Tendon and ligament from the horse: an ultrastructural study of collagen fibrils and elastic fibres as a function of age

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A study has been made of the ultrastructural organization of the collagen fibrils and elastic fibres in tendons and ligaments from horses of ages ranging from 2 months premature to 19 years. Diameter distributions of the collagen fibrils in the common digital extensor tendon, the superficial flexor tendon and the suspensory ligament are unimodal in the foetal tissue and at birth, and at these stages of development the three collagenous tissues are virtually indistinguishable. However, at maturity, the ligament and flexor tendon have bimodal distributions similar to that found for rat-tail tendon. The fibril distribution for extensor tendon remains unimodal until the onset of maturity, beyond which the distribution becomes bimodal. Fibril diameter distributions for ligament, extensor and flexor tendon at old age are, as at birth, virtually identical.

An estimate has been made of fibrillar collagen content in the three tissues as a function of age. As with rat-tail tendon, the collagen content increases steadily from birth to maturity, at which stage the content remains fairly constant though it does drop slowly with increasing age.

The presence of well developed elastic tissue in foetal and immature tendon and ligament shows that the development of the elastic fibres does not parallel the development of the collagen fibrils.

In diseased tissues from a 3 year suspensory ligament and an 8.5 year superficial flexor tendon only immature elastic fibres have been observed. Furthermore, since the collagen fibril diameter distributions in these specimens show a marked change from the norm, it would be expected that the mechanical properties of these tissues would be altered significantly.

1. Introduction

Although connective tissues have been studied by electron microscopy for many years, very little quantitative work has been carried out on the transverse structure of collagen fibrils as a function of age. The mechanical properties and growth mechanism of collagenous tissues may ultimately be understood when sufficient data of this type have been collected and interpreted. Towards this end Parry & Craig (1977, 1978) have recently reported the distribution of collagen fibril diameters for rat-tail tendon from birth to senescence, and this paper presents
data on the transverse structure of the common digital extensor tendon, the superficial flexor tendon and the suspensory ligament of the horse. The role of the suspensory ligament and the flexor tendons in the forelegs of a horse is primarily to resist rotation of the fetlock and coffin joints. The extensor muscle (and its tendon) opposes the action of the flexor muscles (and their tendons) thus forming an antagonistic pair which acts as a brace for these joints. These are necessary requirements for the horse to move and bear its own weight.

Further, observations have been made on the polarity of the collagen fibrils and their axial periodicity, the fibrillar collagen content and the development of elastic fibres. Diseased specimens of superficial flexor tendon and suspensory ligament are compared with their healthy counterparts.

![Histograms showing age-dependent changes in the distribution of collagen fibril diameters and in the distribution of intrafibrillar area seen in transverse section. Measurements were taken from electron micrographs of common digital extensor tendon from a horse.](http://rspb.royalsocietypublishing.org/)
2. Collagen fibril diameter distributions

Since rat-tail tendon had been shown to have a unimodal fibril distribution at birth (Parry & Craig 1977) but a bimodal distribution from a time shortly after birth through maturity to senescence (Parry & Craig 1978) it was of considerable interest to see whether the two tendons and one ligament from horse had similar fibril diameter distributions. The specimens used in this study were removed from the left foreleg of thoroughbred horses within a few hours of death and each was prepared for electron microscopy using the procedures described earlier by Parry & Craig (1977, 1978). Thin transverse sections, post-stained with uranyl acetate and lead citrate, were examined with a Philips EM 200 electron microscope. A replica grating with 2160 lines/mm was used to calibrate the magnification of the microscope. Prints were prepared at a standard magnification of ×65 000 and all measurements were taken directly from the prints.

The distribution of fibril diameters and the distribution of area enclosed by the fibrils are shown in the histograms in Figure 2. Each histogram shows the number of fibrils and the percentage of the total area covered as a function of fibril diameter. The histograms are presented for different age groups: 0.2, 1, 3, 5, and 10 years.

