Joint capsule collagen

Analysis by the study of intra-articular pressure during joint distension

Measurements in the knees of control subjects and patients with rheumatoid arthritis and Ehlers-Danlos syndrome

G. STEER,* M. I. V. JAYSON,*† A. ST. J. DIXON,* AND P. BEIGHTON†

From the *Royal National Hospital for Rheumatic Diseases, Bath, the †Department of Medicine, University of Bristol, and the ‡University of the Witwatersrand, Johannesburg

The joint capsule contains both elastic fibres and collagen tissue (Gardner, 1965). In the normal joint the collagen is relaxed and the stiffness of the tissues is provided by the elastic tissues. When the capsule is stretched the collagen fibres become reorientated, start to bear some of the strain, and produce a considerable increase in the capsular stiffness. By a computerized analysis of the pressures measured during progressive joint distension it has been possible to obtain indices of the joint capsule collagen content and structure.

Subjects studied

Measurements were only performed on volunteers who agreed to these studies after detailed explanations. Adequate data was obtained on fourteen control knees, twelve rheumatoid (RA) knees, and ten knees from patients with Ehlers-Danlos syndrome (EDS) (Table).

Table Subjects studied

Series	No. of cases	Age (yrs)		Sex	
		Mean	Range	Male	Female
CONTROL	14	34·6	20-65	11	3
RA	12	54·7	34-72	5	7
EDS	10	33·3	14-60	6	6

It was unfortunately not possible to match the subjects for age and sex. In nine control knees and five RA knees the data was that previously used by Jayson and Dixon (1970). The ten EDS patients have all been described by Beighton (1970). In one control and one RA knee, the studies were repeated and in both cases the later results corresponded with the earlier measurements.

Method

The intra-articular pressure was recorded at 5-ml. increments of a simulated effusion of 4·3 per cent. dextrose 0·18 per cent. sodium chloride as described by Jayson and Dixon (1970). Distension was continued to the limit of tolerance, which was usually at about 100 ml., although many subjects, and particularly the rheumatoid patients, could accept only much smaller volumes.

Principles of analysis of pressure/volume curves

Distension of a joint by an effusion leads to increase in pressure within the synovial space. The joint elastance may be defined as the pressure change per unit change in effusion volume or dP/dV . The magnitude of the elastance depends principally on the properties of the synovium and joint capsule, and to a lesser extent on other surrounding structures and on the deformability of articular cartilage and bone. Most solids obey Hooke's law when stretched; this states that, within limits, elongation is proportional to the stretching force. For a distended sphere, Laplace's law states that the tension within the wall is related to both the radius and the distending pressure. As the knee cavity is irregular, it is not possible to measure the joint radius, but it follows that the tension of the fibres within the joint capsule is related both to the intra-articular pressure and the effusion volume.

The top diagram in Fig. 1 (overleaf) shows the pressures within the knee joint on progressive joint distension. At low volumes the pressure increases slowly, *i.e.* the elastance is low. Above a certain volume it increases more rapidly. This indicates that the capsule does not consist of fibres of a single kind acting in a uniform manner, but that there is a change in the properties of the capsule with joint distension. This is compatible with hypothesis that when the joint is empty the collagen fibres are lax and coiled and the joint is governed by

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Address for reprints: Dr. M. I. V. Jayson, Royal National Hospital for Rheumatic Diseases, Bath.

FIG. 1 *Principles of analysis of pressure-volume curves.*

elastic tissue. Stretching the capsule reorientates the collagen fibres but requires little force, as elastic tissue presents only little resistance. When the slack in the collagen fibres is taken up, they reach their unstretched lengths and start to bear a share of the tension. As collagen is much stiffer than elastic tissue the pressure then increases much more rapidly for unit volume change. The effusion volume at which this occurs is the volume at which most of the fibres reach their unstretched lengths. The unstretched collagen fibre length in this respect is a hypothetical concept which reflects not only the lengths of the fibres, but also the sum of complexes of cross-linked fibres when reorientated by joint distension. The joint elastance is the slope of the pressure/volume graph and is obtained by differentiating this line as shown in the middle diagram in Fig. 1. The vertical axis now represents the elastance (pressure change for unit change in volume or dP/dV) related to serial increases of fluid in the joint. At small volumes the elastance is low and constant, and at high volumes the elastance is high and constant. At the transitional zone between the two, there is the increase in elastance.

The unstretched lengths of the collagen fibres are best arrived at by further differentiating the equation (bottom diagram, Fig. 1) to show the rate at which the elastance is changing (d^2P/dV^2) at increasing volumes of effusion. At small volumes and at large volumes the elastance does not alter but, in the transitional zone, the rate at which the elastance changes increases to a peak and then falls again to the baseline.

