

Collagen organisation in the interspinous ligament and its relationship to tissue function

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(Accepted 24 February 1987)

INTRODUCTION

Lumbar interspinous ligaments are sheets of tissue which connect spinous processes of adjacent vertebrae, as shown schematically in Figure 1. Anteriorly they meet the ligamenta flava (Warwick & Williams, 1973) while posteriorly they merge with the thoracolumbar fascia (Prestar, 1982; Tesh, Evans & Shaw-Dunn, 1985).

There are conflicting statements in the literature regarding the arrangement of collagen fibres in the interspinous ligaments (Rissanen, 1960; Heylings, 1978). Standard anatomy textbooks disagree to the extent that Heylings (1978) has suggested that some authors have drawn the ligaments upside down. Rissanen (1960) has reviewed many observations published since the early nineteenth century; the majority verdict is that fibres run antero-superior to postero-inferior (Fig. 1*a*), although many of the authors cited do not agree. Indeed his own observations led him to conclude that the fibres run from postero-superior to antero-inferior (Fig. 1*b*). This divergence of opinion is still far from resolved; *Cunningham's Manual of Practical Anatomy* (Romanes, 1968), *Gray's Anatomy* (Warwick & Williams, 1973) and Parke (1975) all describe the fibres as being orientated in a postero-caudal direction (Fig. 1*a*) whereas Grant (1972), Heylings (1978) and Prestar (1982) agree with Rissanen (1960) that the preferred orientation is postero-cranial. (Fig. 1*b*).

The arrangement of collagen fibres helps to determine the mechanical properties of the ligament and hence its suitability for performing its biological function. Collagen fibres provide tensile reinforcement along the directions in which they are orientated and their degree of alignment controls the extensibility of a tissue (Minns, Soden & Jackson, 1973; Hukins, 1982, 1984; Jeronimidis & Vincent, 1984; Hukins & Aspdén, 1985). For example, the collagen fibres in tendon are crimped – when the crimp is straightened (at strains of about 3%), the fibres become highly aligned and the tissue stiffens (Stromberg & Wiederhielm, 1969; Diamant *et al.* 1972). Greater extensibility is conferred by increasing the spread of orientations of collagen fibres about their preferred (mean) directions and also, in some tissues, by the incorporation of a higher proportion of elastic fibres (Minns *et al.* 1973; Hukins, 1984; Hukins & Aspdén, 1985). The ligamentum flavum, for example, contains a high proportion of elastic fibres and its collagen fibres are highly disorientated but become aligned as the ligament extends (Hukins & Aspdén, 1985; Aspdén, 1986).

It has often been assumed that the function of the interspinous ligaments is to stabilise the spine by limiting flexion (e.g. Prestar, 1982; Percy & Tibrewal, 1984). Indeed it has been suggested that the presence of collagen fibres means that the ligaments are almost inextensible (Heylings, 1978). They would then need to be lax in the unflexed spine in order to accommodate flexion at the intervertebral joint; further-

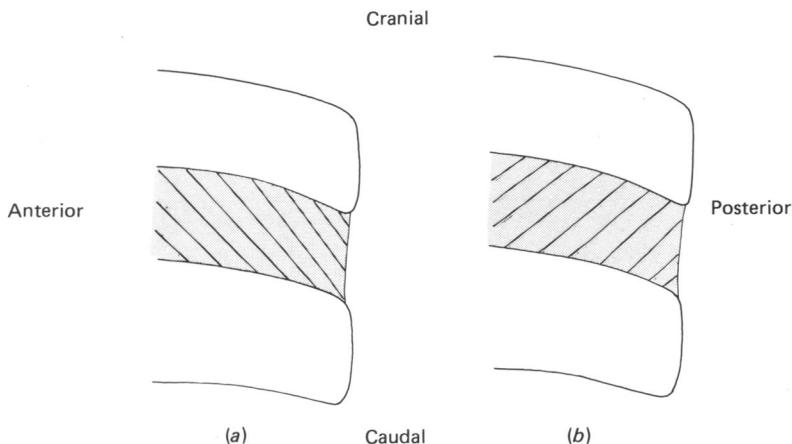


Fig. 1(a-b). Schematic diagram of the interspinous ligaments showing the two main conflicting proposals for the orientation of their collagen fibres: (a) postero-caudal (i.e. antero-superior to postero-inferior) and (b) postero-cranial (i.e. antero-inferior to postero-superior).

