BIOMECHANICAL CHARACTERIZATION OF TISSUES IN DUPUYTREN'S DISEASE

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The aim of this study was to characterize mechanical properties of tissues of Dupuytren's disease and to attempt to identify changes due to cellular activity. Tensile tests confirmed the heterogeneity of Dupuytren's disease tissue with distinct stress-strain curves for the three tissue types normally present, namely, cord, transition zone and nodule. The tensile strength for cord tissue was nearly twice that of nodule tissue, but the latter was nearly twice as stretchable as cord. In contrast, the transition tissue had the tensile strength of cord with the stretchability of nodule. It was found that tensile loading stimulated a cellular response as demonstrated by an increase in the creep strain rate of the tissue at 37°C compared with that at 4°C using Dupuytren's tissue in an in vitro culture test. The creep strain rate for nodule at 37°C was more than seven times that for cord at a nominal creep stress of 0.75 MPa.

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Dupuytren's contracture is a curious disease process in which a cellular connective tissue matrix is deposited at specific anatomical sites in and around the palmar fascia system of the hand. The anatomical location, biological behaviour and treatment have been reviewed by McFarlane et al (1990).

Controversies in treatment have centred around whether it is necessary to excise the cellular masses, clinically termed nodules and cords, or whether it is sufficient to simply divide the tissues to release the contractures (Badois et al, 1993; McGrouther, 1988). Messina and Messina (1991) have developed an innovative approach to treatment by fitting an external fixator to the hand to apply slow traction to the contracted tissue, resulting in elongation of the contracted tissues and improvement in the results of subsequent surgery. Since collagen fibres are virtually inextensible under sub-rupturing loads it seems feasible that this mechanical loading stimulates collagen remodelling, presumably via local cellular activation. Evidence for such an activation of remodelling has been reported as elevated levels of matrix metalloproteinases are present in Dupuytren's tissues, following the Messina treatment (Bailey et al, 1994).

Cord and nodule regions of Dupuytren's tissue are well recognized at gross and histological levels and an intermediate "transition" zone (fleshy bands) has been identified between the two (Flint et al, 1982). The aims of the present study were first, to characterize the mechanical properties of the three distinct Dupuytren's tissues, and second, to develop an in vitro model of Messina's elongation of Dupuytren's tissue, based on the application of constant known mechanical loads to test the creep properties of the tissue. The investigation was centred on how mechanical loading can stimulate cellular responses which themselves result in alterations in the mechanical properties of the tissue. Such information is essential before detailed analyses of associated biochemical changes in the elongated matrix can be performed, which are reported in the following paper. (Tarlton et al, 1998).

MATERIALS AND METHODS

Creep tests

Tissue samples were obtained from 14 patients (average age, 63.2 years) with Dupuytren's disease. The duration of the disease ranged from 9 months to 30 years. The tissue obtained after fasciectomy normally consisted of the cord and/or nodule excised in its entirety. The size of the tissue varied both in cross section and length from patient to patient. The tissue was kept moist for transport, wrapped in a sterile swab moistened with saline and stored at 4°C until tested. The storage time was kept to a minimum but was no longer than 24 hours.

Before testing, the tissue was examined macroscopically and any fatty tissue removed. Zones of tissue were identified and segregated at this stage to give cord, nodule and transition zones (Fig 4a). A cutter block, as shown in Figure 1a, was then used to divide the tissue into manageable strips in a clean environment. The tissue was placed lengthwise on the cutters with the predominant fibre disposition parallel with the blades. A polypropylene cylinder was used to press the tissue into the blades using a rolling action, producing two parallel strips, 1.5 mm thick for each of the creep tests at 4°C and 37°C. These strips were used full width to avoid any further disruption to the fibres and by testing adjacent strips, the effect due to material heterogeneity was minimized. Width measurements were made using a Nikon profile projector with a 20× magnification.