The volume at which the second differential curve reaches its peak is therefore the mean volume at which the collagen fibres reach their unstretched length. The height at this point is an index of the number of collagen fibres of that length, because each increment above the baseline represents a number of collagen fibres reaching their unstretched length and first contributing towards an alteration in the overall joint elastance. Similarly, the shape of the curve represents the frequency distribution of collagen fibre length. The total number of fibres is the sum of the number of fibres of each length and is related to the area under the curve (single and double hatching).

In practice, in many of the studies, insufficient joint distension was obtained so that the second differential curves failed to return to the baseline. In those in which

FIG. 2 *Computer drawn plots from a control knee.*

the curves were complete they were reasonably symmetrical. Analyses were therefore restricted to the first half area shown by double hatching, which gave an indication of half the total number of collagen fibres.

Computer analysis of pressure/volume data

A simple regression equation of the form $P = a + bV$, where P represents the intra-articular pressure and V the volume of simulated effusion and a and b are constants, cannot describe accurately curves of these shapes. A complex polynomial regression of the form $P = a + bV + cV^2 + dV^3 + eV^4 + fV^5$ was necessary to represent the recorded results sufficiently accurately. Each set of observations was therefore fitted to polynomials of the fifth order, the calculations begin performed on the University of Bath computer.

The resultant equations, together with the data, were fed into an X-Y plotter, which was programmed to draw graphs with the experimental data and lines representing the equations, and also the first and second differentials. The areas and half areas beneath the second differential curves were calculated by computer. Fig. 2 is the result from a typical control knee and Fig. 3

from a rheumatoid joint. In the top graphs in both figures, the dots represent the recorded results and the calculated lines pass accurately through these points. The second differential curves show that the unstretched collagen fibre length is considerably reduced, and both the numbers of fibres of this length and the total number of fibres are increased in the rheumatoid joint.

The experimental results are expressed in arbitrary units related to pressure and volume and cannot be converted into absolute figures.

Results

(1) Mean unstretched collagen fibre length (Fig. 4)

The peak of the second differential curve was reached at 73 and 78 ml. for the control and EDS knees, but at only 32 ml. in the RA knees. The difference in the RA joints was highly significant, indicating shorter unstretched collagen fibre lengths.

FIG. 4 Volumes at which the collagen fibres reach their unstretched lengths in control, rheumatoid arthritis, and Ehlers-Danlos syndrome knees

C v. EDS $P < 0.7$

C v. RA $P < 0.001$

EDS v. RA $P < 0.001$

(2) Number of fibres at mean collagen fibre length (Fig. 5, overleaf)

The mean peak height of the second differential curves obtained from the RA knees showed a significant increase compared with the controls. The mean from the EDS knees showed a fall that was not statistically significant.

(3) Total number of collagen fibres (Fig. 6, overleaf)

The total number of fibres was indicated by the total area under the second differential curve. We were able to measure the half area only. There was a fall of borderline significance in the EDS knees, but no increase in the rheumatoid joints.

FIG. 3 Computer drawn plots from a rheumatoid knee.

FIG. 5 Numbers of collagen fibres at mean fibre length
C v. EDS $P < 0.2$
C v. RA $P < 0.001$

Discussion

A study of this type can give only a very crude analysis of the joint collagen. The results reflect the overall joint structure as many tissues other than the joint capsule contribute to the pressures developed on joint distension. However, the results do reflect changes occurring in the whole joint capsule and are not subject to the sampling errors that occur on biopsy.

It was unfortunately not possible to match the three groups for age or sex. This must be considered in interpreting the results, although we failed to obtain any correlation with these features in the control group.

Most studies on collagen have been performed on dermal tissue. Rollhauser (1950), Wenzel (1950), and Grahame (1970) all noted an increase in skin elastance with age. In contrast, Shuster, Raffle, and Bottoms (1967) found a decrease in skin collagen with age on biochemical analysis.

In rheumatoid arthritis, Shuster and others (1967) noted a fall in skin collagen of borderline significance. In the present study the elastance of the rheumatoid joints was increased, the numbers of collagen fibres in the joint capsule were virtually unchanged, and the unstretched lengths of these fibres were considerably decreased. It seems paradoxical that the elastance of normal skin increases even though the amount of collagen decreases with age, while, for rheumatoid joint capsules, the elastance is high yet the total collagen may be normal. The solution to this paradox is that the elastance of collagen depends far more on the intermolecular and intramolecular

FIG. 6 Half total number of collagen fibres
C v. EDS $P < 0.1$
C v. RA $P < 0.4$

cross-linkages than on the total amount of collagen present. Increase in the number of cross-linkages makes the collagen chemically more stable and physically less easy to stretch. Keech (1955) found that digestion of dermal collagen by a collagenase fell with the subject's age, and Bjorksten (1962), Verzár (1963), and Bailey (1969) demonstrated an increase in the cross-linkages in the collagen molecule with age. In lathyrism collagen fibres are fragile and easily broken, but the microscopic appearances of the collagen appear to be unaffected; this change in physical properties is due to cleavage of the cross-linkages (Levene, 1961; Bailey, 1969).

