Changes in the collagen of synovial membrane in rheumatoid arthritis and effect of D-penicillamine

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(Received 16 May 1977; 25 November 1977)

Summary

1. Normal synovial membrane contains approximately equal proportions of two genetically distinct forms of collagen, types I and III. The proportion of these two collagens is unchanged in rheumatoid synovium but in addition a small amount of basement membrane collagen is present. Tissue culture of rheumatoid synovium confirms the synthesis of both type I and III collagens.

2. In young normal synovium both type I and type III collagens are stabilized by a reducible keto cross-link, which is replaced in adult tissue by an as yet unknown non-reducible cross-link. During the proliferation of the collagen in adult rheumatoid synovium a high proportion of the keto cross-link is present. This cross-link is not susceptible to cleavage by D-penicillamine, nor does the drug have any effect on the rate of synthesis in vitro. The mode of action of D-penicillamine in rheumatoid arthritis does not appear to involve a direct effect on the synovial membrane collagen.

Key words: collagen, D-penicillamine, rheumatoid arthritis, synovial membrane.

Introduction

Rheumatoid arthritis is a common disease of unknown aetiology involving connective tissue, the synovial membrane (normally a single or double layer of cells with an underlying weave of loose connective tissue) becoming swollen and congested with the formation of villous processes (Gardner, 1972). The cartilage is destroyed at the interface with the advancing extension of the membrane, the synovial pannus. Although gross thickening of the synovium is one of the histological features of the disease, little is known about the collagen structure in normal synovium, nor of any changes in rheumatoid disease.

Steven (1965, 1966) reported that rheumatoid tissue was more susceptible to pronase as compared with control material, and suggested that there was a deficiency in intermolecular cross-linking. Similarly, Weiss and co-workers (Weiss, Leibovich, Hunter & Cawley, 1974) found that rheumatoid synovium was particularly susceptible to pepsin digestion and contained an abnormal polymeric collagen fraction. These workers have found small amounts of type III collagen in rheumatoid synovium, this type of collagen being absent from normal synovium (Weiss, Shuttleworth, Brown, Sedowfia, Baildam & Hunter, 1975). On the other hand, Eyre & Muir (1975) have reported roughly equal proportions of type III and type I collagen in both normal and rheumatoid synovium.

The inflammatory proliferation of synovial collagen may be an essential part of the disease process. Previous studies on inflammatory processes have shown that the collagen synthesized in response to an acute inflammatory stimulus in the dermis contained a higher proportion of type III collagen than normal skin (Bailey, Sims, Lelous & Bazin, 1975b; Bailey, Bazin, Sims, Lelous,
Nicoletis & Delaunay, 1975a). Four genetically distinct species of collagen have now been identified in mammalian tissues (Miller & Matukas, 1974). It was therefore considered of some importance to elucidate the nature of the fibrous and membranous collagens in normal and rheumatoid synovium.

D-Penicillamine has been used with some success in the treatment of rheumatoid arthritis since 1964 (Jaffe, 1964), although until 1973 no controlled trial of its use had been undertaken (Multicentre Trial Group, 1973). This trial confirmed that D-penicillamine was comparable with gold in the treatment of rheumatoid disease. Despite its undoubted clinical value the mechanism of the action remains obscure; possibilities include the dissociation or inhibition of formation of rheumatoid factor, lowering of serum copper or an anti-viral or anti-bacterial action. D-Penicillamine cleaves the intermolecular cross-links stabilizing newly formed dermal collagen, making the disrupted collagen fibres more susceptible to degradation (Bailey & Lister, 1968; Nimni, 1968); the drug also inhibits collagen synthesis (Herbert, Jayson, Lindberg & Bailey, 1974). For these reasons, D-penicillamine has been used in the treatment of patients with scleroderma in whom there is proliferation of dermal collagen (Herbert et al., 1974). A similar mechanism might apply to the synovial proliferation in rheumatoid disease.