**Figure 2.** Histograms showing age-dependent changes in the distribution of collagen fibril diameters and in the distribution of intrafibrillar area seen in transverse section. Measurements were taken from electron micrographs of superficial flexor tendon from horse.
collagen fibrils for the extensor tendon, the flexor tendon and the ligament are given in figures 1, 2 and 3 respectively (see also tables 1, 2 and 3). A number of features may be noted from these histograms. (a) At birth, the mean diameter and the distribution of diameters of the collagen fibrils are virtually identical for the extensor tendon, the flexor tendon and the ligament. The distribution of diameters,

![Histograms showing age-dependent changes in the distribution of collagen fibril diameters and in the distribution of intrafibrillar area seen in transverse section. Measurements were taken from electron micrographs of suspensory ligament from horse.](http://rspb.royalsocietypublishing.org/)

although unimodal, is broad and in this respect differs from rat-tail tendon which has a sharp unimodal distribution at birth (Parry & Craig 1977). (b) At maturity (ca. 5 years), the extensor tendon still has a unimodal distribution of diameters (figure 4a, plate 1) though the average diameter has increased considerably. In contrast, the flexor tendon and the ligament (figure 4b) clearly have bimodal distributions and are thus similar in form to that found in mature rat-tail tendon. (c) At late maturity, approaching senescence, the fibril diameter distributions for the extensor tendon, the flexor tendon and the ligament are once again almost identical and each is bimodal in form. Rat-tail tendon at senescence exhibited a very similar fibril distribution, the cause of which may be due in part to the breaking down of the larger collagen fibrils (Parry & Craig 1977). A similar explanation may be valid in the case of the three horse specimens discussed here (see, for example, the histogram for the 5 year extensor tendon which shows evidence of the formation of many new small fibrils).
Figure 4. Transverse sections of (a) common digital extensor tendon, and (b) suspensory ligament taken from a 5 year horse. The electron micrographs show the unimodal and bimodal distributions of fibril diameters for (a) and (b) respectively. Sections stained with uranyl acetate and lead citrate. (Magn. x 55000.)
Figure 5. (a–c) Transverse sections of elastic fibres from horse tendon. The growth of the fibres is characterized by initial production of the microfibrillar component with subsequent growth of the elastin core relative to the microfibrillar component. The diameter of the microfibrils is ca. 135Å. Sections stained with uranyl acetate and lead citrate. (Magn. × 75,000.)

(d) Transverse section of typical elastic fibres from the 3 year ligament of a horse suffering from laminitis. The elastin core of the elastic fibres is much reduced in size and is, in many cases, completely absent leaving the elastic fibre composed entirely of the microfibrillar component. Sections stained with uranyl acetate and lead citrate. (Magn. × 75,000.)
In the bimodal distributions, the inner peak was designated A and the outer peak B (Parry & Craig 1977, 1978). For the flexor tendon and the ligament it is possible to assess the percentage of the fibrils in both the A and B peaks (tables 2 and 3). The percentage of the fibrils in the A peak appears to increase steadily from birth while the percentage of fibrils in the B peak decreases concomitantly.

**Table 1. Structural information on collagen fibrils and elastic fibres in the common digital extensor tendon of horse**

<table>
<thead>
<tr>
<th>age</th>
<th>mean diam. of collagen fibrils (Å)</th>
<th>total no. of measurements of collagen fibril diameters</th>
<th>mean no. of fibrils per print†</th>
<th>percent-age of area covered by collagen fibrils in transverse sections devoid of cellular components</th>
<th>length of D-period (Å)</th>
<th>mean diam. of micro-fibrillar component of elastic fibres (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>year</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1050</td>
<td>700</td>
<td>460</td>
<td>48</td>
<td>675</td>
<td>140</td>
</tr>
<tr>
<td>1.5</td>
<td>1700</td>
<td>736</td>
<td>245</td>
<td>58</td>
<td>690</td>
<td>136</td>
</tr>
<tr>
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<td>1850</td>
<td>2100</td>
<td>200</td>
<td>65</td>
<td>620</td>
<td>125</td>
</tr>
<tr>
<td>5</td>
<td>1650</td>
<td>865</td>
<td>290</td>
<td>72</td>
<td>675</td>
<td>130</td>
</tr>
<tr>
<td>19</td>
<td>1600</td>
<td>1516</td>
<td>250</td>
<td>66</td>
<td>660</td>
<td>130</td>
</tr>
<tr>
<td>250  and 1750</td>
<td>952</td>
<td>2000</td>
<td>51</td>
<td>690</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Prints are produced at a magnification of × 65000 and are each 20 cm × 20 cm.

