THE COLLAGEN OF THE INTERVERTEBRAL DISC IN ADOLESCENT
IDIOPATHIC SCOLIOSIS

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A defect in the collagenous matrix of the intervertebral disc has been proposed as a contributory factor in the pathogenesis or progression, or both, of the scoliotic deformity. In an attempt to resolve these questions the collagen extractability and distribution across normal and scoliotic discs was investigated. The collagen content of the scoliotic nucleus pulposus was found to be higher than normal, particularly at the apex of the curve, but no consistent correlation was found with the spinal mobility or degree of curvature. The collagen content of the annulus fibrosus from scoliotic discs was shown to be abnormally distributed, again only in those discs encompassed by the curve. Since these abnormalities in absolute collagen distribution are dependent on location within the spine it is considered that they represent a consequence of the curvature rather than the cause. These results contrast with the pepsin extractability of collagen in the annulus fibrosus, which was abnormal in all the scoliotic discs examined, and found to be independent of location. While the precise interpretation of these latter findings is complex, it would seem that a subtle defect in collagen does exist within the scoliotic disc which, coupled with extraspinal influences, may play an important role in progression of the scoliotic curve.

By far the most common form of scoliosis is the adolescent idiopathic curvature, for which the precise aetiology has not been established. A genetic background to this disorder has been clearly demonstrated (Riseborough and Wynne-Davies 1973) but the mode of expression remains obscure. Thus, while initiation of the curve would appear to be genetically determined in many instances, other factors—for example, growth rates—are clearly implicated in its progression.

Scoliosis is a feature of some inherited disorders of connective tissue in which abnormalities of collagen have been demonstrated, and these have been reviewed by Uitto and Lichtenstein (1976). In Ehlers–Danlos syndrome Type VI a hydroxylysine deficiency has been observed (Pinnelle et al. 1972; Sussman et al. 1974) which results in a defect in the intermolecular cross-linking of collagen (Eyre and Glimcher 1972). Collagen metabolism is also impaired in Marfan's syndrome (Krieg and Müller 1977) where there are high levels of urinary hydroxyproline (Laitinen et al. 1968; Priest, Moinuddin and Priest 1973). In homocystinuria, collagen in the skin is more soluble than normal and a cross-linking defect, due to the accumulation of homocysteine, has been demonstrated (Kang and Trelstad 1973; Siegel 1975). A disturbance in the types of collagen synthesised seems to be implicated in the clinical manifestations of osteogenesis imperfecta (Penttinen et al. 1975; Sykes, Francis and Smith 1977), along with a defect in cross-linking (Fujii et al. 1977; Trelstad, Rubin and Gross 1977). In the severe form of osteogenesis imperfecta, in which scoliosis is common, there is a decreased stability of polymeric collagen in the skin (Smith, Francis and Bauze 1975).

In contrast to the scoliosis associated with these syndromes, the idiopathic type is not generally considered to be a manifestation of connective-tissue disorder. There is, however, some recent evidence to suggest that both the collagen and glycosaminoglycans of some connective tissues in scoliosis are abnormal. Francis, Sanderson and Smith (1976) examined the stability of polymeric collagen of the skin from patients with idiopathic and congenital scoliosis. The stability of the polymeric collagen from adolescents with idiopathic scoliosis was found to be significantly less than that of control subjects, except for two patients with mature skeletons, where the polymeric collagen was normal. One of three patients with congenital scoliosis also had polymeric collagen of low stability.

Glycosaminoglycans in serum (Balaba 1972; Kaz'min and Merkur'eva 1971) and in the intervertebral disc (Pedrini, Ponseti and Dohrman 1973; Ghosh et al. 1979) are reported to be abnormal in idiopathic

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scoliosis, but it has not been possible to determine if these changes are primary, or secondary to the development of the curve. Harrington (1977) proposed that a collagen defect in the intervertebral disc might be responsible for the initiation of the scoliotic curve. The present work describes our further investigations of the extractability and distribution of collagen across normal discs and those from scoliotic patients, and demonstrates an abnormality which could account for the development of the curve.