more, they could only function when the spine is in extreme flexion by preventing further movement (Heylings, 1978; Percy & Tibrewal, 1984). However, the greatest strength would then be provided if the fibres were oriented to link directly the spinous processes i.e. in the cranio-caudal direction, which is the direction of the fibres in the longitudinal ligaments of the spine once their crimp is straightened (Shah, Jayson & Hampson, 1979).

An alternative function which has recently been proposed for the interspinous ligaments is to anchor the thoracolumbar fascia to the spine (Tesh *et al.* 1985). Collagen fibres of the interspinous ligaments merge with those of the thoracolumbar fascia (Prestar, 1982; Tesh *et al.* 1985). Experimental and theoretical studies (Fairbank & O'Brien, 1980; Gracovetsky, Farfan & Lamy, 1981) have led to the suggestion that tension in the fascia, produced by contraction of the abdominal muscles, may produce extension of the lumbar spine thus providing support and contributing to stability during lifting (Tesh *et al.* 1985). The interspinous ligament would then be most effective if its collagen fibres tended to be oriented in the antero-posterior direction so that they could transmit tension directly from the fascia to the spine.

Thus there are two distinct proposals for the function of the interspinous ligaments which lead to completely different optimal orientations for their collagen fibres; neither of these simple predictions accords exactly with the published accounts of the arrangement of collagen fibres in the ligament. Here we present the results of a detailed determination of collagen fibre orientation in pig and human lumbar interspinous ligaments, when the spine is flexed as well as relaxed, using X-ray diffraction and by determining their optical anisotropy. These techniques yield fibre orientation in full-thickness samples of ligament and not just the superficial arrangement of fibres. Our results allow us to distinguish which is the more likely of the two proposals for the function of the ligament.

MATERIALS AND METHODS

Materials

Lumbar spines were obtained from four adult mini-pigs and three human cadavers (one male aged 30 years; two females aged 32 and 75 years). Specimens were stored

deep frozen (-20°C) as this does not affect the orientation of collagen fibres in fibrocartilage (Hickey & Hukins, 1979) and so, presumably, in ligaments.

X-ray diffraction

X-ray diffraction allows the orientations of the collagen fibres in a sample of tissue to be determined with respect to an axis which is defined in the specimen (Aspden & Hukins, 1979; Hukins, 1984). Orientations are determined throughout the thickness of the sample traversed by the X-ray beam and within the cross sectional area of the beam (about 1 mm^2). Diffraction patterns were recorded using a horizontal beam of X-rays which was perpendicular to the plane of a ligament using a Searle X-Ray Diffraction Camera (Marconi-Elliott, Borehamwood, Herts.) equipped with a toroidal focusing mirror (Elliott, 1965). Copper K_{α} X-rays (wavelength 0.154 nm) were used to record the diffraction pattern on Kodak industrial X-ray film on which a vertical reference was marked (Aspden & Hukins, 1979). A travelling microscope was used to measure the angle of the spinous processes to the vertical. Thus an X-ray diffraction pattern could be used to measure the preferred orientation of the collagen fibres, in the tissue site from which it was recorded, with respect to the vertical direction and, hence, to the spinous processes; furthermore, the orientation distribution function, $g(\phi)$, which here represents the probability of finding a fibre at an angle ϕ to this preferred direction, could also be computed (Aspden & Hukins, 1979; Hukins, 1984). This function represents an average through the thickness of the specimen and is not influenced by any bifid structure in the ligament (Heylings, 1978).

One pig spine was sectioned transversely through each spinous process and vertebral body, from L1 to L5, to leave the interspinous ligaments intact between their bony attachments. Each ligament was mounted in an airtight specimen cell whose interior was saturated with vapour from physiological saline; Melinex windows in the cell allowed X-rays to pass through (Aspden & Hukins, 1979). Diffraction patterns, each taking 2 hours, were then recorded from each of the ligaments L1-2 to L4-5 in a fully hydrated state.