In order to make reliable measurements of creep properties of Dupuytren's tissues over prolonged time periods it was necessary to design and produce a custom device. Critical to this instrument was the development of a mechanically sound means of tissue attachment. This consisted of two clamping plates, held together by a screw and drilled to allow the tissue to be passed between the plates and out (Fig 1b). This allowed a mechanically secure fixation to be made with minimal stress concentrations. Each sample was then mounted into the creep

device (Fig 1c) in such a way that the tissue was fully immersed in the culture medium, anchored at the lower end and attached to a pivoting bar at the upper end. After allowing the tissue to reach the test temperature, predetermined weights, corresponding to a stress level of either 0.5 or 0.75 MPa, were added to the opposite end of the pivot to produce the desired load and hence stress level. Any displacement under load was monitored electronically using a linear voltage differential transformer (LVDT) and recorded as displacement-time curves on an analogue chart recorder.

One sample strip from each specimen was maintained in a humidified incubator at 37°C for 18 h throughout the test and the other in a refrigerator at 4°C. Specimens were maintained in Dulbecco's modified Eagle's medium (DMEM, Paisley, Scotland) supplemented with penicillin (500 U/ml) and streptomycin (500 µg/ml).

Tensile testing

The remaining tissue, after two adjacent specimens were obtained for creep testing, was further divided using the cutter block. By repeating the cutting process on appropriate cutter blocks, strips of tissue of a given width were obtained. Thirty-eight samples from 12 patients were tested in tension. The average age of the patients was 63.7 years and the duration of the disease varied from 9 months to 30 years. The clamping system for creep testing was used to mount the specimens for tensile testing in a Testometric 220M screw testing machine fitted with a 50 N load cell. An extension rate of 2 mm/min was used throughout the tensile tests. The gauge length for the tests varied depending on the length of each specimen. All tensile tests were done at room temperature with the specimen immersed in phosphate buffered saline at pH 7. Extension of the specimen under load was monitored with a LVDT with a 10 mm stroke and load-extension curves obtained from an XY plotter.

RESULTS

Creep tests

Tissue from the 14 patients yielded ten cord specimens and four nodule specimens. Six of the cord specimens were tested at a stress of 0.75 MPa and four at 0.5 MPa. The nodule specimens were equally divided between the