MATERIALS AND METHODS

Preparation of tissues. Five normal human spines (aged thirteen to eighteen years; three boys, two girls) were obtained at necropsy and were stored at −20 degrees Celsius for use as control material. Discs were obtained from seven scoliotic patients with "idiopathic" thoracolumbar curves at the time of anterior correction and fusion by the Dwyer technique (Table I). Annular tissue was carefully selected from segments of the disc nearest the concave, convex and anterior sides of the scoliotic curve (Fig. 1). The nucleus pulposus was sampled centrally. Control discs from four of the normal spines were dissected in a similar manner. The scoliotic tissues were immediately washed with physiological saline and all tissues were stored at −20 degrees Celsius. One whole scoliotic spine was also obtained at necropsy from a fifteen-year-old girl (see Fig. 3) and zonally dissected into thirteen regions (Fig. 1); the fifth normal spine was similarly dissected. All specimens were finely diced and freeze-dried before chemical analysis and extraction. The water content of the zonally extracted discs was determined as a percentage of the original wet weight by weighing the samples before and after lyophilisation.

Mobility measurements. On films taken with the patient erect and bending sideways, lines were drawn parallel to the adjacent upper and lower vertebral surfaces. The angle at each interspace was measured at the intersection of these lines.

Extraction of collagen from freeze-dried tissues. The tissues were first extracted with 100 volumes of 0.5M sodium chloride in 0.02M sodium phosphate at pH 7.4 for three periods of twenty-four hours at 4 degrees Celsius with constant stirring (Fielding 1976). The three extracts were combined and the insoluble residues were treated with 100 volumes of 0.1 per cent pepsin (Merck) in 0.5M acetic acid for two periods of twenty-four hours at 4 degrees Celsius (Miller 1972). The two extracts were combined and the insoluble residues were thoroughly washed three times with distilled water (the washes were discarded) before further extraction with 1M sodium chloride in 0.05M Tris hydrochloric acid at pH 7.5 for three periods of twenty-four hours at 4 degrees Celsius (Eyre and Muir 1975). These three extracts were also combined.

Analyses. The hydroxyproline content of the freeze-dried tissues and of the extracts was determined by the method of Stegemann and Stalder (1967) and was multiplied by the factor of 7.4 to give the collagen content. The amount of collagen extracted from the tissues by each treatment was expressed as a percentage of the amount in the original dried tissues. Statistical analysis of results was performed using Student’s t-test.

RESULTS

Nucleus pulposus

Collagen content. The collagen content of the nucleus pulposus in scoliotic patients has been reported to be higher than that of age-matched control tissues (Pedrini et al. 1973). Our results confirm this observation when the analyses from all spinal levels examined (T9–10 to L3–4) are pooled (Table II). When each spinal level is examined separately, however, this difference is statistically significant at only three (T12–L1, L1–2, L2–3) of the seven levels analysed. As all but one of the scoliotic curves examined here had apices at T12–L1, L1–2 or L2–3 (the other had its apex at T11–12) it would appear that the collagen content of the nucleus pulposus is higher than normal only in those discs which are at or close to the apex of the scoliotic curve.

An attempt was made to correlate the collagen content of the nucleus pulposus with various radiographic features before operation. For five of the seven patients who had mobility measurements taken, a correlation was found between the collagen content of the nucleus pulposus and mobility. However, this correlation was not statistically significant.
correlation was not consistent, since four of the five patients showed a negative correlation, that is an increasing collagen content with decreasing mobility, this correlation being statistically significant in three of these four curves. The fifth patient, however, showed a significant positive correlation.

**Collagen extraction.** The amount of collagen which was extractable from the nuclei pulposi by the sequential extraction procedure is shown in Table III. Using 0.5M sodium chloride in 0.02M sodium phosphate at pH 7.4, the amount of collagen extracted from the sciotic tissues was significantly less than that extracted from the normal. In contrast, the amount extracted by pepsin and subsequently by 1M sodium chloride in 0.05M Tris hydrochloric acid at pH 7.5 was the same for the controls and the sciotic tissues.

**Annulus fibrosus**

**Collagen content.** Analysis of the left and right lateral segments (Fig. 1) and the anterior portion of the annulus fibrosus from control tissue showed no consistent difference in collagen content. Results from these various segments from four control spines were therefore pooled for each disc (Fig. 2). From the segmental analysis of the discs removed from six sciotic patients (Fig. 2)—there being insufficient material available from the seventh—it is apparent that each patient has an individual pattern, but some general observations can be made. The collagen content of the segment nearest the concave aspect of the sciotic curve was lower than or the same as that from the segment nearest the convex side in all but the one patient (Case 4) who had a double major curve: in her profile, one disc (L2–3) gave the reverse analysis. Furthermore, all patients had at least one disc within the curvature where the collagen content of the concave aspect was significantly less than that of the convex. It is also clear that when the analyses from each patient were compared with pooled control results at the appropriate disc levels, at least one disc of five of the patients exhibited a collagen content on the concave aspect which was less than that of control tissues. In one patient (Case 2) two of the discs had a collagen content on the convex aspect which was higher than that of the control values.