We now present evidence that normal synovial collagen comprises both type I and type III collagen and possesses stable reducible cross-links in young tissue and non-reducible links in old tissue. In active rheumatoid synovium, despite active synthesis of collagen, the proportions of type I and type III collagens are unchanged, but there was also a significant proportion of type IV collagen. All the collagens are stabilized by the same reducible keto cross-link, which is not cleaved by D-penicillamine. The rate of collagen synthesis during the tissue culture of rheumatoid synovium was unaffected by D-penicillamine, suggesting that D-penicillamine has little, if any, direct effect on the synovial collagen in rheumatoid arthritis.

Materials and methods

Synovium

Synovium was obtained at operation from the knees of ten rheumatoid patients, aged 50–70 years. The patients gave informed consent to surgery, which was performed for the treatment of their condition. Only samples showing histological evidence of villus formation, with chronic inflammatory changes, were studied. Synovium was dissected carefully from the infrapatellar fat pads of knees obtained at necropsy of patients with no evidence of rheumatic disease.

The synovium was dissected clean of adhering fat and cartilage, and defatted by extraction with methanol/chloroform (1:3, v/v).

Total collagen and elastin content

The total collagen content of the washed synovium was determined by estimation of hydroxyproline in a freeze-dried and hydrolysed (6 mol/l HCl) sample with the automated Technicon system described by Grant (1964). The elastin content was estimated from the amount of insoluble residue after extraction with NaOH (200 mmol/l) at 90°C for 1 h. The desmosine content of an acid hydrolysate (Partridge, 1973) of the residue was determined with the Jeol 6 AH-DK amino acid analyser to demonstrate the purity of residue as elastin. The solubility of the collagen was assessed both by extraction of the samples with acetic acid (500 mmol/l) for 2 days at 4°C and by heating with water at 80°C for 2 h. The dry weight of the insoluble residue was determined, and the amount of collagen solubilized confirmed by hydroxyproline estimations on both the supernatants and the residue.

Identification of reducible cross-links

Rheumatoid synovium from subjects aged 50–70 years, and normal synovium covering the age range 0–80 years, was shredded and washed with copious amounts of NaCl solution (150 mmol/l; saline) at pH 7.4. The wet weight of each specimen was approximately 100 mg. The samples were reduced simultaneously with equal volumes of a solution of tritiated potassium borohydride (300 µCi/mg) dissolved in buffered saline (4°C) to give a collagen:borohydride ratio of 30:1 for each sample. After 1 h the reaction was terminated by the addition of acetic acid to pH 4-0. The reduced fibres were then dialysed overnight against distilled water, freeze-dried and weighed. The fibres were then hydrolysed by refluxing for 24 h with redistilled constant-boiling HCl (6 mol/l), which was then removed by evaporation in vacuo at 40°C.

The amino acids and radioactive cross-linking components were separated with a Technicon Autanalyzer adapted for use with volatile buffers (Robins, Shimokomaki & Bailey, 1973). The
compounds were eluted on a pH gradient formed by running pyridine/formate buffer (1 mol/l; pH 5-0) into a mixing flask containing 350 ml of pyridine/formate (100 mmol/l; pH 2-9). Fractions (5 ml) were collected and a portion (0-2 ml) of each fraction was dissolved in 3 ml of Bray’s (1960) solution and the tritium radioactivity determined in a liquid scintillation counter (Packard 3375). The identity of the radioactive cross-links was confirmed by comparison with authentic standards on an extended (60 cm) basic column of the Beckman amino acid analyser (Bailey et al., 1970).