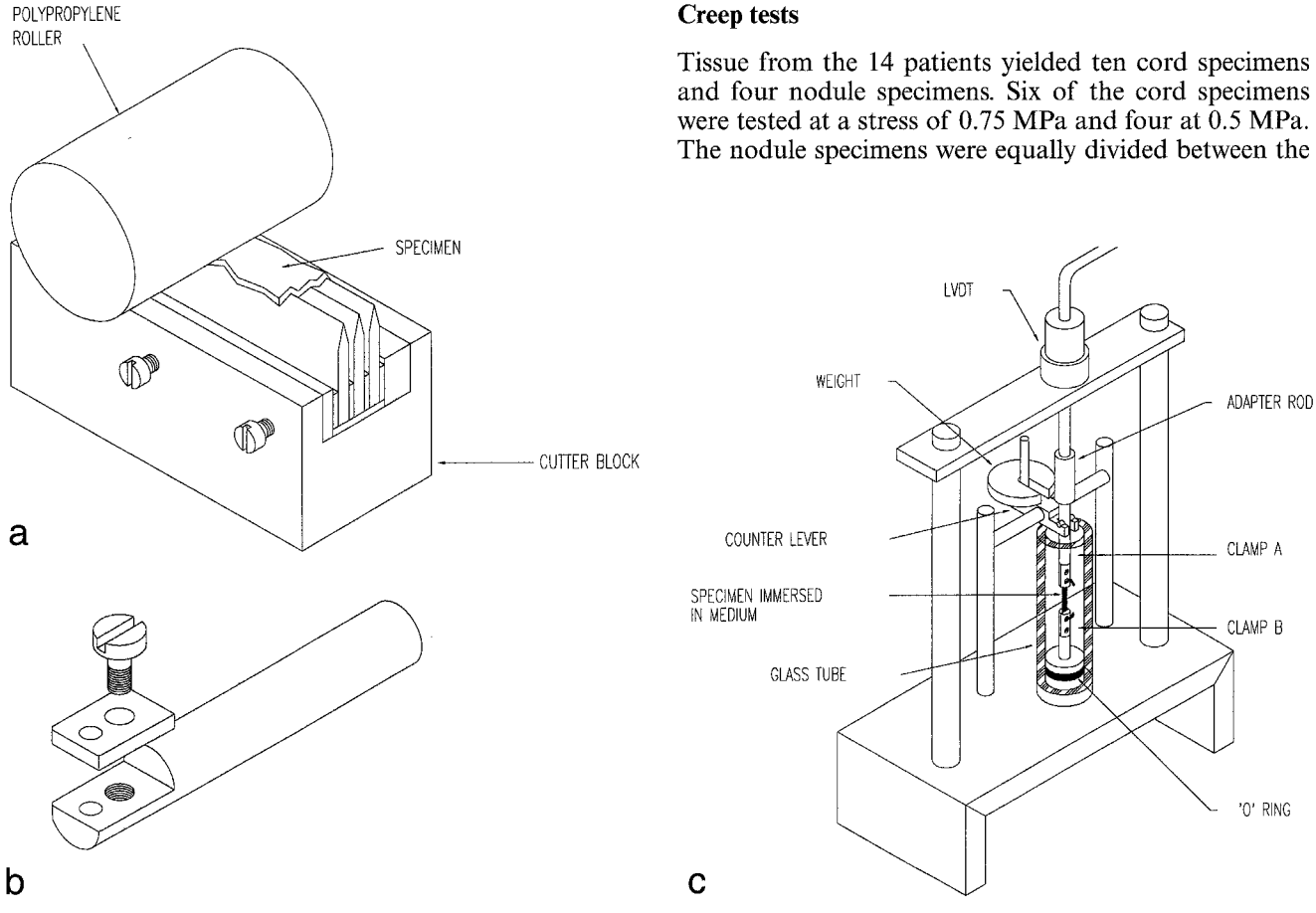


Fig 1 (a) Roller cutting device used to prepare strips of Dupuytren's tissue of fixed thickness for mechanical analysis. (b) Clamping assembly to retain the Dupuytren's tissue between the creep instrument jaws. (c) Creep testing device showing the mounting arrangement for the specimen held in a bath of culture medium and retained at 37°C. The LDVT mounted above the specimen pivot detected any movement of the beam, feeding the displacement to a chart recorder.

