We studied the vascular pattern of human posterior tibial tendons by injection techniques and immunohistochemically using antibodies against laminin. The intravascular volume of the posterior tibial tendon was determined using a new method of injection of a solution of $^{99m}$Tc and gelatin ink into the lower legs of cadavers. Three segments of 1 cm length from different regions of the human posterior tibial tendon were measured using a gamma well counter.

The main blood supply arises from the posterior tibial artery. Blood vessels enter the paratenon of the posterior tibial tendon via a mesotenon from the posterior aspect. From the paratenon, the blood vessels penetrate the posterior tibial tendon and Anastomose with a longitudinally orientated intratendinous network. The number of vessels in the substance of the tendon is consistently less than that in the surrounding paratenon. The distribution of blood vessels within the posterior tibial tendon is not homogeneous. In the retromalleolar region the intravascular volume was significantly reduced with a mean value of $15 \mu l/g$ tendon tissue. There was no significant difference between the mean intravascular volumes of the proximal and distal areas (distal, $27.7 \mu l/g$ tendon tissue; proximal, $30 \mu l/g$ tendon tissue). The immunohistochemical investigation showed that there was no immunostaining for laminin in the anterior part of the tendon in the region where it passes behind the medial malleolus. This region is avascular.

The most frequent site of rupture of the posterior tibial tendon is in the region behind the medial malleolus. A potential endogenous risk factor may be the limited healing potential of avascular tissue.

Recent clinical studies have shown that dysfunction of the posterior tibial tendon may cause flat foot, chronic pain and the need for surgical treatment. Reduced vascularity is an important factor contributing to degeneration and possible rupture of the tendon under strain.

There are very few reports of the distribution of blood vessels in the posterior tibial tendon. Frey, Shereff and Greenidge described the microvasculature qualitatively using conventional injection methods such as the Spalteholz technique. They found no evidence for an avascular zone but suggested that the tendon has a hypovascular zone in the retromalleolar region. However, qualitative data obtained by analysing vascular injections of tissues cleared by the Spalteholz technique are highly subjective and such techniques may give false-positive and false-negative results.

Laminin is a basic component of the basement membrane and immunohistochemical staining reliably detects blood vessels in dense connective tissue.

We therefore assessed the intravascular volume of the posterior tibial tendon in cadavers by injection of $^{99m}$Tc and studied the vascular pattern using immunohistochemical methods.

Materials and Methods

We obtained the cadavers from the Departments of Anatomy and Pathology of the Christian-Albrechts University of Kiel. All the tendons studied were free from macroscopically visible degenerative changes. 

Techniques of injection. The lower legs of the fresh frozen cadavers whose mean age was 67 years (47 to 83) were injected with a solution of 20 MBq of $^{99m}$Tc, Indian Ink (1%) and gelatin (10%) simultaneously into the anterior and posterior tibial arteries and into the peroneal artery under continuous manual pressure at a temperature of 37°C for the limb and the injection solution. The injection pressure (120 mmHg = 160 Pa) which was similar to arterial blood...
pressure was controlled by a pressure gauge. We performed clinical evaluation and radiography of the lower limb in two planes to exclude specimens with major arteriosclerosis from the study.

The tendons were dissected after injection and the paratenon and, if necessary, the musculature were removed. The tendon was cut into three segments each of 1 cm in length (Fig. 1). These sections were placed in test-tubes and weighed. We measured first the activity (counts per minute, cpm/μl) of the injection solution in a gamma well counter (Multilogger LB 5310; Dr Berthold, Hannover) and then the activity (cpm/mg) of the test-tubes. After correction for the half-life time of $^{99m}$Tc the injected intravascular volume ($\mu l/g_{tendon}$) was calculated as follows:

$$t_0cpm \text{ test tube} \times (\frac{\mu l_{injection-solution}}{cpm_{inj-sol}} \times \frac{g_{test \text{ tubes}}}{t_0cpm_{test \text{ tube}}})$$

$$= \mu l_{intravascular \text{ volume}}/g_{tendon}$$

As a control group ten other cadavers with a mean age of 72 years (45 to 87) were injected with the same medium using the same protocol as for measurement of the intravascular volume. They were analysed macroscopically and cleared according to the method of Spalteholz.

For statistical analysis of the results, we used the Wilcoxon test.