Separation of genetic types of collagen

Synovium was obtained from rheumatoid patients and from normal subjects aged 50–70 years. The synovial collagen was solubilized by limited pepsin (EC 3.4.4.1) digestion, and fractional precipitation of the digest was carried out to isolate the polymorphic forms of collagen present in the tissue (Chung & Miller, 1974). Briefly, the tissues were extracted with NaCl solution (1 mol/l) and with acetic acid (500 mmol/l) and then insoluble residue was incubated in acetic acid (500 mmol/l) with pepsin at a substrate : enzyme ratio of 10:1 for 24 h at 4°C and 18°C. After precipitation of collagen types I and III with NaCl solution (900 mmol/l) the precipitate was either dialysed against acetic acid (500 mmol/l) and freeze-dried, or redissolved in NaCl solution (1 mol/l)/Tris (50 mmol/l), pH 7.5. The resolubilized collagen was then subjected to fractional precipitation by stepwise increase in the NaCl concentration to obtain precipitates at 1.5 mol/l (type III) and 2.5 mol/l (type I). The NaCl (900 mmol/l) supernatant was dialysed extensively against Na2HPO4 (200 mmol/l) to precipitate any basement membrane collagen (Chung, Rhodes & Miller, 1976). The fractionated precipitates obtained were dialysed against acetic acid (500 mmol/l) and freeze-dried.

Sodium dodecyl sulphate-polyacrylamide-gel electrophoresis

Samples of the total pepsin digests and of the type I and type III collagen precipitates were each redissolved, denatured in sodium dodecyl sulphate (20 g/l) at 38°C, with and without mercaptoethanol, to cleave the intramolecular disulphide bonds of type III collagen. The incubated mixture was then analysed for α-chain composition by polyacrylamide-gel electrophoresis in Tris/borate buffer by the flat-bed technique described in detail by Sykes & Bailey (1971). In view of the similar mobilities of human α-type III and α-type I the electrophoresis was interrupted after 15 min and mercaptoethanol added to convert the γ-type III to α-type III chain, as suggested by Sykes, Puddle, Francis & Smith (1976).

Carboxymethyl cellulose chromatography

The collagen solubilized by pepsin treatment was dialysed against sodium acetate solution (400 mmol/l), heated to 50°C for 10 min and then applied to a CM-cellulose column (2-5 cm × 20 cm) equilibrated with sodium acetate (40 mol/l)/urea (2 mol/l) and eluted with a linear NaCl gradient of 0–100 mmol/l over a total volume of 400 ml at 45°C (Bellamy & Bornstein, 1971).

Cyanogen bromide cleavage

Samples of the washed synovial membranes were extracted with guanidine hydrochloride (4 mol/l) at 4°C and the insoluble collagenous residue was freeze-dried and digested with an equal weight of cyanogen bromide in 70% formic acid under nitrogen at 25°C for 4 h. The digestes were centrifuged, diluted with deionized water, concentrated by rotary evaporation and freeze-dried. Analysis of the cyanogen bromide peptides was carried out by sodium dodecyl sulphate-polyacrylamide disc-gel electrophoresis with the phosphate buffer system (Furthmayer & Timpl, 1971), and by chromatography on CM-cellulose. Columns of CM-cellulose (Whatman CM 52), 0-9 cm × 10 cm or 1·8 cm × 12 cm, were equilibrated with sodium formate (200 mmol/l)/NaCl solution (200 mmol/l) at pH 3.6; they were eluted with a linear gradient of NaCl (0–17 mmol/l) superimposed on the starting buffer over total volumes of 500 or 1000 ml respectively at flow rates of 60 ml/h or 110 ml/h respectively (A. C. Nicholls, unpublished work). Column effluent was monitored at 230 nm with a Cecil spectrophotometer.

Synthesis of collagen by synoviurn in vitro

Immediately after removal of the rheumatoid synovium from the patient at surgery the tissue was shredded and cultured in suspension with Dulbecco–Vogt medium. The following additives were included: ferrous sulphate (0·1 μmol/l); vitamin C (0·3 mmol/l); β-aminopropionitrile (50 μg/ml of medium); [3H]proline (100 μCi); penicillin (50 μg/ml); and streptomycin (50 μg/ml). The
tissue was incubated in a water bath at 38°C in a sterile laminar flow cabinet for 48 h. The contents of the flask were then homogenized, adjusted to pH 3.1 with acetic acid and treated with pepsin (substrate : enzyme ratio 10 : 1) for 6 h at 18°C. The solution was then raised to pH 8.0 to inactivate the pepsin, centrifuged, dialysed against acetic acid (500 mmol/l) and then against acetate (400 mmol/l)/urea (2 mol/l), pH 4.8. The solution was then applied to the CM-cellulose column for separation of the collagen chains.