The distribution of the areas occupied by the collagen fibrils in transverse sections have been calculated from the fibril diameter distributions and plotted in figures 1, 2 and 3. The dominant feature of these area distributions, which are of course proportional to the volume and mass distributions, is that although the percentage of collagen fibrils in the A peak is often very high, the area (i.e. mass) associated with these fibrils is usually fairly small. The significance of these features in terms of the mechanical attributes of a tendon or ligament are discussed in the following paper (Parry, Barnes & Craig 1978).

3. **Longitudinal structure of collagen fibrils**

The axial period of stained collagen fibrils (the D-period) has a value in vivo of 668 Å† as determined by X-ray diffraction (A. Miller, D. A. D. Parry & J. S. Wray, unpublished data). The D-periods of all specimens studied in this work, which were measured in longitudinal sections cut perpendicular to the long axes of the collagen fibrils, had means of ca. 670 Å. The electron microscope procedures

† 1 Å = 10⁻¹⁰m = 10⁻¹ nm.
Table 2. Structural information on collagen fibrils and elastic fibres in the superficial flexor tendon of horse

<table>
<thead>
<tr>
<th>age year</th>
<th>mean diameter of collagen fibrils/Å</th>
<th>percentage of collagen fibrils in</th>
<th>percentage of area covered by collagen fibrils in</th>
<th>total no. of measurements of collagen fibril diameters</th>
<th>mean no. of collagen fibrils per print†</th>
<th>mean diameter of microfibrillar component of elastic fibres Å</th>
<th>length of D-period Å</th>
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<tr>
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<td>0–100 0–100</td>
<td>0–100 0–100</td>
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<td>47</td>
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<td>133</td>
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<tr>
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<td>55–60 40–45</td>
<td>10 90</td>
<td>1378 460</td>
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<tr>
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<td>350 1650</td>
<td>90 10</td>
<td>30 70</td>
<td>1293 1300</td>
<td>49</td>
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<tr>
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<td>90 10</td>
<td>25 75</td>
<td>1116 1250</td>
<td>48</td>
<td>655</td>
<td>135</td>
</tr>
<tr>
<td>8.5‡</td>
<td>(1650) (1650)</td>
<td>(0–100) (0–100)</td>
<td>(0–100) (0–100)</td>
<td>(720) (200)</td>
<td>(49)</td>
<td>(400–900)</td>
<td>—</td>
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<tr>
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<td>250 1750</td>
<td>95 5</td>
<td>40 60</td>
<td>1031 2000</td>
<td>40</td>
<td>670</td>
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</tr>
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</table>

† Prints are produced at a magnification of ×65 000 and are each 20 cm x 20 cm.
‡ Specimen taken from a horse suffering from the condition known as 'contracted tendon'.
Table 3. Structural information on collagen fibrils and elastic fibres in the suspensory ligament of horse

<table>
<thead>
<tr>
<th>age (year)</th>
<th>mean diameter of collagen fibrils (Å)</th>
<th>percentage of collagen fibrils in</th>
<th>percentage of area covered by collagen fibrils</th>
<th>total no. of measurements of collagen fibrils</th>
<th>mean no. of cellular components per print†</th>
<th>length of D-period (Å)</th>
<th>mean diameter of microfibrillar component of elastic fibres (Å)</th>
</tr>
</thead>
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<tr>
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<td>B peak</td>
<td>A peak</td>
<td>B peak</td>
<td>A peak</td>
<td>B peak</td>
<td></td>
</tr>
<tr>
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<td>1100</td>
<td>1100</td>
<td>0–100</td>
<td>0–100</td>
<td>0–100</td>
<td>0–100</td>
<td>879</td>
</tr>
<tr>
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<td>400</td>
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<td>50</td>
<td>50</td>
<td>5</td>
<td>95</td>
<td>1186</td>
</tr>
<tr>
<td>3‡</td>
<td>(250)</td>
<td>(1600)</td>
<td>(95)</td>
<td>(5)</td>
<td>(45)</td>
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<td>(888)</td>
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<tr>
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<tr>
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<td>1750</td>
<td>90–95</td>
<td>5–10</td>
<td>30</td>
<td>70</td>
<td>825</td>
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</tbody>
</table>

