Molecular abnormalities of collagen

F. M. POPE AND A. C. NICHOLLS

From the Clinical Research Centre, Northwick Park Hospital, Harrow, Middlesex

Others have described the structure and synthesis of collagen and the techniques of collagen analysis (D. S. Jackson (Jackson, 1978) at page 44 and A. J. Bailey (Bailey, 1978) at page 49. Collectively these techniques have already increased the molecular understanding of both the inherited and acquired collagen defects. We shall discuss here the question of heredity and collagen abnormalities.

Inherited diseases of collagen

Many inherited abnormalities of collagen are likely and may be listed under the following headings as proved, probable, or possible. Proved Ehlers-Danlos syndrome (EDS) (types III, IV, V, VI, VII); osteogenesis imperfecta (broad-boned type); thanatophoric dwarfism; dermatosparaxis (animal equivalent of EDS VII); blotchy mice (animal equivalent of EDS V).

Probable Marfan syndrome; pseudoxanthoma elasticum (some types); epidermolysis bullosa (some types).

Possible Progeria; Werner's syndrome; neurofibromatosis; mongolism.

Known defects have affected either collagen cross-linking or production of types I and III. Basement membrane defects await identification. Molecular abnormalities of collagen have been convincingly shown in several types of Ehlers-Danlos syndrome.

EHLERS-DANLOS SYNDROME

This disorder has proved to be as heterogeneous biochemically as it is clinically (Table). Originally considered one disease (Ehlers, 1901; Johnson and Falls, 1949), two (McKusick, 1959), three (Barabas, 1967), five (Barabas, 1967), and then seven abnormalities (Lichtenstein et al., 1974; Pinnell et al., 1972) have been recognised. Even this classification is incomplete and we have recently studied a probable eighth clinical type (Pope, 1977). EDS type IV is thought to represent at least two disorders and EDS type III may also be heterogeneous. Eight types of procollagen peptidase deficiency (EDS VII) are theoretically possible. Anomalous steps in the collagen biosynthetic pathway have been identified. Failed synthesis of complete chains (Pope et al., 1975), cross-linking abnormalities (Pinnell et al., 1972; Lichtenstein et al., 1974; DiFerrante et al., 1975), mishydroxylation of hydroxylsine (Pinnell et al., 1972), procollagen cleavage impairment (Lichtenstein et al., 1974), and alteration in collagen ratios have all been described (Pope et al., 1975; Penttinen et al., 1975; Sykes et al., 1977). Doubtless other defects await identification.

EDS TYPE I (GRAVIS)

Hyperextensible skin, loose jointedness, facial scarring (typically under the chin), epicanthic folds, blue sclerae, paper-tissue scars, bruising, pectus excavatum, and soft, smooth, easily torn skin are the typical features of this disorder (Fig. 1) (Beighton, 1970). It is the classical form of the syndrome typical of the early descriptions of EDS. Aortic incompetence and aortic rupture are rare but mitral valve prolapse is not. Inheritance is an autosomal dominant but the precise biochemical

Table Classification of Ehlers-Danlos syndrome (EDS)

<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
<th>Classical features</th>
<th>Inheritance</th>
<th>Chemical defect</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>EDS gravis</td>
<td>Classical severe</td>
<td>Autosomal dominant</td>
<td>Type I collagen substitution?</td>
</tr>
<tr>
<td>II</td>
<td>EDS mitis</td>
<td>Classical mild</td>
<td>Autosomal dominant</td>
<td>Unknown</td>
</tr>
<tr>
<td>III</td>
<td>EDS benign</td>
<td>Joint hypermobility</td>
<td>Autosomal dominant</td>
<td>Unknown</td>
</tr>
<tr>
<td>IV</td>
<td>EDS hypermobile</td>
<td>Lethal arterial rupture</td>
<td>Autosomal recessive</td>
<td>Deficiency type III collagen ? two types</td>
</tr>
<tr>
<td>V</td>
<td>EDS linked</td>
<td>Mild classical</td>
<td>Sex linked</td>
<td>Lysyl oxidase deficient</td>
</tr>
<tr>
<td>VI</td>
<td>EDS (ocular form)</td>
<td>Retinal detachment, scoliosis</td>
<td>Autosomal recessive</td>
<td>Lysyl hydroxylase deficient</td>
</tr>
<tr>
<td>VII</td>
<td>EDS (arthrogryphosis multiplier)</td>
<td>Severe joint laxity, shortness</td>
<td>Autosomal recessive</td>
<td>Procollagen peptidase deficient</td>
</tr>
<tr>
<td>VIII</td>
<td>EDS gravis</td>
<td>Tall stature, foot deformity</td>
<td>Autosomal recessive</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
defect is unidentified. Collagen cross-linking may be faulty and increased solubility of tissue collagens has been provisionally described. Amino-acid substitutions are suspected (Steinmann et al., 1977).

**EDS TYPE II (MITIS)**
Scarring is less severe (Beighton, 1970), but bruising is especially common. Skin is hyperextensible and there is limited hypermobility. Inheritance is as an autosomal dominant. The basic defect is unidentified. Prognosis is good.