Rates of collagen synthesis in vitro

The synovium was divided into two portions, shredded, placed on sterile stainless-steel grids and suspended over a central well containing medium A or B (see below) in sterile plastic organ culture dishes (Herbert et al., 1974). The samples were maintained at 37°C in a modified MacIntosh–Fildes jar in an atmosphere of O₂/CO₂/N₂ (20 : 5 : 75, by vol.) at 37°C for 24 h. After 24 h the tissue and medium were dialysed separately against frequent changes of distilled water at 4°C for 4–5 days, freeze-dried and hydrolysed in HCl (6 mol/l) at 110°C for 24 h. The HCl was removed by evaporation in vacuo, and the residue dissolved in 1 ml of water. The hydroxyproline content of a portion (0.5 ml) was estimated by the automated procedure of Grant (1964) as modified by Pennock, Moore & Hoyle (1970); a mixture of non-radioactive proline and hydroxyproline was added to another portion (0.5 ml) to act as markers and the mixture separated on the Amino Acid Analyzer with pyridine/formate buffers. The fractions were counted for 3H radioactivity as described above, and the amounts of [3H]hydroxyproline in the three different systems compared. A proline-free medium, Trowell T8 (Flow Laboratories, Ayrshire, Scotland), was chosen, to which the following were added: medium A: 10% foetal calf serum, ascorbic acid (0.3 mmol/l), ferrous sulphate (0.1 µmol/l), penicillin (100 units/ml), streptomycin (100 µg/ml), [3H]proline (50 µCi/ml; The Radiochemical Centre, Amersham, Bucks., U.K.); medium B: all above additives plus D-penicillamine hydrochloride (50 µg/ml) (Dista Products).

Results

Total collagen and elastin content

The collagen content of the synovium varied somewhat but on average the tissue contained 50% collagen. Only a small proportion of elastin, less than 1% (w/w) was present. Both the normal and rheumatoid synovial collagen were found to be almost insoluble in non-denaturing solvents, only 1% and 5% (w/w) respectively being soluble in dilute acetic acid. Even under denaturing conditions in hot water only 0.8% and 3.6% could be dissolved from the normal and rheumatoid synovium respectively.

Genetic types of collagen

Pepsin digestion. The normal synovium was found to be resistant to pepsin digestion at 4°C, only 5% of the collagen being solubilized. Incubation at 18°C increased the yield to 40%. In view of the small amounts of synovium available from normal human knees the solubilized collagen was not fractionally precipitated but analysed directly for the presence of type III collagen by CM-cellulose chromatography and sodium dodecyl sulphate-polyacrylamide-gel electrophoresis (Fig. 1).

In contrast to the control synovium, over 60% of the rheumatoid synovial collagen was solubilized by pepsin digestion at 4°C. Fractional precipitation of the solubilized material yielded a precipitate at 1.5 mol of NaCl/l and a second at 2.5 mol of NaCl/l. Confirmation of the identity of the two precipitates as type III and type I collagen was obtained by CM-cellulose chromatography and sodium dodecyl sulphate-polyacrylamide-gel electrophoresis. The type III eluted as a γ-component on the CM-cellulose and sodium dodecyl sulphate gels but after incubation with mercaptoethanol to cleave the intramolecular disulphide bonds the type III had the mobility of α-chains (Fig. 1). These analyses demonstrate the presence of both type I and type III collagen in synovial membrane.

The ratio of the type III to type I collagen based on the weights of the precipitates from four patients varied somewhat, but on average was about 3:2. Assuming the solubilized material to be representative of the whole tissue, then rheumatoid synovium contains about 60% of type III collagen.