† Prints are produced at a magnification of ×65000 and are each 20 cm × 20 cm.
‡ Specimen taken from a horse suffering from 'laminitis'.
for the horse tendons and ligament have therefore not affected the axial periodicity of the collagen fibrils. In rat-tail tendon, the observed D-period was ca. 610Å from birth to the onset of maturity (13–14 weeks) but ca. 670Å at about 13 months and 2 years (Parry & Craig 1977, 1978).

The collagen fibrils in the extensor tendon, the flexor tendon and the ligament all exhibit a polar banding pattern and furthermore, there is no relation between the directionality of neighbouring collagen fibrils, i.e. the fibrils randomly point ‘up’ or ‘down’, as found in rat-tail tendon.

4. **Fibrillar collagen content**

Parry & Craig (1978) have used a method by which the percentage of collagen in a transverse section largely devoid of cellular material can be estimated. Such values have been calculated as a function of age for the extensor tendon, the flexor tendon and the ligament (tables 1, 2 and 3). Several sources of error are inherent in this type of calculation but the trend in results with age should remain valid (Parry & Craig 1978). In rat-tail tendon, the percentage of tendon that is collagenous is known as a function of age (Torp, Baer & Friedman 1975) but no corresponding figures are available for horse tendon or ligament. From the data on fibrillar collagen content in regions devoid of cellular material, it is apparent that the content for both extensor tendon and ligament increases steadily from birth until maturity (tables 1 and 3), beyond which the content drops slightly. For flexor tendon, the fibrillar collagen content remains constant until late maturity, when it decreases. If the cellular content of these tissues is about 50% at birth and only a small percentage at maturity (as in rat-tail tendon), then the true collagen content would vary from about 25% at birth for the three tissues studied to values at maturity of 50%, 60% and 70%, respectively for flexor tendon, ligament and extensor tendon (see tables 1, 2 and 3).

5. **Elastic fibres**

Elastic fibres are found in most connective tissues and, although often present in minor quantity, are an important constituent. The elastic fibres consist of a central core of elastin and a peripheral layer composed of a loose arrangement of microfibrils (Greenlee, Ross & Hartman 1966). These microfibrils have a measured diameter of 100Å (Ross & Bornstein 1969), 130Å (Fahrenbach, Sandberg & Cleary 1966), or 140Å (Parry & Craig 1977, 1978). In this work the microfibrillar component of the elastic fibres of common digital extensor tendon, superficial flexor tendon and suspensory ligament had a mean diameter of 135Å (tables 1, 2 and 3). The variance between these values may be attributed to the staining and measurement procedures employed.

It is known that immature elastic fibres are composed entirely of a microfibrillar component and that as the development of the fibre proceeds, a central
amorphous core of elastin is laid down (Greenlee, Ross & Hartman 1966; Greenlee & Ross 1967). Immature and mature elastic fibres from horse tendon are shown in figure 5(a–c), plate 2.

The largest elastic fibres are usually found in the vicinity of cellular components or at the periphery of the tendonous mass while the smaller elastic fibres are more often found within the bulk of the collagenous tissue.

![Diagram showing fibril distribution](image)

**Figure 6.** Histograms showing the distributions of collagen fibril diameters and the distributions of intrafibrillar area seen in transverse section for 5 year superficial flexor tendon from a normal horse and for 8.5 year superficial flexor tendon from a horse suffering from the condition known as ‘contracted tendon’. The fibril diameter distribution for the diseased specimen is unimodal whereas normal superficial flexor tendon is bimodal. The fibrillar collagen content in the ‘contracted tendon’ is close to that expected for normal superficial flexor tendon.

