagen synthesis in skin. *Clinica Chimica Acta*, **23**, 341-346.
- Keiser, H. R., Stein, H. D., and Sjoerdsma, A. (1971). Increased procollagen proline hydroxylase activity in sclerodermatous skin. *Archives of Dermatology*, **104**, 57-60.
- Kivirikko, K. I., and Risteli, L. (1976). Biosynthesis of collagen and its alterations in pathological states. *Medical Biology*, **54**, 159-186.
- Klemperer, P., Pollack, A. D., and Baehr, G. (1942). Diffuse collagen disease. Acute disseminated lupus erythematosus and diffuse scleroderma. *Journal of the American Medical Association*, **119**, 331-332.
- Kovacs, C. J., and Fleishmajer, R. (1974). Properties of scleroderma fibroblasts in culture. *Journal of Investigative Dermatology*, **63**, 456-460.
- Krane, S. M., Pinnell, S. R., and Erbe, R. W. (1972). Lysyl procollagen hydroxylase deficiency in fibroblasts from siblings with hydroxylysine-deficient collagen. *Proceedings of the National Academy of Sciences of the United States of America*, **69**, 2899-2903.
- Laitinen, O., Uitto, J., Iivanainen, M., Hannuksela, M., and Kivirikko, K. I. (1968). Collagen metabolism in the skin in Marfan's Syndrome. *Clinica Chimica Acta*, **21**, 321-326.
- Layman, D. L., Epstein, E. H., Jr., Dodson, R. F., and Titus, L. J. (1977). Biosynthesis of type I and III collagens by cultured smooth muscle cells from human aorta. *Proceedings of the National Academy of Sciences of the United States of America*, **74**, 671-675.
- Layman, D. L., Narayanan, A. S., and Martin, G. R. (1972). The production of lysyl oxidase by human fibroblasts in culture. *Archives of Biochemistry and Biophysics*, **149**, 97-101.
- Lenaers, A., Ansay, M., Nusgens, B. V., and Lapière, C. M. (1972). Collagen made of extended α -chains procollagen in genetically-defective dermatosparaxical calves. *European Journal of Biochemistry*, **23**, 533-543.
- Leroy, E. C. (1972). Connective tissue synthesis by scleroderma skin fibroblasts in cell culture. *Journal of Experimental Medicine*, **135**, 1351-1362.
- Levene, C. I. (1962). Studies on the mode of action of lathyrogenic compounds. *Journal of Experimental Medicine*, **116**, 119-130.
- Levene, C. I. (1973). Lathyrism. In *Molecular Pathology of Connective Tissues*, edited by R. Pérez-Tamayo and M. Rojkind, pp. 175-228. Dekker, New York.
- Levene, C. I., and Bates, C. J. (1975). Ascorbic acid and

- collagen synthesis in cultured fibroblasts. *Annals of the New York Academy of Sciences*, **258**, 288-306.
- Levene, C. I., Bates, C. J., and Bailey, A. J. (1972). Biosynthesis of collagen cross-links in cultured 3T6 fibroblasts: effect of lathyrogens and ascorbic acid. *Biochimica et Biophysica Acta*, **263**, 574-584.
- Levene, C. I., Bye, I., and Saffioti, U. (1968). The effect of β -aminopropionitrile on silicotic pulmonary fibrosis in the rat. *British Journal of Experimental Pathology*, **49**, 152-159.
- Levene, C. I., and Gross, J. (1959). Alterations in state of molecular aggregation of collagen induced in chick embryos by β -aminopropionitrile (Lathyrus factor). *Journal of Experimental Medicine*, **110**, 771-790.
- Lichtenstein, J. R., Bauer, E. A., Hoyt, R., and Wedner, H. J. (1976). Immunologic characterization of the membrane-bound collagen in normal human fibroblasts; identification of a distinct membrane collagen. *Journal of Experimental Medicine*, **144**, 145-154.