Two additional bands were present in the total pepsin digest of rheumatoid synovium (Fig. 1b). Their mobilities were identical with those of basement membrane chains (α₅ and α₆) isolated from placental membranes (Duance, Restall, Beard, Bourne & Bailey, 1977), and similar to those reported by Burgeson, El Adli, Kaitila & Hollister (1976). Analysis of the Na₂HPO₄ (20 mmol/l) precipitate revealed only the α₅ and α₆ bands on the
Fig. 1. Sodium dodecyl sulphate-polyacrylamide-gel electrophoresis patterns of total pepsin digests of normal and rheumatoid synovium. (a) Normal synovium: (i) without mercaptoethanol, (ii) with mercaptoethanol added 10 min after start of electrophoresis to resolve αIII, (iii) with mercaptoethanol. (b) Rheumatoid synovium: (i) without mercaptoethanol, (ii) with mercaptoethanol added 10 min after start to resolve αIII, (iii) with mercaptoethanol, (iv) placenta basement membrane, (v) placenta basement membrane with mercaptoethanol. Note that unless interrupted electrophoresis was carried out human αI and αIII chains do not resolve.
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**FIG. 2.** Comparison of the densitometric traces of disc electrophoretograms of cyanogen bromide (CNBr)-derived peptides in sodium dodecyl sulphate-polyacrylamide gels.

(a) Total CNBr peptide digest of normal synovial membrane. (b) Total CNBr peptide digest of rheumatoid synovial membrane. Gels were stained with Coomassie blue and scanned with a Joyce-Loebl Chromoscan. The formulae represent CNBr peptides of $\alpha_1$ and $\alpha_2$ chains of type I collagen (prefixed $\alpha_1$ and $\alpha_2$) and chains of type III collagen (prefixed $\alpha_1$, (III)) (Traub & Piez, 1971).

polyacrylamide gels and the amino acid analysis of this material was characteristic of basement membrane collagen, including 3-hydroxyproline and a high hydroxylysine:lysine ratio. The analyses were similar to those obtained by Burgeson *et al.* (1976) and Chung *et al.* (1976), but insufficient material was available to analyse the $\alpha_1$ and $\alpha_2$ chains separately.

**Cyanogen bromide peptides.** Confirmation of the relative proportions of type I and III collagens was achieved by digestion of the total collagen with cyanogen bromide. Comparison of the sodium dodecyl sulphate-polyacrylamide gels from normal and rheumatoid synovium (Fig. 2) revealed almost identical patterns, demonstrating little change in the proportions of type I and type III. Comparison with gel patterns from pure type I and pure type III indicated a 1 : 1 ratio of the two types in synovial collagen. Other attempts to characterize the cyanogen bromide peptides by CM-cellulose chromatography and the phosphocellulose technique employed by Eyre & Muir (1976) were unsuccessful owing to interference from other matrix components, which could not be removed by normal methods. The poorly resolved CM-cellulose patterns of normal synovium are shown in Fig. 3, and compared with the more typical resolution of peptides obtained from human uterine fibromyoma (Fig. 3). Similar poorly resolved CM-cellulose patterns for other tissues, e.g. spleen and placenta, have been reported (Epstein & Munderloh, 1975).

**Reducible cross-links in normal synovia and changes with age**

Borohydride reduction of young normal synovium revealed the presence of two major reduced
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they were barely detectable. There was an increase in the two minor components $A_1$ and $A_2$ with increasing age (Fig. 4); these compounds are derived from condensation products of lysine and hexoses and are not cross-links (Robins & Bailey, 1972).

**Reducible cross-links in rheumatoid synovium**

Analysis of adult patients with rheumatoid arthritis showed that dihydroxylysinonorleucine was the major reducible component, indicating a reappearance of the major cross-link present in young growing synovial tissue. The high proportion of this cross-link clearly indicates that the rate of collagen synthesis is increased in rheumatoid synovium. Borohydride reductions of a series of different areas of synovium from three rheumatoid patients revealed the same pattern.