The L1-2 ligament was then mounted in a device which allowed it to be stretched within the specimen cell. It was stretched in a direction corresponding to cranio-caudal, in order to simulate the effects of flexion. The maximum strain of 30% was reached in intervals of 5%; all strains were measured using a travelling microscope. An X-ray diffraction pattern was recorded in the relaxed state and at each stage of stretching in an exposure time of 2 hours.

Diffraction patterns of L1-2, L2-3 and L3-4 interspinous ligaments were recorded from all three spines. The entire lumbar spine was maintained intact and unstrained and was enclosed within a special cover built for the X-ray diffraction camera. A travelling microscope was used to measure the angle of the spinous processes to the vertical axis, which was marked on the X-ray film as before. No appreciable drying of the specimen occurred during the time required ($\frac{1}{2}$ hour) to record a diffraction pattern. Each of the three ligaments could be examined by simply shifting the spine. However, the L4-5 and L5-S1 ligaments were inaccessible because there was little or no space between the adjacent spinous processes through which an X-ray beam could pass.

Optical anisotropy

The arrangements of collagen fibres in the interspinous ligaments of the three remaining pig spines were inferred from the optical anisotropy of the ligaments. A long exposure time (2 hours) was required to record each X-ray diffraction pattern from pig ligament and several (typically 5) patterns had to be recorded to ensure a representative sample of tissue sites. Determining the directions of the optic axes in the

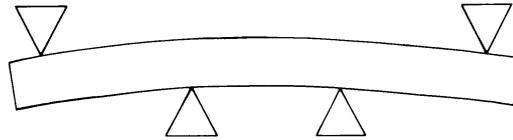


Fig. 2. Schematic diagram of the four-point bending frame in which forces applied in the directions indicated apply a pure bending moment to the spine.

ligaments allowed the preferred orientations of the collagen fibres to be identified in a much shorter time, but this technique could not be adopted for human interspinous ligaments which are too thick to transmit light.

Spines were left intact and each ligament was examined in its entirety with a dissecting microscope equipped with polarisers. Ligaments were examined between 'crossed' polarisers so that the direction of their collagen fibres could be determined in exactly the same way as conventional polarised light microscopy can be used to determine preferred orientations of microscopic fibres in thin sections of tissue (Bennett, 1950; Diamant *et al.* 1972; Hukins, 1984; for details of the theory see Hartshorne & Stuart, 1950). We used this technique to measure the directions of preferred orientations only, which corresponds to the peak in $g(\phi)$. The width of $g(\phi)$ can only be measured in thin sections and involves impregnating the tissue with glycerol (Yarker, Aspdén & Hukins, 1983). However, the determination of preferred orientations has proved adequate for determining the arrangement of collagen fibres in the meniscus (Aspdén, Yarker & Hukins, 1985) and for observing collagen fibre reorientation during stretching of tendon (Diamant *et al.* 1972) and so the same approach was adopted here.

The ligaments were also examined when these three pig spines were flexed in a four-point bending frame, shown schematically in Figure 2. This method of loading a beam subjects it to a pure bending moment without the application of shear (Higdon *et al.* 1976).

RESULTS

Pig lumbar interspinous ligaments appeared as thin, translucent sheets stretched between the spinous processes; human ligaments were much thicker and virtually opaque. Although fibres could not be discerned in the pig ligaments with the unaided eye, the human interspinous ligaments had an obviously fibrous appearance in which the fibres were oriented predominantly in the antero-posterior direction (Fig. 3). Some fibres also followed the curvature of the processes where they joined to the laminae. In both pig and human, all the interspinous ligaments appeared to be under some tension even when the spine was not flexed. No folding of the ligament out of the sagittal plane was observed, even when the spine was extended, indicating that none of the ligaments was lax.