- Lichtenstein, J. R., Byers, P. H., Smith, B. D., and Martin, G. R. (1975). Identification of the collagenous proteins synthesized by cultured cells from human skin. *Biochemistry*, **14**, 1589-1594.
- Lichtenstein, J. R., Martin, G. R., Kohn, L. D., Byers, P. H., and McKusick, V. A. (1973). Defect in conversion of procollagen to collagen in a form of Ehlers-Danlos syndrome. *Science*, **182**, 298-300.
- McCullagh, K. A., and Balian, G. (1975). Collagen characterisation and cell transformation in human atherosclerosis. *Nature (London)*, **258**, 73-75.
- McKusick, V. A. (1972). *Heritable Disorders of Connective Tissue*, 4th edition, pp. 61-28, 292-470. Mosby, St Louis.
- Martin, G. R., Layman, D. L., Narayanan, A. S., Nigra, T. P., and Siegel, R. C. (1971). Collagen synthesis by cultured human fibroblasts. *Israel Journal of Medical Sciences*, **7**, 455-456.
- Mayne, R., Vail, M. S., Mayne, P. M., and Miller, E. J. (1976). Changes in type of collagen synthesized as clones of chick chondrocytes grow and eventually lose division capacity. *Proceedings of the National Academy of Sciences of the United States of America*, **73**, 1674-1678.
- Mayne, R., Vail, M. S., and Miller, E. J. (1975). Analysis of changes in collagen biosynthesis that occur when chick chondrocytes are grown in 5-bromo-2'-deoxyuridine. *Proceedings of the National Academy of Sciences of the United States of America*, **72**, 4511-4515.
- Meigel, W. N., Gay, S., and Weber, L. (1977). Dermal architecture and collagen type distribution. *Archives of Dermatological Research*, **259**, 1-10.
- Meigel, W. N., Müller, P. K., Pontz, B. F., Sörensen, N., and Spranger, J. (1974). A constitutional disorder of connective tissue suggesting a defect in collagen biosynthesis. *Klinische Wochenschrift*, **52**, 906-912.
- Menkes, J. H., Alter, M., Steigleder, G. K., Weakley, D. R., and Sung, J. H. (1962). A sex-linked recessive disorder with retardation of growth, peculiar hair, and focal cerebral and cerebellar degeneration. *Pediatrics*, **29**, 764-779.
- Menzel, J., Steffen, C., Kolarz, G., Eberl, R., Frank, O., and Thumb, N. (1976). Demonstration of antibodies to collagen and of collagen-anticollagen immune complexes in rheumatoid arthritis synovial fluids. *Annals of the Rheumatic Diseases*, **35**, 446-450.
- Michaeli, D., and Fudenberg, H. H. (1974). The incidence and antigenic specificity of antibodies against denatured human collagen in rheumatoid arthritis. *Clinical Immunology and Immunopathology*, **2**, 153-159.
- Milch, R. A. (1961). Studies of alcaptonuria; collagenase degradation of homogentisic-tanned hide powder collagen. *Proceedings of the Society for Experimental Biology and Medicine*, **107**, 183-185.
- Miller, E. J., Epstein, E. H., and Piez, K. A. (1971). Identification of three genetically distinct collagens by cyanogen bromide cleavage of insoluble human skin and cartilage collagen. *Biochemical and Biophysical Research Communications*, **42**, 1024-1029.
- Miller, E. J., and Robertson, P. E. (1973). The stability of collagen cross-links when derived from hydroxylysyl residues. *Biochemical and Biophysical Research Communications*, **54**, 432-439.
- Müller, P. K., Lemmen, C., Gay, S., and Meigel, W. N. (1975). Disturbance in the regulation of the type of collagen synthesized in a form of osteogenesis imperfecta. *European Journal of Biochemistry*, **59**, 97-104.
- Müller, P. K., Raisch, K., Matzen, K., and Gay, S. (1977). Presence of type III collagen in bone from a patient with osteogenesis imperfecta. *European Journal of Pediatrics*, **125**, 29-37.