In addition to untreated rheumatoid synovium we have also carried out some preliminary analyses of tissues from patients on long-term $\delta$-penicillamine therapy. Synovium was obtained from a 62 year old lady with a 4 year history of seropositive rheumatoid disease with vasculitis and peripheral neuropathy. Two years before synovectomy, treatment with $\delta$-penicillamine base (1250 mg/day) was commenced and the dose continued until operation. In this patient, an identical reducible cross-link pattern to that of the other rheumatoid patients was obtained, indicating that $\delta$-penicillamine had minimal, if any, effect on the proliferation of collagen in rheumatoid synovium. Similar results were obtained from four other patients who had received $\delta$-penicillamine for a period of between 7 and 16 months. (We are grateful to Dr Hilary Hill, Stoke Mandeville Hospital, for supplying the synovium from these cases.)

**Synthesis of synovial collagen in vitro**

Analysis of the total pepsin digest on CM-cellulose (Fig. 5) shows the peak eluting unretarded to contain proline-labelled non-collagenous proteins. The second peak elutes in the position of $\alpha_1$-type I and the third in the position of $\alpha$-type III. The identity of peak 2 as $\alpha_1$-type I was confirmed by sodium dodecyl sulphate-polyacrylamide-gel electrophoresis. The third peak when analysed by electrophoresis revealed the presence of $\alpha$ components derived from type III and $\alpha_2$ derived from type I. From the relative proportions of the type I and type III collagen it is clear that the synovium...
Fig. 5. Elution patterns \(^{3}H\)-proline-labelled collagen synthesized in vitro and separated on CM-cellulose columns. (a) Total pepsin digests of tissue and medium after incubation with \(^{3}H\)-proline. (b) Type I and type III collagens isolated from pepsin digest by salt fractionation. ---, 1.5 mol of NaCl/l precipitate (type III); ----, 2.5 mol of NaCl/l precipitate (type I).

was synthesizing both types in significant quantities.

Rates of total collagen synthesis in vitro: effect of D-penicillamine

There was marked variation in the rates of collagen synthesis in different tissue cultures; however, within one experiment, no significant alteration of the rate of synthesis, as expressed by amount of radioactive hydroxyproline/total hydroxyproline, could be detected on addition of D-penicillamine to the medium.

Discussion

The loose connective tissue of synovial membrane supporting the synovial cells is fibrous, highly vascularized and merges with the cellular layer without a true basement membrane. The fibres enmeshing the synovial cells have the properties of reticulin, whereas the underlying supporting layer is composed of a dense meshwork of coarse collagen fibres. The type III collagen may be associated with the reticulin, or the blood vessels or the coarse supporting fibres. It is unlikely that the high proportion of type III collagen in normal synovium is derived from blood vessels as no basement membrane collagen could be detected.

In pepsin digests of rheumatoid synovium, however, two additional bands were detectable, resembling the \(\alpha_\alpha\) and \(\alpha_\beta\) bands obtained from foetal amnion and chorion (Burgeson et al., 1976) and from placental membranes (Duance et al., 1977). Similar collagenous components have been obtained from skin, liver and large blood vessels by Chung et al. (1976), who conclude that these components are derived from basement membrane (type IV) collagen. These latter chemical analyses are supported by the specific staining of basement membranes by antibodies raised against the \(\alpha_\alpha\) and \(\alpha_\beta\) chains (Duance et al., 1977). The type IV collagen detected in rheumatoid synovium may reflect the increased vascularity of the tissue, although localization by immunofluorescence is clearly required.

In rheumatoid synovium there appears to be little change in the relative proportions of collagen types I and III and this was supported by tissue culture in vitro, when approximately equal proportions of both types of collagen were synthesized. During proliferation the synovium thickens and forms extensive outgrowths, i.e. pannus, into the joints. Studies on experimental granulation tissue have demonstrated that there is usually an increase in the proportion of type III collagen synthesized (Bailey et al., 1975b) and this may also occur in the pannus.