**Immunohistochemical studies.** We obtained 20 posterior tibial tendons together with their bony attachment sites at post-mortems performed within 48 hours after death. The age of the subjects ranged from 39 to 80 years. The tendons were divided into 2 cm segments (Fig. 1) and the samples were shock-frozen. We used a monoclonal laminin antibody (Chemicon, Hamburg, Germany) and the respective secondary antibody, fluorescein-thiocyanate-conjugated (FITC) goat anti-rabbit IgG (Medac, Hamburg). Control sections were labelled only with the FITC-conjugated antibody. We used positive controls including a tissue with defined antigen sites (skeletal muscle). Five sections of each segment were evaluated qualitatively by fluorescence microscopy. The presence of positive immunoreactions in each quarter of the tendon was noted. If there was one positive immunoreaction, it was counted as a positive result.

**Results**

**Macroscopic anatomy.** Examination of the injected specimens showed that the major blood supply of the posterior tibial tendon was from the posterior tibial artery. Most of the tendon was covered by a paratenon in which the blood vessels formed a web-like network. From the paratenon,
they penetrated the tendon tissue and anastomosed with an intratendinous arterial network which was inhomogeneous. In the region where the tendon passed around the medial malleolus, the longitudinally orientated intratendinous vascular network was interrupted and the tendon was avascular in the anterior part, directed towards the malleolus (Fig. 2).

Measurements with radionuclides. The mean intravascular volume varied from one segment to another (Fig. 3). The middle segment had the lowest volume (15.05 μl/g, SD 4.7) whereas in the proximal and distal segments the volumes were 30.02 μl/g (SD 7.9) and 27.68 μl/g (SD 4.8), respectively. The Wilcoxon test showed that the differences between segments I and II and III and II are highly significant.

Immunohistochemical findings. The immunohistochemical demonstration of laminin in the wall of blood vessels was positive in all the examined tendons. All negative controls remained negative. The findings confirmed those of the injection technique. The posterior tibial tendon was covered by a well-vascularised paratenon. Within the tendon, the blood vessels were located in the loose connective tissue between the bundles of longitudinal fibres.

The distribution of blood vessels within the posterior tibial tendon was inhomogeneous (Fig. 4, Table I). There was no immunohistochemical evidence of laminin in the anterior half of the segment which was located behind the medial malleolus (segment II in Figure 1). Positive immunoreaction could only be found in the posterior quarter of the retromalleolar gliding zone (Table I).

Discussion

Many attempts have been made to clarify the natural history of the spontaneous rupture of tendons. In most, degenerative changes can be found with no distinct aetiological explanation. A key factor in the degeneration of tendon tissue may be hypoxia.

Our observations on the gross blood supply of the posterior tibial tendon agree with previous findings. There has been only one previous study on the intratendinous blood supply of the posterior tibial tendon. Our observations on the distribution of blood vessels within the posterior tibial tendon agree with previous findings.

Table I. The total number of cases with positive immunoreactivity in the various segments of the tendon

<table>
<thead>
<tr>
<th>Tendon quarter</th>
<th>Segment</th>
<th>II (retromalleolar)</th>
<th>III (distal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (proximal)</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>9</td>
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<tr>
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Immunohistochemically stained tendon (FITC conjugated antibody ×14).
supply of the posterior tibial tendon in which a hypovascular zone was found. Qualitative data obtained by analysing vascular injections of tissues cleared by the Spalteholz technique are highly subjective. Our aim therefore was to study quantitatively the vascular status of the posterior tibial tendon by determination of the intravascular volume using a radioisotope method. Our study has shown that the tendon has a significantly reduced intravascular volume in the retromalleolar region compared with that in the distal and proximal areas. Injection techniques, however, have to be interpreted with caution because of false-negative or false-positive results.

Laminin is a basic component of the basement membrane and immunohistochemical staining reliably detects blood vessels in dense connective tissue. Our study indicated that there was no laminin within the avascular zone. The discrepancy between our results and those of Frey et al may arise from the accuracy of the methods used.

Avascular zones consisting of fibrocartilage occur in several gliding tendons in regions where the tendons run around a bony pulley. The posterior tibial tendon is a typical gliding tendon and Vogel et al have shown that the area where this tendon changes its direction resembles fibrocartilage with a high content of acid glycosaminoglycans. The location of the avascular zones found in our study corresponds to these fibrocartilaginous areas.

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References