- Murray, J. C. (1976). Evidence for the role of pyridoxal phosphate as a cofactor of 'lysyl oxidase'. Ph.D. thesis, Cambridge University.
- Murray, J. C., Fraser, D. F., and Levene, C. I. (1978). The effect of pyridoxine deficiency on lysyl oxidase activity in the chick. *Journal of Experimental and Molecular Pathology*, in press.
- Murray, J. C., and Levene, C. I. (1977). Evidence for the role of vitamin B6 as a cofactor of lysyl oxidase. *Biochemical Journal*, **167**, 463-467.
- Murray, J. C., Lindberg, K. A., and Pinnell, S. R. (1977). *In-vitro* inhibition of chick embryo lysyl hydroxylase by homogentisic acid. *Journal of Clinical Investigation*, **59**, 1071-1079.
- Narayanan, A. S., and Page, R. C. (1976). Biochemical characterization of collagens synthesized by fibroblasts derived from normal and diseased human gingiva. *Journal of Biological Chemistry*, **251**, 5464-5471.
- Nimni, M. E. (1977). Mechanism of inhibition of collagen crosslinking by penicillamine. *Proceedings of the Royal Society of Medicine*, **70**, Supplement 3, 65-72.
- Nimni, M. E., and Deshmukh, K. (1973). Differences in collagen metabolism between normal and osteoarthritic human articular cartilage. *Science*, **181**, 751-752.
- Oakes, B. W., Danks, D. M., and Campbell, P. E. (1976). Human copper deficiency: ultrastructural studies of the aorta and skin in a child with Menkes' syndrome. *Experimental Molecular Pathology*, **25**, 82-98.
- O'Dell, B. L., Hardwick, B. C., Reynolds, G., and

- Savage, J. E. (1961). Connective tissue defect in the chick resulting from copper deficiency. *Proceedings of the Society for Experimental Biology and Medicine*, **108**, 402-405.
- O'Hara, P. J., Read, W. K., Romane, W. M., and Bridges, C. H. (1970). A collagenous tissue dysplasia of calves. *Laboratory Investigation*, **23**, 307-314.
- Penttinen, R. P., Lichtenstein, J. R., Martin, G. R., and McKusick, V. A. (1975). Abnormal collagen metabolism in cultured cells in osteogenesis imperfecta. *Proceedings of the National Academy of Sciences of the United States of America*, **72**, 586-589.
- Pinnell, S. R., Krane, S. M., Kenzora, J. E., and Glimcher, M. J. (1972). A heritable disorder of connective tissue, hydroxylysine-deficient collagen disease. *New England Journal of Medicine*, **286**, 1013-1020.
- Pinnell, S. R., and Martin, G. R. (1968). The cross-linking of collagen and elastin: enzymatic conversion of lysine in peptide linkage to α -amino-adipic- δ -semi-aldehyde (allysine) by an extract from bone. *Proceedings of the National Academy of Sciences of the United States of America*, **61**, 708-716.
- Pope, F. M., Martin, G. R., Lichtenstein, J. R., Penttinen, R. P., Gerson, B., Rowe, D. W., and McKusick, V. A. (1975). Patients with Ehlers-Danlos syndrome type IV lack type III collagen. *Proceedings of the National Academy of Sciences of the United States of America*, **72**, 1314-1316.
- Priest, R. E., Moinuddin, J. F., and Priest, J. H. (1973). Collagen of Marfan syndrome is abnormally soluble. *Nature (London)*, **245**, 264-266.
- Prockop, D. J., Berg, R. A., Kivirikko, K. I., and Uitto, J. (1976). Intracellular steps in the biosynthesis of collagen. In *Biochemistry of Collagen*, edited by G. N. Ramachandran and A. H. Reddi, pp. 163-273. Plenum, New York.