Case 1 is of particular interest, as the collagen content of the concave aspect for all discs in the curve was significantly lower than that of the control spines (Fig. 2). The difference between the collagen content of the two sides of the discs was greater in this patient than in all the others studied. This girl (unlike the other patients) had worn a brace consistently for eighteen months before operation. Whether or not the immobilisation contributed to these findings is not yet established, but is a distinct possibility.

**Zonal analysis.** The collagen content (on a dry weight basis) across a lateral axis of control discs showed a

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**Table II.** Collagen content of nucleus pulposus as a percentage of the dry weight

<table>
<thead>
<tr>
<th>Disc</th>
<th>Control Mean ±SEM (n)</th>
<th>Scoliotic Mean ±SEM (n)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>T9–10</td>
<td>12.14 ± 0.27 (4)</td>
<td>21.98 (1)</td>
<td>NS</td>
</tr>
<tr>
<td>T10–11</td>
<td>13.14 ± 1.84 (3)</td>
<td>26.33 ± 5.73 (3)</td>
<td>NS</td>
</tr>
<tr>
<td>T11–12</td>
<td>14.77 ± 3.77 (3)</td>
<td>21.77 ± 2.40 (7)</td>
<td>NS</td>
</tr>
<tr>
<td>T12–L1</td>
<td>13.05 ± 0.80 (4)</td>
<td>22.30 ± 2.10 (8)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>L1–2</td>
<td>10.88 ± 1.10 (5)</td>
<td>19.65 ± 2.15 (8)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>L2–3</td>
<td>12.18 ± 0.52 (5)</td>
<td>20.39 ± 2.95 (7)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>L3–4</td>
<td>15.34 ± 1.72 (5)</td>
<td>29.08 ± 6.76 (4)</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Table III.** Percentage of collagen extractable from the nucleus pulposus in control and sciotic spines

<table>
<thead>
<tr>
<th></th>
<th>Percentage of collagen extracted (mean ±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5M NaCl</td>
</tr>
<tr>
<td>Controls (15)</td>
<td>3.99 ± 0.30</td>
</tr>
<tr>
<td>Scoliotics (16)</td>
<td>2.39 ± 0.28</td>
</tr>
<tr>
<td>Significance</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

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Fig. 2

Collagen content of annulus fibrosus (segmentally dissected). Results from six sciotic patients are compared with the pooled results from control tissues (.....) for each spinal level.
symmetrical distribution about the nucleus pulposus (zone 7 in Fig. 3). The outer regions of the annulus fibrosus contained more collagen than the inner regions. This was also found for the anterior portion of the disc, and a gradual decline in collagen content from the outer anterior region inwards to the nucleus pulposus was observed. When collagen distribution was calculated on a wet weight basis a similar symmetrical profile was found for normal discs. Analysis of the zones from the scoliotic spine (Fig. 3) demonstrated that those discs in the centre of the lumbar curve (L1-2, L2-3 and L3-4) showed an asymmetric collagen distribution contrasting with the symmetrical distribution observed for normal discs. It was also apparent that the distribution of collagen for each disc was different, and dependent on its spinal level and thus curvature. The two discs at the extremity of the lumbar curve (L4-5 and T12-L1) showed almost normal profiles of collagen content.

The anterior and posterior segments of these discs were also analysed zonally for collagen distribution, but no significant differences were observed between the tissues of the scoliotic and the control spines.

When expressed as percentage collagen on a wet weight basis, the L1-2, L2-3 and L3-4 discs from the scoliotic spine showed asymmetric profiles (similar to Fig. 3).

Collagen extraction. Less than 3 per cent of the total collagen present was extracted from tissues of the annulus fibrosus by 0.5M sodium chloride in 0.02M sodium phosphate at pH 7.4. The amount extracted from the outer regions was less than that extracted from the inner regions or the transitional zone. There did not appear to be any significant difference between the amount extracted from the controls compared to the scoliotics (but errors, which were large, would conceal small differences if these existed).

Subsequent pepsin extraction of the tissue residues revealed that markedly less collagen was extracted from the inner and transitional regions of the scoliotic tissue than from the same zones of the control discs (Fig. 4).

The extraction from the various zones of the control discs showed a symmetrical profile about the nucleus pulposus, and was inversely related to the collagen content (Fig. 5). No such inverse relationship was observed for scoliotic tissues (Fig. 6), neither from necropsy nor from operative specimens.