Figure 4 is an X-ray diffraction pattern which is typical of those obtained from both human and pig interspinous ligaments; it shows clearly that the preferred direction of orientation of collagen fibres is parallel to the spinous processes, within a few degrees, and is, therefore, in an antero-posterior direction. The direction of preferred orientation can be readily identified from such a diffraction pattern since it is perpendicular to the equator EE' (Fig. 4): Figure 5 shows the orientation distribution function, $g(\phi)$, computed from Figure 4 (Aspdén & Hukins, 1979); the value of $g(\phi)$ at a given angle,

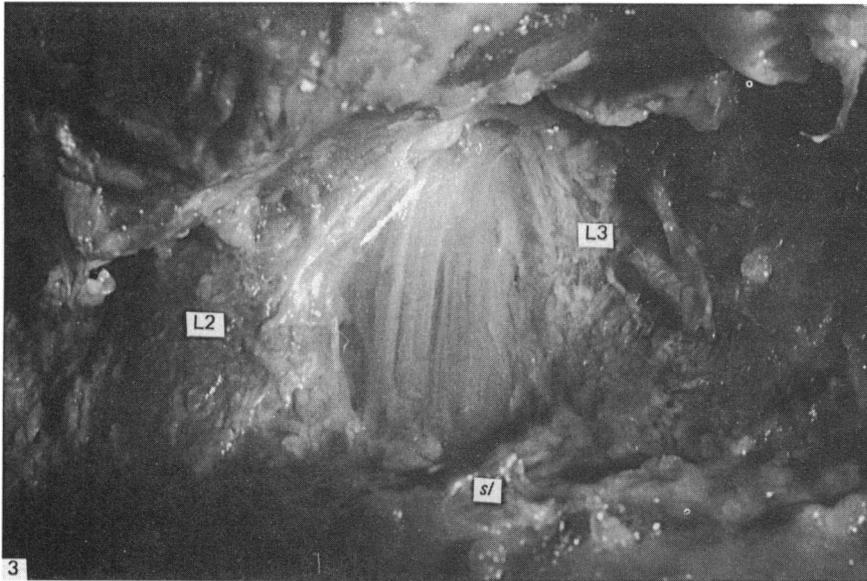


Fig. 3. View of the L2-3 interspinous ligament of an 80 years old human male cadaver. Collagen fibres can be seen to be oriented roughly parallel to the spinous processes. *sl*, supraspinous ligament; *L2*, *L3*, spinous processes of L2 and L3 vertebrae.

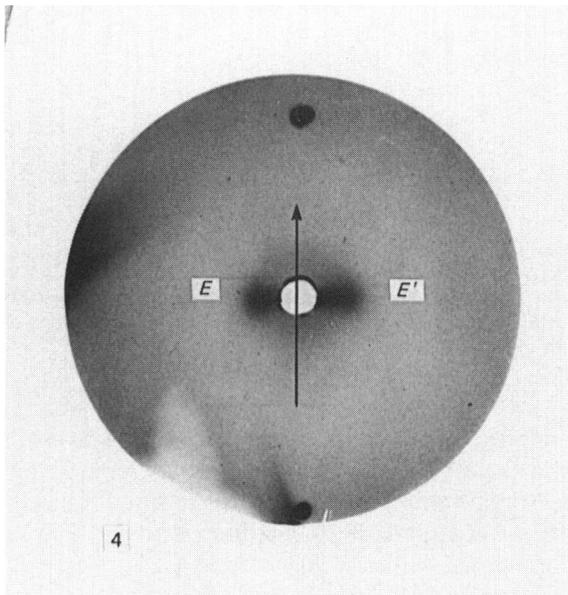


Fig. 4. X-ray diffraction pattern recorded from the L3-4 interspinous ligament of an 80 years old human male cadaver, with the X-ray beam passing perpendicular to the plane of the ligament. The axis of the spine was approximately horizontal and the spinous processes were pointing vertically downwards. The direction of preferred orientation of the collagen fibrils is perpendicular to the equator (*EE'*) of the diffraction pattern and is indicated by an arrow.