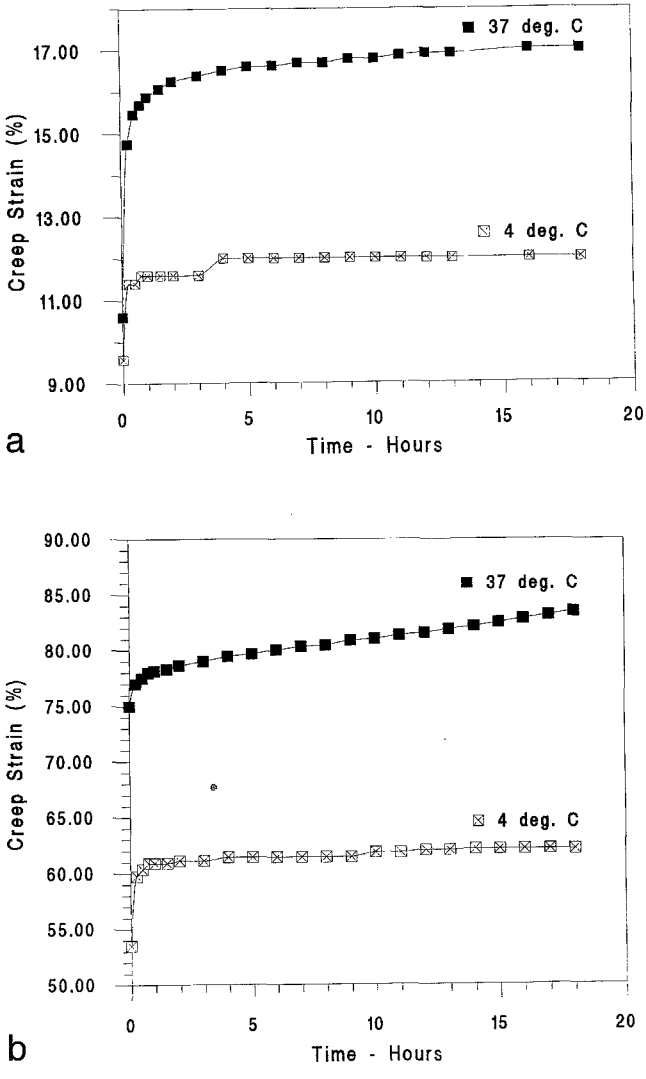
two stresses. To enable direct comparisons among the specimens, the extension-time curves from the chart recorder were converted to creep strain-time curves. Figures 2a and 2b show the typical creep strain-time curves for cord and nodule from different patients at 0.75 MPa, tested at 4°C and 37°C, respectively.

These curves displayed the characteristic primary (representing the initial stretch of the tissue under load), secondary (representing the effect of creep) and tertiary (representing tissue failure) phases of creep curves (Fig 2c). During the 18-h test only the primary phase, which lasted for 4 to 5 hours, and secondary phase were observed in all specimens tested. However, for one nodule specimen tested at 0.75 MPa, the creep curve had all three phases and failed after 12.25 h at 37°C. At 4°C the secondary phase for cord showed no increase in strain whereas at 37°C there was a gradual increase in strain with time. The trend was similar in the nodule except that there was a perceptible increase in strain with time at 4°C and a marked increase at 37°C. For comparison, the strain at 0.25 hour (representing the instantaneous strain) and the gradient of the secondary phase (representing the creep rate) for each specimen were measured. For creep stress of 0.5 MPa, the average creep strain rates per hour for cord and nodule were 0 and 0.08% at 4°C and 0.14% and 0.36% at 37°C respectively. Increasing the stress to 0.75 MPa had no effect on the creep strain rates of cord and nodule at 4°C and the creep rates at 37°C were 0.05% and 0.36% respectively. The ratios of creep strain rate for nodule to cord at 37°C were 2.5 ($P = 0.112$) for stresses of 0.5 MPa and 7.2 ($P = 0.0002$) for stresses of 0.75 MPa. The creep strain rate ratios for other test parameters were all statistically significant except for that of stress level, 0.75 MPa to 0.5 MPa, for nodule at 37°C. The ratios for the instantaneous strain for all the test parameters were not statistically significant.

Figure 3 shows the histogram comparing the creep rates at 4°C and 37°C for both tissues at stresses of 0.5 and 0.75 MPa respectively.

Tensile tests

Four distinctive tissue types were observed macroscopically from the 38 specimens tested in tension. These were: nodule (ten), nodule-nodule transition region (two), nodule-cord transition region (five) and cord (21). Although not all samples contained clearly defined transition tissues, it was fortuitous in this study that all four types of tissue were present in one patient. Figure 4a shows a schematic representation of that tissue. Figures 4b, c and d show the stress-strain curves obtained from the four types of tissue derived from the same patient; these were typical of curves for the respective tissue zones obtained from other patients.