- Prockop, D. J., Dehm, P., Olsen, B. R., Berg, R. A., Grant, M. E., Uitto, J., and Kivirikko, K. I. (1973). Recent studies on the biosynthesis of collagen. In *Biology of the Fibroblast*, edited by E. Kulonen and J. Pikkariainen, pp. 311-320. Academic Press, London.
- Quinn, R. S., and Krane, S. M. (1976). Abnormal properties of collagen lysyl hydroxylase from skin fibroblasts of siblings with hydroxylysine-deficient collagen. *Journal of Clinical Investigation*, **57**, 83-93.
- Rauterberg, J., Allam, S., Brehmer, U., Wirth, W., and Hauss, W. H. (1977). Characterization of the collagen synthesized by cultured human smooth muscle cells from fetal and adult aorta. *Hoppe-Seyler's Zeitschrift für physiologische Chemie*, **358**, 401-407.
- Rauterberg, J., and Bassewitz, D. B. von (1975). Electron microscopic investigations of type III collagen segment-long-spacing crystallites of type III collagen from calf aorta and fetal calf skin. *Hoppe-Seyler's Zeitschrift für physiologische Chemie*, **356**, 95-100.
- Read, A. E. (1977). Practolol peritonitis. *Annals of the Rheumatic Diseases*, **36**, Supplement 2, 37-38.
- Rowe, D. W., McGoodwin, E. B., Martin, G. R., Sussman, M. D., Grahn, D., Faris, B., and Franzblau, C. (1974). A sex-linked defect in the cross-linking of collagen and elastin associated with the mottled locus in mice. *Journal of Experimental Medicine*, **139**, 180-192.
- Scott, D. M., Harwood, R., Grant, M. E., and Jackson, D. S. (1977). Characterization of the major collagen species present in porcine aortae and the synthesis of their precursors by smooth muscle cells in culture. *Connective Tissue Research*, **5**, 7-13.
- Seyer, J. M., Hutcheson, E. T., and Kang, A. H. (1976). Collagen polymorphism in idiopathic chronic pulmonary fibrosis. *Journal of Clinical Investigation*, **57**, 1498-1507.
- Seyer, J. M., Hutcheson, E. T., and Kang, A. H. (1977). Collagen polymorphism in normal and cirrhotic human liver. *Journal of Clinical Investigation*, **59**, 241-248.
- Shields, G. S., Coulson, W. F., Kimball, D. A., Carnes, W. H., Cartwright, G. E., and Wintrobe, M. M. (1962). Studies on copper metabolism. 32. Cardiovascular lesions in copper-deficient swine. *American Journal of Pathology*, **41**, 603-621.
- Shinkai, H., Hirabayashi, O., Tamaki, A., Matsubayashi, S., and Sano, S. (1976). Connective tissue metabolism in culture fibroblasts of a patient with Ehlers-Danlos syndrome type I. *Archives of Dermatological Research*, **257**, 113-123.
- Siegel, R. C. (1974). Biosynthesis of collagen cross-links: increased activity of purified lysyl oxidase with reconstituted collagen fibrils. *Proceedings of the National Academy of Sciences of the United States of America*, **71**, 4826-4830.
- Siegel, R. C. (1975). The connective tissue defect in homocystinuria (HS). (Abstract). *Clinical Research*, **23**, 263A.
- Siegel, R. C., Pinnell, S. R., and Martin, G. R. (1970). Cross-linking of collagen and elastin. Properties of lysyl oxidase. *Biochemistry*, **9**, 4486-4492.
- Simar, L. J., and Betz, E. H. (1971). Dermatosparaxis of the calf; a genetic defect of the connective tissue. 2. Ultrastructural study of the skin. (Abstract). *Hoppe-Seyler's Zeitschrift für physiologische Chemie*, **352**, 13.
- Spiro, R. G., and Spiro, M. J. (1971). Studies on the biosynthesis of the hydroxylysine-linked disaccharide unit of basement membranes and collagens. III. Tissue and subcellular distribution of glycosyltransferases and the effect of various conditions on the enzyme levels. *Journal of Biological Chemistry*, **246**, 4919-4925.