Well developed elastic fibres, having a large elastin component, are frequently found in foetal and immature tendon and ligament from the horse. It then follows that the development of the elastic fibres in horse tendon and ligament does not parallel the development of the collagen fibrils.

6. Diseased superficial flexor tendon

A superficial flexor tendon of an 8.5 year horse suffering from the condition known as ‘contracted tendon’ was so affected by the disease that the horse could barely walk and was consequently destroyed. The aetiology of this disorder, although unknown, may lie in the superficial flexor tendon, its muscle or in abnormal bone growth.

The fibril diameter distribution of this tendon was measured and shown to be unimodal whereas that for normal flexor tendon is bimodal (figure 6). However, the percentage area of the tendon occupied by collagen was the same as that expected from normal tendon (table 2). It follows that the cause of the anomalous unimodal distribution of fibril diameters lies in some factor other than the total collagen content.
Longitudinal sections of the 'contracted' flexor tendon showed that many fibrils were ruptured and that the D-period varied widely from 400 to 900Å.

In addition, transverse sections indicated that the tendon was almost completely devoid of elastic fibres and much of the damage in the connective tissue may ultimately be associated with this factor. It may be concluded that the main features of this tendon were the anomalous unimodal distribution of fibril diameters, the variability of the D-period and the greatly reduced number of elastic fibres. These factors were undoubtedly related to the changed mechanical properties of the superficial flexor tendon in the 'contracted' specimen.

![Histograms showing the distribution of collagen fibril diameters and the distributions of intrafibrillar area seen in transverse section for 1 and 5 year suspensory ligament from normal horses and for 3 year suspensory ligament from a horse suffering from laminitis. A marked reduction in the magnitude of the B peak relative to the A peak is apparent in the ligament from the diseased horse. Also, the fibrillar collagen content of the suspensory ligament from the diseased horse is about two-thirds of that expected.](image)

**Figure 7.** Histograms showing the distribution of collagen fibril diameters and the distributions of intrafibrillar area seen in transverse section for 1 and 5 year suspensory ligament from normal horses and for 3 year suspensory ligament from a horse suffering from laminitis. A marked reduction in the magnitude of the B peak relative to the A peak is apparent in the ligament from the diseased horse. Also, the fibrillar collagen content of the suspensory ligament from the diseased horse is about two-thirds of that expected.

**7. Diseased suspensory ligament**

A suspensory ligament from a 3 year horse suffering from laminitis was studied as previously described. The fibril diameter distribution is similar in general form to that expected from a ligament of that age (see figure 7). However, the percentage of the fibrils in the B peak in the diseased ligament is only about 5% compared with the expected value of about 50% (table 3). In addition, the fibrillar collagen content is about 40% which is about two-thirds of the expected value.

The elastic fibres in the ligament are, in many instances, composed entirely of the microfibrillar component. The elastin core is invariably missing or much reduced in size (figure 5d).
The axial structure of the collagen fibrils is normal and there is no evidence of the specimen having suffered excessive axial strain since the fibrils remain intact and have the usual D-period of ca. 670Å.

In this case, the diseased ligament is characterized by an unusual collagen fibril distribution, a low collagen content and elastic fibres similar to those seen in their early stages of development. These features imply a modified mechanical property for the ligament.

8. Discussion

The results described in this work have yielded information on the growth and development of fibrils in three type I collagen tissues from the horse. At birth, the fibril diameter distributions are all unimodal, the collagen contents are similar and the mean diameters are the same. At maturity, only the common digital extensor tendon fibril distribution has remained unimodal whereas those from the superficial flexor tendon and the suspensory ligament are both bimodal. The bimodal distribution at senescence may arise from fibril breakdown.

The significance of the unimodal and bimodal peaks in the fibril diameter distributions is discussed in the following paper (Parry, Barnes & Craig 1978) in relation to the distributions from other collagenous tissues. The results from the two diseased specimens imply that the mechanical properties of connective tissue are intimately related to the collagen fibril diameter distribution, to the fibrillar collagen content and the presence (or absence) of elastic fibres.

The authors are grateful to Mr R. Parsons for his considerable help and expertise in the preparation of the histograms.

References

(following paper).