Extraction of the pepsin-insoluble disc fractions by
1M sodium chloride in 0.05M Tris hydrochloric acid at pH 7.5 revealed a similar symmetrical profile about the nucleus pulposus, and an inverse relationship between the percentage of collagen content and amount extracted, but in contrast to the pepsin extractions no differences were apparent between scoliotic \( r = -0.68 \) and control \( r = -0.89 \) tissues.

**DISCUSSION**

The equilibrium and stability of the spinal column, particularly during growth, depends upon a number of complex interrelated factors including the development and maturation of muscles, ligaments, vertebrae and the intervertebral discs. Scoliosis has been produced in animals by a number of methods, such as scarifying the laminae (Somerville 1952), excising the ribs, the intercostal muscles, the costotransverse ligaments (Langenskiöld and Michelsson 1962), or the erector spinae muscles (Silva 1969), but no direct evidence has yet been provided to implicate a primary intrinsic abnormality in the structure or development of these tissues as a cause of adolescent idiopathic scoliosis. Studies on spinal muscles and nerve activity on both sides of scoliotic spines have revealed abnormalities in intramyofibre type, calcium levels, and neuromuscular development (Zuk 1962; Spencer 1974; Hoogmartens and Basmajian 1976; Spencer and Eccles 1976; Spencer and Zorab 1976; Yarom, Robin and Gorodetsky 1978). It is, however, uncertain whether these abnormalities are primary or a consequence of the development of the curve, of operative trauma or of previous management. Direct measures of collagen content and mechanical properties of tendons and interspinous ligaments from forty patients with idiopathic scoliosis failed to demonstrate abnormalities relative to age-matched control tissues (Nordwall 1973). Harrington (1977) and Ponseti et al. (1976) have suggested that a defect in the intervertebral disc is a principal factor in the pathogenesis of the curve. Some evidence to support an abnormality of collagen which could result in altered stability of the disc has recently been presented (Bushell et al. 1978) and it is significant that the potential for correction of the curve decreases with the extent to which the disc becomes wedge-shaped (MacEwen 1971; Nordwall 1973).

Differences in the extractability of collagen from the annulus fibrosus of the spines of scoliotic patients compared with controls were evident only on using pepsin. Extraction of the various zones with this enzyme demonstrated that the differences were independent of location (concave or convex side of the scoliosis), and thus apparently not a consequence of different mechanical environments. This is particularly evident in the L4-5 disc of the necropsy specimen, where, although the collagen distribution is normal (Fig. 3), the pepsin extractability is not (Fig. 4). It was most marked for the inner and transitional zones (regions 6 and 8, Fig. 4). It is perhaps significant that the transitional zone of the disc is the region of maximal metabolic activity in the growing rabbit and pig (Hansen and Ullberg 1960; Souter and Taylor 1970; Ghosh, Taylor and Horsburgh 1975) and this preferential activity extends into adult life in the rabbit (Souter 1971; Taylor et al. 1977). Precise interpretation of these results in molecular terms is difficult as there are a number of equally plausible explanations. It has been reported (Osebold and Pedrini 1976; Eyre and Muir 1977) and confirmed by us (unpublished observations) that pepsin preferentially extracts Type II collagen from the annulus fibrosus. The
pepsin extraction obtained for normal discs (Fig. 4) correlates with the known distribution of Type II collagen across this tissue. Eyre and Muir (1977) reported that Type II collagen was not present in the outer annulus but that its proportion gradually increased to 100 per cent in the transitional zone and in the nucleus pulposus. Thus, the pepsin extraction profile obtained for discs from scoliotic patients (Fig. 4) could be accounted for by a decrease in the proportion of Type II collagen, relative to Type I, present in the inner regions of the annulus fibrosus. Alternatively, as the major effect of pepsin on collagen at 4 degrees Celsius is to cleave only the non-helical telopeptide fraction (Rubin et al. 1965; Leibovich and Weiss 1970; Zimmerman et al. 1970; Miller 1972; Bannister 1975) the results obtained for scoliotic tissues may arise from structural abnormalities in this fraction of the collagen molecule. The telopeptide fraction is an important site of collagen cross-linkage (Bailey, Robins and Balian 1974) and in this context it is significant that scoliosis frequently occurs in a wide variety of connective-tissue disorders, such as Ehlers–Danlos syndrome (Type VI) where cross-linking is known to be deficient (Uitto and Lichtenstein 1976).