- Steffen, C. (1970). Consideration of pathogenesis of rheumatoid arthritis as collagen autoimmunity. *Zeitschrift für Immunitätsforschung Allergie und klinische Immunologie*, **139**, 219-227.
- Steinmann, B., Gitzelmann, R., Vogel, A., Grant, M. E., Harwood, R., and Sear, C. H. J. (1975). Ehlers-Danlos syndrome in two siblings with deficient lysyl hydroxylase activity in cultured skin fibroblasts but only mild hydroxylysine deficit in skin. *Helvetica Paediatrica Acta*, **30**, 255-274.
- Stuart, J. M., Postlethwaite, A. E., and Kang, A. H. (1976). Evidence for cell-mediated immunity to collagen in progressive systemic sclerosis. *Journal of Laboratory and Clinical Medicine*, **88**, 601-607.
- Sussman, M., Lichtenstein, J. R., Nigra, T. P., Martin, G. R., and McKusick, V. A. (1974). Hydroxylysine-deficient skin collagen in a patient with a form of

- Ehlers-Danlos syndrome. *Journal of Bone and Joint Surgery*, American Volume, **56-A**, 1228-1234.
- Sykes, B., Francis, M. J. O., and Smith, R. (1977). Altered relation of two collagen types in osteogenesis imperfecta. *New England Journal of Medicine*, **296**, 1200-1203.
- Tane, N., Takeda, T., Shioji, T., Ohyama, H., Itoh, H., and Matsumura, Y. (1976). Effect of vitamin B6 deficiency on collagen metabolism in rats. *Journal of Nutritional Science and Vitaminology*, **22**, 105-114.
- Tanzer, M. L. (1973). Cross-linking of collagen. *Science*, **180**, 561-566.
- Trelstad, R. L. (1974). Human aorta collagens: evidence for three distinct species. *Biochemical and Biophysical Research Communications*, **57**, 717-725.
- Trelstad, R. L., Rubin, D., and Gross, J. (1977). Osteogenesis imperfecta congenita. Evidence for a generalized molecular disorder of collagen. *Laboratory Investigation*, **36**, 501-508.
- Uhlendorf, B. W., and Mudd, S. H. (1968). Cystathionine synthetase in tissue culture derived from human skin; enzyme defect in homocysteinuria. *Science*, **160**, 1007-1009.
- Uitto, J. (1971). Collagen biosynthesis in human skin. A review with emphasis on scleroderma. *Annals of Clinical Research*, **3**, 250-258.
- Uitto, J., Halme, J., Hannuksela, M., Peltokollia, P., and Kivirikko, K. I. (1969). Protocollagen proline hydroxylase activity in the skin of normal human subjects and of patients with scleroderma. *Scandinavian Journal of Clinical Laboratory Investigation*, **23**, 241-247.
- Uitto, J., Hannuksela, M., and Rasmussen, O. G. (1970a). Protocollagen proline hydroxylase activity in scleroderma and other connective tissue disorders. *Annals of Clinical Research*, **2**, 235-239.
- Uitto, J., Helin, P., Rasmussen, O., and Lorenzen, I. (1970b). Skin collagen in patients with scleroderma: biosynthesis and maturation *in vitro* and the effect of D-penicillamine. *Annals of Clinical Research*, **2**, 228-234.
- Uitto, J., and Lichtenstein, J. R. (1976). Defects in the biochemistry of collagen in diseases of connective tissue. *Journal of Investigative Dermatology*, **66**, 56-79.
- Walshe, J. M. (1977). Brief observations on the management of Wilson's disease. *Proceedings of the Royal Society of Medicine*, **70**, Supplement 3, 1-3.
- Ziff, M. (1974). Autoimmune processes in rheumatoid arthritis. In *Progress in Immunology II*, vol. 5, edited by L. J. Brent and E. J. Holborow, pp. 37-46. North-Holland Publishing, Amsterdam.