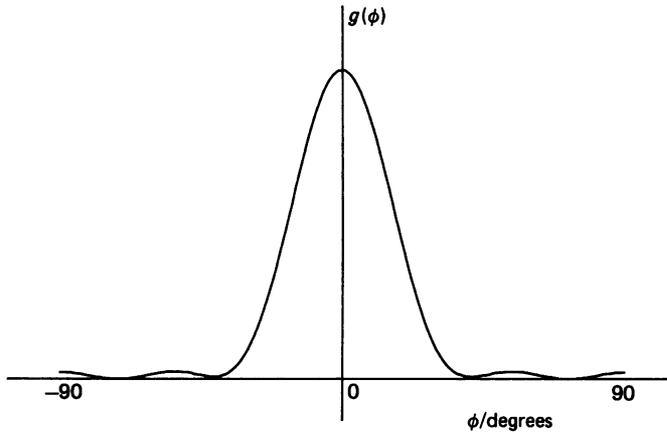


Fig. 5. Orientation distribution function, $g(\phi)$, computed from the X-ray diffraction pattern of Figure 4. This function represents the probability of finding a fibre oriented at an angle, ϕ , to the preferred direction which is here defined to have $\phi = 0^\circ$.

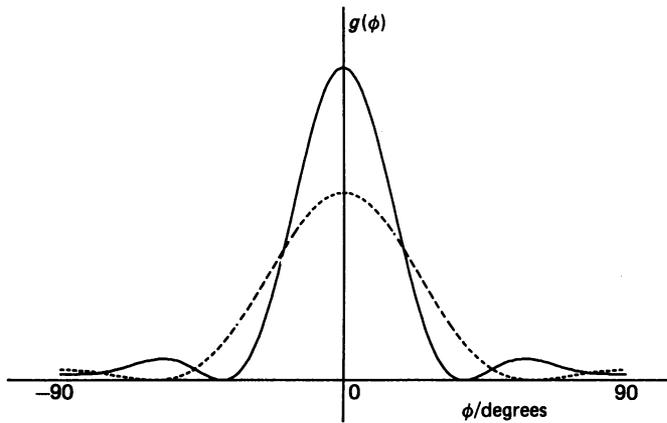


Fig. 6. Orientation distribution function, $g(\phi)$, computed from X-ray diffraction patterns of a pig L1-2 interspinous ligament when unstrained (continuous line) and subjected to a 30% strain (dashed line). The zero value of ϕ corresponds to the preferred orientation of the collagen fibres which remained unchanged when the ligament was stretched in the cranio-caudal direction to simulate the effects of flexion of the spine.

ϕ , represents the probability of finding a fibre oriented at that angle to the preferred direction. The narrower the distribution, the more highly aligned are the fibres. Indeed the spread of the fibre orientations about the preferred direction can be represented by $\Delta\phi$ which is the width of $g(\phi)$ at half its maximum height. For the distribution of Figure 5, $\Delta\phi = 36^\circ$. Combined values of $\Delta\phi$ were $39 \pm 5^\circ$ (mean \pm standard deviation) for all human ligaments and $36 \pm 3^\circ$ for the pig specimens.

Stretching an isolated pig interspinous ligament along the cranio-caudal direction, to stimulate the effect of flexion, did not change the direction of preferred orientation of the collagen fibres but led to an increase in $\Delta\phi$, i.e. the fibres became less highly aligned. Before the ligament was stretched its X-ray diffraction pattern was very similar to that in Figure 4. Figure 6 shows that stretching the ligament increases the width of $g(\phi)$, so that $\Delta\phi$ increased from 34° at a strain of 0% (i.e. unstretched) to 52° at a 30% strain.