**EDS TYPE III**
Scarring is slight, but loose-jointedness is especially obvious (Beighton, 1970). The skin is usually soft and hyperextensible. Early osteoarthrosis and spontaneous dislocation of large joints is common. This group may be heterogeneous and is probably the commonest variety of EDS. Inheritance is as an autosomal dominant.

**EDS TYPE IV**
This especially interesting autosomal recessive defect (Fig. 2) has been given different names in various clinical specialties and regarded as distinct entities by each.

European dermatologists first described EDS IV as a premature ageing defect, acrogeria (Gottron, 1941; Basex and Dupré, 1955). The Textbook of Dermatology (1972) categorically separates it from EDS. Then Barabas (1967) and later Beighton (1970) recognised the heterogeneity of EDS and described the dangerous ecchymotic or arterial type which McKusick (1972) later called EDS IV. Beighton and McKusicks' illustrations are identical with those of the patients described as acrogeric. McKusick now acknowledges them to be one and the same (McKusick, 1975).

We have studied several affected patients and there has been a complete lack of type III collagen in tissues and cultured skin fibroblasts from most of
Molecular abnormalities of collagen

**EDS Type V (Lysyl Oxidase Deficiency)**
This is inherited as a sex-linked recessive trait (affected sons with carrier mothers). DiFerrante et al. (1975) showed that lysyl oxidase (involved in cross-linking) is deficient in the skin fibroblasts. The features are those of classical EDS but scarring is less severe. The disorder is benign in humans, but oxidase-deficient, blotchy mice often die from spontaneous aortic rupture (Rowe et al., 1974). Lysyl oxidase is probably polymorphic so that different tissue-specific mutations have widely disparate effects. Close analogies to the mouse model may therefore be expected in humans.

**EDS Type VI (Hydroxyllysine Deficiency)**
This variant was the first molecular defect of collagen to be identified. Retinal detachment, severe kyphoscoliosis (Fig. 4), and premature aortic rupture complicate the usual clinical signs of EDS (McKusick, 1972). Pinnell and colleagues (1972) detected hydroxyllysine deficiency in skin biopsies from such patients and Sussman et al. (1974) confirmed that a lysyl hydroxylase defect is responsible for the deficiency. The enzyme hydroxylates lysine residues in the collagen chain after its ribosomal translation. In the face of the defect, faulty or unusual cross-linking presumably results. Alternatively, adequate cross-links occur between the non-hydroxylated lysines and impaired glycosylation explains the various clinical signs.

**EDS Type VII (Procollagen Peptidase Deficiency)**
This variety has extreme joint laxity and short stature in addition to the usual features of EDS. Lichtenstein et al. (1974) showed that tissues from such patients contained only procollagen, the precursor of collagen (Fig. 5). This fault follows deficiency of procollagen peptidase, the enzyme responsible for removing the extension peptides and the conversion of procollagen to collagen. The effect of procollagen persistence is to interfere with fibril formation. In sheep and cattle this is lethal (Helle and Nes, 1972; Lenaers et al., 1971). In man the defect is relatively benign. Possibly different peptidases act upon amino and carboxy terminal extensions of each particular collagen type. Eight separate abnormalities are theoretically possible, one for every two extensions of the four collagen types. This may explain the difference between the human and animal disorders.

**EDS Type VIII**
We have recently investigated an EDS patient with additional tall stature and orthopaedic defects but otherwise indistinguishable from EDS I. Inheritance appears to be as an autosomal recessive characteris-
tic in this case. Work is in progress to identify the molecular abnormality.

OSTEOGENESIS IMPERFECTA (OI)
Autosomal dominant and recessive variants of this defect are likely and genetic heterogeneity probable for each (McKusick, 1972; Pope, 1976). The disease (Fig. 6) can vary from very mild, with few fractures, to extremely severe. In the latter slight damage from coughing or sudden movement are sufficient to fracture bones. Overlap with the Ehlers-Danlos syndrome occurs and we have recently seen a 21-year-old woman with pronounced joint laxity combined with otherwise typical, mild OI. Severe patterns of disease occur in which there is enlargement of the head, prominent blue sclerae, and dwarfism with soft silky skin and mild loose-jointedness. Radiographs show general osteoporosis with delicate, twisted bones. Parents of such individuals have the general features of inherited connective tissue diseases with bruising, hyperextensible skin, and loose-jointedness. If such individuals can be identified as heterozygotes a very real advance will be possible in the genetic counselling of this OI variant. Penttinen et al. (1975) advanced the molecular understanding of OI when they showed that cultured skin fibroblasts from the lethal, broad-boned variety synthesised reduced amounts of type I collagen. The distribution of collagens in tissues was not fully investigated. Inheritance of this lethal variant is probably as an autosomal recessive characteristic, although autosomal dominance and genetic heterogeneity are possible.