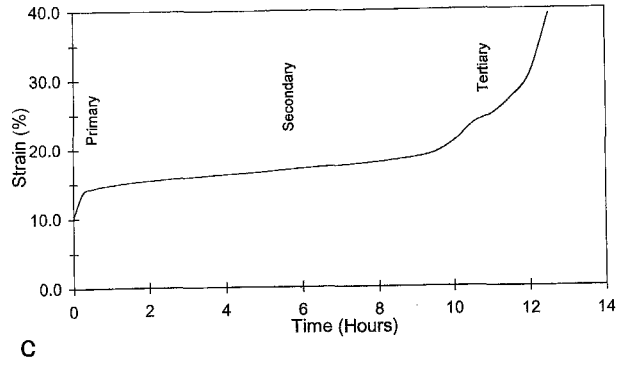


Fig 2 (a,b) Representative traces of creep tests for cord and nodule tissues, respectively measured at 37°C and 4°C and 0.75 MPa. Selected from traces of two patients. (c) Characteristic creep curve showing the primary, secondary and tertiary phases.

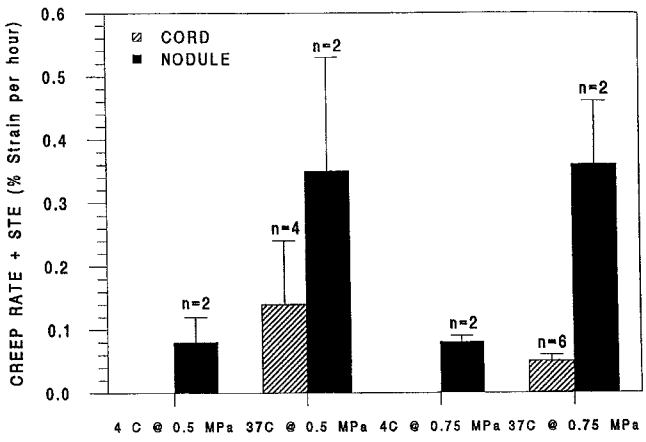


Fig 3 Histogram comparing the mean creep rates of cord and nodule tissues at 4°C and 37°C for stresses of 0.5 MPa and 0.75 MPa respectively. (n = number of samples and bar represents standard error).

Two striking features emerged from this family of curves:

1. There was a saw tooth failure mode of the nodule, which suggested a random fibre orientation in the tissue. This feature was most pronounced in the nodule and least evident in the cord.
2. The initial toe region was far more pronounced in cord and transition-cord (over the same stress range) than for nodule and transition-nodule (note the scale on the stress axis). The sequence was cord ≥ transition-cord > transition-nodule ≫ nodule consistent with greater extension in the nodule.

Two parameters were chosen from the load-extension curves, the maximum tensile strength and the strain to