Studies by Herbert et al. (1975) on collagen cross-linking in human intervertebral discs during ageing and in degenerative disc disease showed that there was variation in reducible cross-links in discs at different spinal levels. The more caudal discs contained fewer reducible cross-links suggesting a more “mature” type of collagen. It has been suggested that this result may be related to the higher mechanical stress acting in this region of the spine (Bailey, Herbert and Jayson 1976). An abnormality in the collagenous component of the disc in scoliotics could be expected to result in a structure less capable than normal of maintaining stability for normal spinal development. Such a nascent structural defect would become potent during a growth spurt where a minor musculoskeletal asymmetry in the vertebral axis would be amplified. It is significant, in this regard, that scoliotic girls have an abnormal pattern of skeletal development and sexual maturation (Willner 1974, 1975a,b; Nordwall and Willner 1975; Low et al. 1978). Burwell (1978) has demonstrated an increased incidence of joint laxity in the upper limbs of girls with adolescent idiopathic scoliosis compared with a control population. Our observations of Australian girls with idiopathic scoliosis are in general accordence with Burwell’s findings. Regrettably, there is no reliable clinical method yet available for assessing and measuring ligamentous laxity and the methods currently employed, such as hyperextension of the elbow, are relatively crude. The Australian girls who show evidence of hypermobility of the joints in the upper limbs rarely exhibit comparable increases in the lower limbs—an inexplicable phenomenon. While abnormality in normal cross-link development within the collagen of the disc in scoliotics would seem a likely explanation of our results, we cannot exclude the possibility of the synthesis of different collagen types, either alone or in combination with abnormal proteoglycans (Ghosh et al. 1979).

From the results of the extractability experiments with 0.5m sodium chloride it would appear that the nucleus pulposus in scoliotics contains less newly synthesised collagen than normal, even though the total collagen content is higher than in control tissue. This observation, while consistent with the known advanced skeletal age of scoliotic girls (Nordwall and Willner 1975; Willner 1975a,b) may also reflect hormonal imbalance involving interplay between mediators of pituitary, ovarian and adrenal origins. There is abundant experimental evidence to indicate the important modulatory role of these agents in connective-tissue growth and development (Silberberg and Silberberg 1965; Silberberg 1973, 1974; Langeland 1977; Canalis and Raisz 1978; Dearden, Mosier and Espinosa 1978).

While the pepsin extraction results were independent of location (concave or convex side of the curve) the collagen content of both segmentally derived and zonally dissected disc material was clearly related to spinal curvature. Pedrini et al. (1973) reported that in scoliosis the collagen content of the nucleus pulposus of the disc was elevated, but we find this true only at or immediately adjacent to the apex of the curve. As the moisture content of the nucleus pulposus is essentially normal this result holds true for both dry and wet weight determination. The collagen distribution results for surgical material were complex and no uniform correlation was found with various mechanical factors. Only the mobility showed a reasonable correlation with the collagen content of the nucleus pulposus and these results were inconsistent. One of the major difficulties in attempting to correlate biochemical and radiographic results is that a radiograph is a two-dimensional representation of a three-dimensional body, and lack of consistent correlation may simply mean that the two-dimensional information is an insufficient description of the overall deformity. In addition to this problem, there is the possibility that within the group of patients labelled as “idiopathic” scoliotics, there may be a number of subgroups, differing not only in the aetiology of their disorder, but perhaps also in their response to management.

In attempting to correlate the distribution of collagen in the annulus fibrosus with spinal mobility or the degree of curvature, it was apparent that the collagen content in the concave segment was often very different from the convex counterpart. There was, however, a large range of values found in many instances for a single piece of tissue. This variability was clearly shown by the use of zonal analysis (Fig. 3) to be due to the heterogeneity in the tissue samples. By the use of this dissection procedure it was established that in normal discs, a gradual decline in collagen content from the
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outer annular regions to the nucleus existed (Fig. 3). Similar analysis of sciotic discs about the same coronal axis demonstrated an asymmetrical distribution of collagen in those discs which were encompassed by the curve whereas those discs at the upper and lower extremities displayed profiles similar to the controls (Fig. 3). This finding strongly suggests that collagen content is directly related to the degree of curvature and in this regard is consistent with the general response of connective tissues to an altered mechanical environment (van der Hooff 1964; Scapinelli and Little 1970; Flint 1972; Gillard et al. 1977).

These observations on collagen distribution neither support nor refute the contention that changes in the disc are primary to the aetiology of idiopathic scoliosis, but they are more consistent with secondary rather than primary events. This contrasts with pepsin extractability where the results are independent of location. Do these results reflect an abnormality in collagen which is the much-sought-after primary connective tissue defect in idiopathic scoliosis? Even if this defect is not totally responsible for the initiation of the curve it could, coupled with extraspinal influences, play a paramount role in its progression.