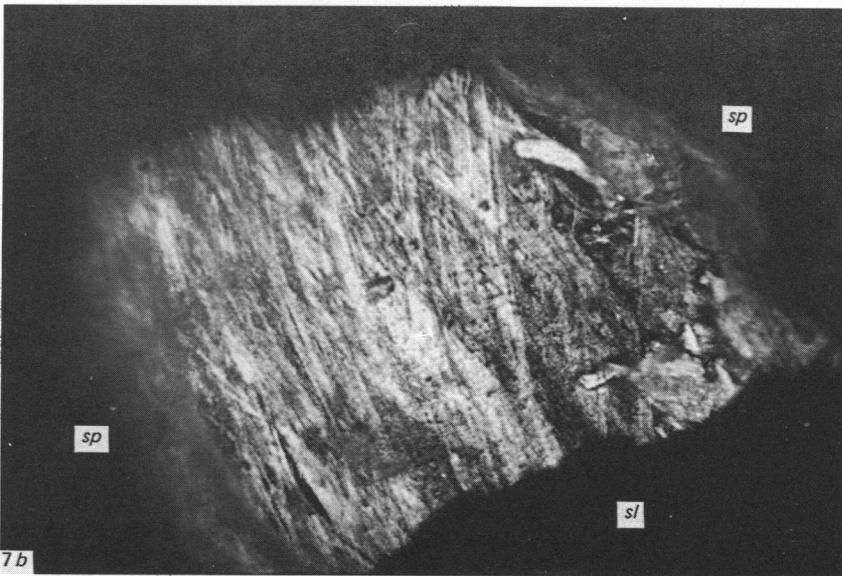
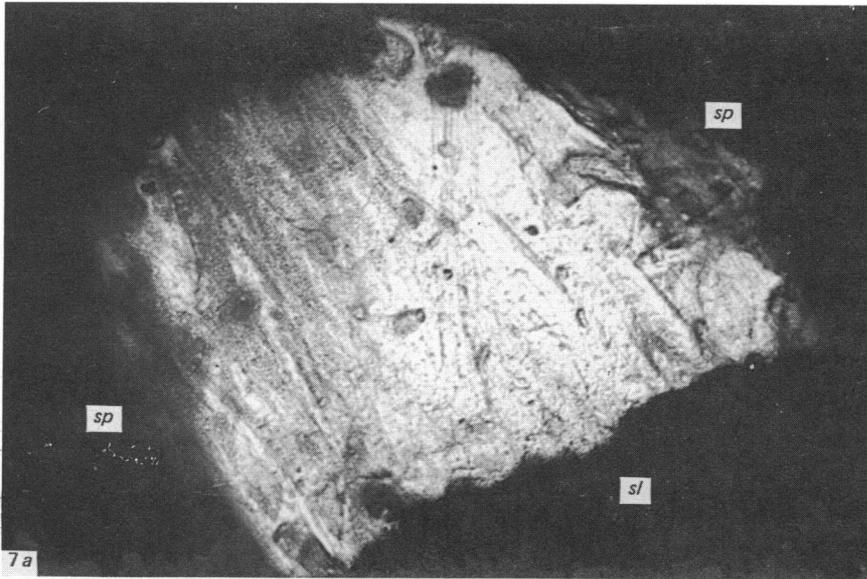


Fig. 7 (*a-b*). Pig L3-4 interspinous ligament viewed under a dissecting microscope. In (*a*) the ligament is viewed in unpolarised transmitted light; in (*b*) the ligament is also examined with transmitted light but it is in a position of minimum brightness between crossed polarisers. As a result the fibres oriented in the preferred direction (parallel to the optic axis of the first polariser) do not appear in (*b*). The spinous processes were roughly parallel to the optic axis of the first polariser. *sl*, supraspinous ligament; *sp*, spinous process.

The optical anisotropy of pig interspinous ligaments is consistent with their collagen fibres being predominantly oriented parallel to the spinous processes. Figure 7*a* shows a pig L3-4 interspinous ligament viewed in the dissecting microscope but without the polarisers in position. Although there is a spread of fibre orientations, the predominant direction appears to be parallel to the spinous processes. In Figure 7*b* the ligament is



Fig. 8 (*a-b*). Fig L3-4 interspinous ligament viewed between crossed polarisers and in a position of minimum brightness when the spine is (*a*) unflexed and (*b*) flexed. At the L3-4 joint the ligament strain was about 25%. The preferred orientation of the collagen fibres remains unchanged but the angle between the remaining minority fibres, which are visible in this Figure, has increased. *sp*, spinous process; *sl*, supraspinous ligament.

seen between crossed polars in a position of minimum brightness, i.e. when the fibres oriented in the preferred direction disappear. This position occurred when the spinous processes were roughly parallel to the optic axis of the first polariser, i.e. the observed optical anisotropy is consistent with the preferred orientation measured by X-ray diffraction. The fibres which do appear in Figure 7*b* are those which are distributed about the preferred orientation. These minority orientations can be more readily appreciated when the fibres aligned in the preferred direction are made to disappear.