Even more heterogeneity of OI is likely. Sykes et al. (1977) have suggested that the measurement of type I:III collagen ratios may help to identify such defects. Levin et al. (1978) claim that tooth pattern can be used to separate OI phenotypes and that dentinogenesis imperfecta breeds true within OI families. Different collagen patterns can be expected in such situations.

Collagen defects waiting identification

MARFAN SYNDROME
Tall stature, arachnodactyly, long extremities, dis-
Molecular abnormalities of collagen

Fig. 3 (a) Procollagen patterns of homozygous normal, abnormal, and heterozygotes as produced by DEAE chromatography of labelled cultured human fibroblasts (by permission of Journal of Medical Genetics). (b) Cyanogen bromide patterns of: from left, uncleaved collagen, EDS IV skin, normal skin, and α1 (III) CB8. (c) Cyanogen bromide patterns of EDS IV heterozygote skin of, from left, normal, twice normal, and thrice normal concentration. The fourth gel is α1 (III) CB8. (d) Cyanogen bromide patterns of: from left, normal, long-lived, and short-lived EDS IV. (e) CM cellulose chromatography of cyanogen bromide cleaved EDS IV whole skin (note little or no type III peptides). (f) Pattern of normal whole skin for comparison.
located lens, and vascular problems are typical of the Marfan syndrome (Fig. 7). Classical (McKusick, 1972), heavy (McKusick, 1976), and loose-jointed (Marfanoid) (Walker et al., 1969) variants of the Marfan syndrome have been described. The first two are inherited as autosomal dominant characteristics, the latter as an autosomal recessive. We have recently seen a family with the typical Marfan syndrome but probable autosomal recessive inheritance; Fried and Krakowsky (1977) have reported a similar family. Dislocated lenses are typical of the classical variant, less common in the heavy, and absent in the Marfanoid type. Aortic rupture complicates all, especially the heavy variety. Idiopathic autosomal dominant aortic rupture is probably another variant of the syndrome. Specific biochemical defects wait identification. Homozygous cystinuria was separated from the Marfan syndrome when the amino-aciduria of the former was identified. Two types, vitamin B6 responders and non-responders, occur (Kang et al., 1970).

PSEUDOXANTHOMA ELASTICUM (PXE)
Two autosomal dominant (Pope, 1974a) and two autosomal recessive types (Pope, 1974b) of PXE have been described. Some or all could be molecular abnormalities of collagen. Many of the clinical features of inherited connective tissue disorders are detected, including hyperextensible skin, high arched palate, loose joints, blue sclerae, and skeletal deformities. Alterations in collagen ratios and patterns await identification.

INHERITED ABNORMALITIES OF CARTILAGE
Numerous clinical defects having in common dwarfism and degeneration or dislocation of large joints with various associated bony abnormalities are well recognised. The disorders include achondroplasia, pseudoachondroplasia, hypochondroplasia, and thanatophoric dwarfism. There are reviews by McKusick (1972), Spranger and Langer (1970), and Maroteaux and Lamy (1965). Evidence is beginning to accumulate that cartilage collagen may be affected in some of these syndromes (Byers et al., 1978; Holbrook et al., 1977). Horton et al. (1977) have preliminary evidence that abnormal cartilage collagens are produced by cultured chondrocytes from thanatophoric dwarves and recognise three subtypes. Sewell et al. (1977), on the other hand, suggest that the glycosaminoglycans and not collagen are at fault.
Possible inherited defects of connective tissue

NEUROFIBROMATOSIS
Neurofibromatosis may be heterogeneous. Some patients have clinical features reminiscent of inherited connective tissue defects (with extensible joints and skin, blue sclerae, and skeletal abnormalities). The perineurium contains type I and III collagens and cultured neurofibroma cells produce type I collagen (Penttinen et al, 1977). Further studies are needed.

PROGERIA AND WERNER'S SYNDROME
Cutaneous atrophy, premature vascular disease, and osteoporosis suggest a possible inherited collagen defect (Fig. 8). Clinical resemblances between progeria and acrogeria indicate that collagen synthesis or maturation may be faulty in the former. The latter has a specific deficiency of type III collagen.

GENERAL DISORDERS OF COLLAGEN PRODUCTION
These disorders, which are more fully discussed on other pages, include cirrhosis of the liver, pulmonary fibrosis, atheroma, keloid scarring, and systemic sclerosis. All have in common excessive deposition of collagen. One or more could follow the overproduction of collagen caused by unidentified stimulating factors. Collagen stimulators could be distributed in serum or tissue or liberated by cells such as lymphocytes or macrophages (Allison et al., 1977). McGee has described a collagen stimulator contained by cirrhotic livers (McGee et al., 1973; J. O'D. McGee and A. Fallon (McGee and Fallon, 1978) at p. 150).

Other pertinent problems that may reflect defects in the inheritance of connective tissue include
susceptibility to varicose veins, herniae, and cerebral or other aneurysms. Although these characteristics are often graded, specific genetic susceptibility should be seriously considered in the explanation of some cases. The investigation of this inheritance is amenable to the analytical techniques outlined above.

References

Molecular abnormalities of collagen


Fig. 8 Facies of patient with progeria.


communication