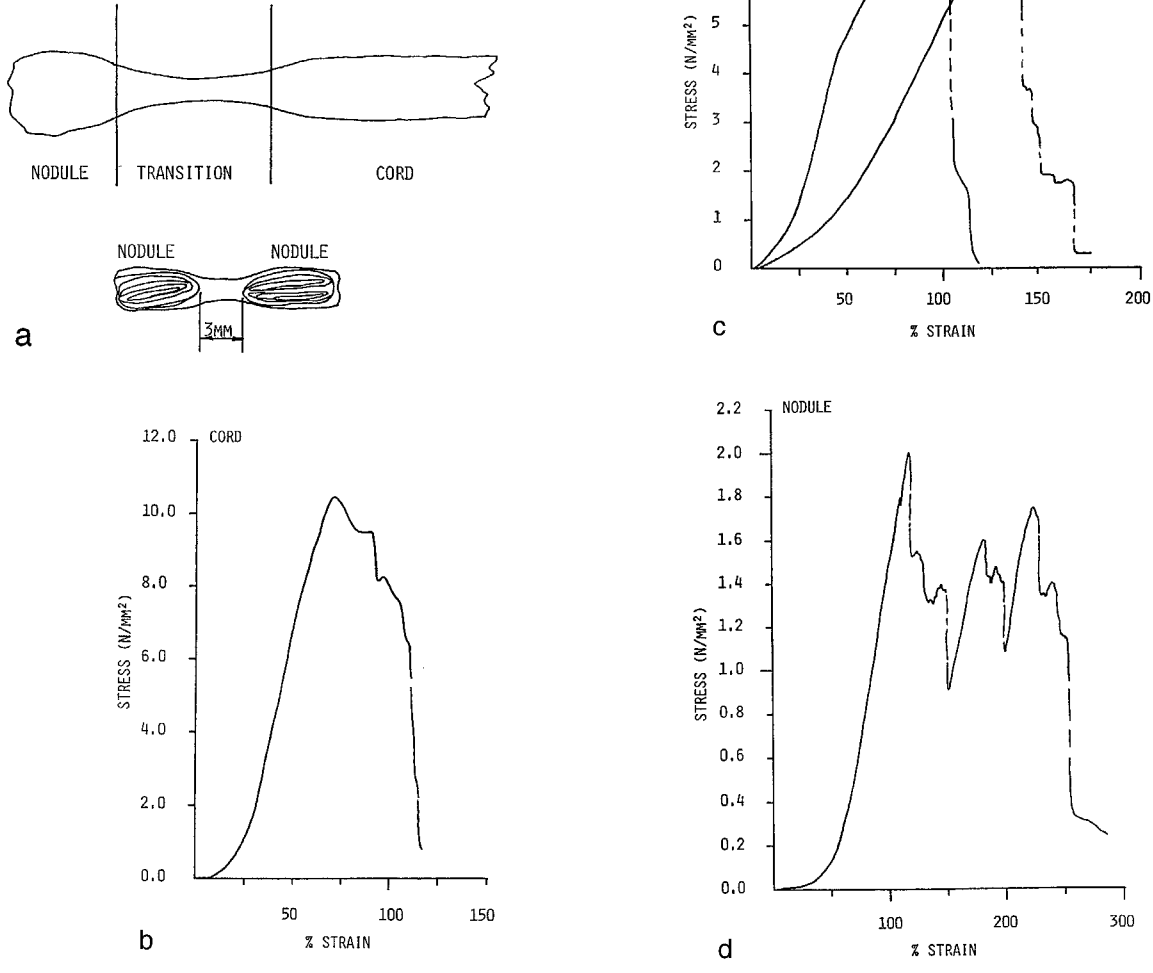


Fig 4 (a) Schematic representation of the Dupuytren's tissue containing nodule, transition and cord tissue type. The sketch below represents the transition region between nodules. (b,c,d) Representative traces of tensile tests for cord, transition region and nodule tissues, respectively. Selected from traces of one patient.

maximum tensile strength (which is a measure of the tissue stretchability). For statistical purposes, data from the two types of transition zone were pooled together. Figures 5a and 5b show the histograms comparing the mean values of maximum strength and the mean stretchability for the three tissue types. These indicated that cord was strong and non-stretchable whilst nodule was almost 60% as strong and 1.7 times more stretchable than cord.

The tensile results confirmed the heterogeneity of Dupuytren's tissue with transition tissue type having the highest tensile strength followed by cord and nodule respectively. The ratio of tensile strength for transition to nodule (2.1, $P = 0.009$) was significant, but those for cord to nodule (1.65, $P = 0.25$) and transition to cord (1.26, $P = 0.56$) were not. However, the stretchability of the tissue was highest for nodule and least for cord with statistically significant ratios for nodule to cord (1.75, $P = 0.0001$) and for transition to cord (1.64, $P \ll 0.00001$).

DISCUSSION

Creep tests

The 0.5 and 0.75 MPa stresses used for the creep tests represent 15.1% and 23.4% of the maximum tensile strength for nodule and 9.4% and 14.1% for cord respectively. Interestingly, the creep strain rates for both nodule and cord were similar at these stress levels at 4°C. At this temperature, it can be assumed that cellular activity would be minimal and that any creep measured would be a reflection of material properties. Increasing the temperature to 37°C caused more than four-fold increase in the creep strain rate for both stresses in nodule.