When the spine was flexed the preferred orientation of the collagen fibres in the interspinous ligaments remained unchanged but the angle between the minority fibres increased. This result, obtained by detecting the optical anisotropy of the ligaments while the intact lumbar spine was bent in a four-point bending frame (Fig. 2), is consistent with the X-ray diffraction results which showed an increase in $\Delta\phi$ when an isolated ligament was stretched to simulate the effects of flexion. Figure 8 compares the same L3-4 ligament in a pig spine in an unflexed (Fig. 8*a*) and a flexed (Fig. 8*b*) position. This Figure was recorded with the specimen between crossed polars in a position of minimum brightness, i.e. with the collagen fibres oriented in the preferred direction extinguished. In both Figures 8*a* and *b* the position of extinction is the same (with the spinous processes roughly parallel to the optic axis of the first polariser) so that the preferred orientation of the fibres is unchanged by flexion of the spine. However, it can be seen that flexion of the intervertebral joint has increased the angle between the minority fibres from about 20° in the unstretched ligament to about 30° when strained by about 25%.

DISCUSSION

Our results show that the preferred orientation of collagen fibres in the interspinous ligaments is parallel to the spinous processes; however, not all the fibres are oriented in this direction. The spread of collagen fibre orientations, computed from X-ray diffraction patterns has a standard deviation of around 16° ($\Delta\phi/2.35$; Aspden, 1981). Figure 7*b*, which shows those fibres that are not oriented in the preferred direction, may provide some explanation for the wide divergence of results which have been previously published. These minority fibres tend to be oriented in postero-cranial and postero-caudal directions which have been described as the preferred directions by other authors who were simply examining the superficial appearance of the ligaments. The techniques we have used are sensitive to fibre orientation throughout the thickness of the ligament and are capable of providing quantitative results. Both these techniques yield comparable results. Though these techniques take no account of the reported bifid nature of the ligament (Heylings, 1978) this does not affect the conclusion drawn here as it is the preferred direction of the collagen fibres throughout the thickness of the ligament that determines the directions in which the tissue can best withstand tensile stress.

Flexion of the spine does not change the preferred orientation of the collagen fibres in the interspinous ligament. However, the angular distribution of fibres increases in width so that at least some of the fibres spread out, rather like the leaves of a fan, when the ligament is stretched by flexion of the spine.

Our results are inconsistent with the interspinous ligaments being reinforced to resist flexion of the spine; however, the ligaments are well adapted for withstanding tension in an antero-posterior direction which is appropriate for transmitting tensile stresses developed in the thoracolumbar fascia. Determination of collagen fibre orientations provides a means of helping to decide between two conflicting views of the function of the ligaments and strongly suggests that their primary role is to anchor the thoracolumbar fascia to the spine.

The pig, in common with man, has a well developed thoracolumbar fascia. In both cases the posterior lamina of this fascia is attached to the tips of the spinous processes and to the interspinous ligaments. The mechanical role of the interspinous ligaments is therefore similar and this is evidenced by their having very similar collagen fibre organisation.

SUMMARY

X-ray diffraction and determination of optical anisotropy show that collagen fibres in pig and human lumbar interspinous ligaments tend to be orientated parallel to the spinous processes. There is a distribution of fibre orientations about this preferred direction. Flexion of the spine does not change the direction of preferred orientation but the angular spread of fibres increases.

This pattern of collagen fibre orientations is consistent with the interspinous ligaments being able to transmit tension from the thoracolumbar fascia to the vertebrae. Since the collagen fibres tend to be aligned antero-posteriorly, they provide a high efficiency of reinforcement in this direction. The lack of fibres orientated perpendicular to the spinous processes will lead to low strength in the cranio-caudal direction so that the ligament can provide little resistance to flexion of the spine.

We thank Miss K. E. Davies for help with photography and Mr M. C. Kirby for making measurements on X-ray diffraction patterns as well as the Medical Research Council for financial support.

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