In contrast Weiss et al. (1975) have reported that normal synovium contains only type I collagen; inflamed synovial tissues, however, contain a small proportion of type III collagen, the amount of which reflects the degree of inflammation. On the other hand, our findings support those of Eyre & Muir (1975), who, using more accurate quantification by cyanogen bromide peptides, demonstrated that both normal and rheumatoid synovium contain type I and III collagens in roughly equal proportions.

The stability of the collagen fibres of the synovium depends on the presence of specific lysine-derived intermolecular cross-links between the tropocollagen molecules making up the fibre. The major cross-link detected on borohydride reduction of young normal synovium is dihydroxy-
lysinonorleucine derived via an Amadori rearrange-
ment of the Schiff-base form, thereby producing a
stable cross-link in vivo. A similar rearrangement
occurs with a proportion of hydroxylysinonor-
leucine, both cross-links thereby being very stable.

The borohydride-reducible cross-links detectable
in young collagenous tissues are intermediates and
are modified to some as yet unknown structure
during tissue maturation. In dermal collagen these
reducible cross-links are barely detectable after
maturity, when the synthesis of collagen is very
low, the turnover time being extremely long
(Herbert et al., 1974; Bailey & Robins, 1973).
Similarly, the reducible cross-links present in
synovial collagen disappear, and cannot be detec-
ted in adult tissue. However, in actively proliferat-
ing rheumatoid synovium dihydroxylysinonor-
leucine, both cross-links thereby being very stable.

Similarly, the reducible cross-links present in
synovial collagen disappear, and cannot be detec-
ted in adult tissue. However, in actively proliferat-
ing rheumatoid synovium dihydroxylysinonor-
leucine is again the major reducible cross-link. The
presence of this stable cross-link is important as it
is not susceptible to cleavage by D-penicillamine,
unlike the labile Schiff-base cross-link present in
dermal collagen.

The marked difference in the ability of pepsin to
solubilize normal and rheumatoid synovial collagen
cannot be attributed to a difference in the
molecular type of collagen present, but may be
accounted for by differing extents of maturation of
the fibres. During maturation of collagen there is a
change in the chemistry of the cross-link and inter-
action with other matrix components; the collagen
becomes less soluble and is less susceptible to
proteolytic enzymes. Rheumatoid synovium con-
tains a higher proportion of newly synthesized
 collagen than normal synovium and these immu-
nature collagen fibres are more susceptible to pro-
 teolytic enzymes such as pepsin.

The value of D-penicillamine in rheumatoid
disease does not depend upon a direct effect on
synovial collagen. Rheumatoid synovial collagen
possesses a stable reducible cross-link which is
resistant to cleavage by D-penicillamine. The drug
does not inhibit collagen synthesis by rheumatoid
synovium in vitro. Furthermore, there is no
difference in the cross-link pattern of patients after
long-term D-penicillamine therapy.

We conclude that both normal and rheumatoid
synovial membranes contain type I and III collagen
in roughly equal proportions; in addition rheumatoid synovium contains a small amount of
basement membrane (type IV) collagen. Young
normal synovial collagen is stabilized by a
reducible keto cross-link, which is replaced in the
adult by an as yet unknown cross-link. In
rheumatoid synovium the reducible cross-link is
again detectable. Finally, we conclude that D-
penicillamine does not act directly on collagen
proliferation in rheumatoid synovial membrane.

Acknowledgments

We thank the Consultant Surgeons at Winford
Orthopaedic Hospital for providing specimens, Mr
Douglas Wakefield for providing post-mortem
specimens, and Mrs A. Swan and Mrs M. Gibson
for expert technical assistance. We are also
indebted to Dr D. Eyre for helpful advice on
analysis of cyanogen bromide peptide patterns and
to Dr R. Pentinnen on tissue culture technique.
We are grateful to the Hadwen Trust and the
Nuffield Foundation for financial support.

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