Thus, in the rheumatoid joint, the increase in the stiffness of the joint capsule appears to be due to increased numbers of inter- and intramolecular cross-linkages producing effective shortening of the fibre length.

In spite of the dramatic clinical stigmata of the Ehlers-Danlos syndrome, the nature of the basic defect has remained enigmatic. On light and electron microscopy (Tunbridge, Tattersall, Hall, Astbury, and Reed, 1952; Wechsler and Fisher, 1964; Goltz and Hult, 1965) and on measurement of its physical properties (Nordschow and Marsolais, 1969), the collagen appears to be normal, but both Tunbridge and others (1962) and Wechsler and Fisher (1964) found a significant reduction in the total quantity of collagen present. Bland, Lipson, Dunihue, Kusserow, Clemmons, and Williams (1965) found increased urinary hydroxyproline excretion, suggesting an increased rate of collagen turnover. However, in a further study of eight patients, the 24-hour urinary hydroxyproline excretion was normal (Beighton, Price, Lord, and Dickson, 1969).

Jansen (1955) suggested that the basic defect in the EDS lay in an abnormality of the cross-linking of collagen fibrils. Measurements of the physical properties of the skin have supported this hypotheses (Grahame and Beighton, 1969).

The results of the present study indicates that the unstretched lengths of fibres of joint capsule collagen are normal, and therefore suggest that there is no increase in cross-linkages. There may however be a decrease in the total number of collagen fibres.

Summary

By a computerized study of pressure/volume data obtained during distension of the knee joint, it has been possible to obtain indices of the unstretched lengths of the collagen fibres and the numbers of fibres. In rheumatoid arthritis there is a significant fall in the collagen fibre length, but no real increase in the number of fibres. In the Ehlers-Danlos syndrome the collagen fibre length is unaltered, but there is a possible decrease in the numbers of fibres.

These results have been interpreted in terms of the intermolecular and intramolecular cross-linkages of collagen.

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DISCUSSION

DR. G. NUKI (*Glasgow*) It would seem that the hypothesis depends on the assumption that the inflection in the stress/strain graph represents the taking up of slack in the collagen fibres of the joint capsule. Do you think it possible that similar changes could result from changes in compliance in the articular cartilage or in the intercollagenous ground substance? If this were so, it would invalidate any conclusions regarding changes in the collagen of the capsule.

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DR. JAYSON I think that the cartilage is so rigid when compared with the capsule that it plays little part in the pressure volume characteristics. We are studying the cross-linkages in collagen biochemically in an attempt to verify this hypothesis.

DR. D. A. BREWERTON (*London*) I was interested that you suggested that other tissues were so rigid that they need not be included in your calculations. You seem to be leaving out the synovium. Surely synovial proliferation could diminish the volume of the joints and this synovium could be compressed by the fluid you injected. Can you exclude the influence of proliferative synovium on your calculations; and have you compared those rheumatoid patients who have grossly thickened synovium with those who have only an effusion?

DR. JAYSON By using a double differentiation technique, we measure the change in the elastance during distension. We have assumed that synovium behaves according to Hooke's law. Any collagen present would of course be reflected in the result as from the joint capsule.

DR. R. GRAHAME (*London*) One of the authors of this paper has pointed out elsewhere that the Ehlers-Danlos syndrome is not a homeogenous entity but appears in various forms, with varying degrees of joint and skin involvement. In particular, the hypermobility varies very much in the different forms. Did the cases selected for this study particularly show joint changes? The work that I did with Dr. Beighton indicated that taking up the slack in the skin was delayed along the stress/strain curve. Was there any evidence in this work that this was so in the joints also?

DR. JAYSON The Ehlers-Danlos patients were not selected specifically. We asked many patients if they would care to participate in this study and some agreed to do so. We examined the data to see if it could be correlated with the different clinical patterns that Dr. Beighton has described, and we could not find any correlation. Our results contrast with yours in the skin since you suggested that there was delay in the uptake of slack in the stress/strain curve, whereas in joints we obtained the converse result.

DR. A. G. S. HILL (*Stoke Mandeville*) It is difficult to see that there could be much difference in volunteers and non-volunteers.

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