Creep results show that at 0.75 MPa and at 37°C, nodule creeps 7.2 times faster than cord. This can again be attributed to matrix changes mediated by cells as they are strained since nodules have greater cellularity than cord (Shum and McFarlane, 1988). Indeed, increased levels of degradative enzymes have been reported in stretched Dupuytren's tissue following extension by the Messina technique (Bailey et al, 1994). The absence of nodules after traction treatment would further support the finding that nodule creeps in preference to cord since it is likely that nodular areas would be preferentially extended, thus losing their nodular morphology.

The creep strain rate of 0.36% strain per hour for a 15 mm nodule at 0.75 MPa results in an extension of 1.29 mm over 24 hours, which is within the 2 mm daily extension used in the Messina technique. The method can therefore be used to investigate the fundamental mechanism causing the breakdown of the collagen fibres.

Tensile tests

The toe region of the stress-strain curves can be attributed to the alignment of the collagen fibres with the direction of the applied load. This was more pronounced in the nodule, which macroscopically looked like a whorl, and on fracture was reminiscent of a lattice network. In contrast, the transition tissue was translucent and gel-like in appearance with a less pronounced toe region, suggesting a more aligned collagen network. Although the mean tensile strength of the transition tissue was higher than that of cord, the difference was not statistically significant.

From the specimen (Fig 4a) containing all three tissue types, it was estimated that the ratio of the cross sectional areas for nodule to transition to cord was 9 : 1 : 4.

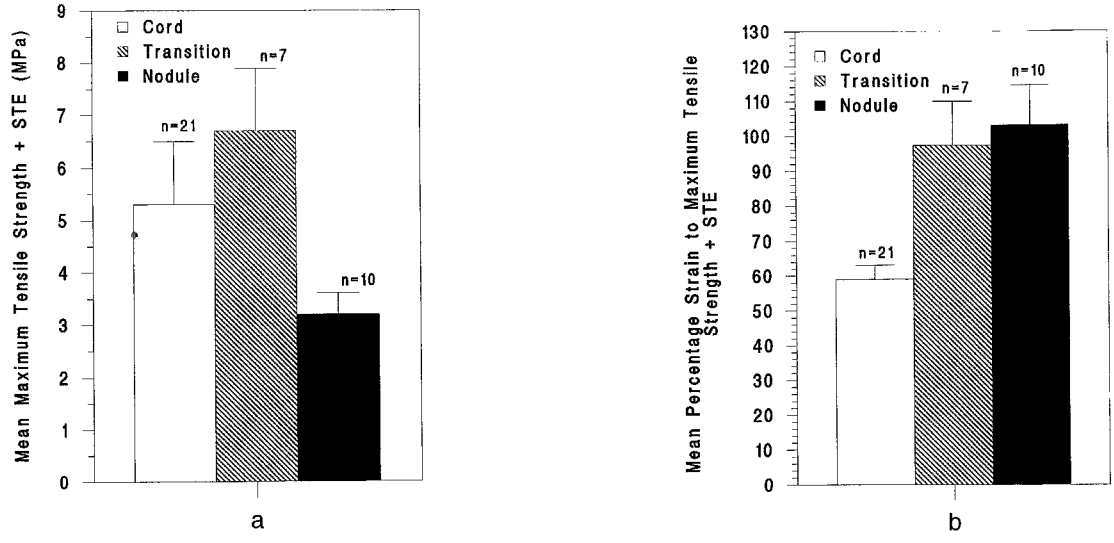


Fig 5 (a,b) Histograms comparing the mean maximum tensile strength and mean percentage strain with maximum strength for cord, transition region and nodule respectively. (n = number of samples and bar represents the standard error).

Given that the ratio of the average tensile strength for the three tissue types is 0.48 : 1 : 0.8, this gives a load ratio of 4.3 : 1 : 3.2. This ratio suggests that any stretching of the contracture from externally applied force such as that of Messina's device (Messina and Messina, 1991) will occur first in the transition tissue. Using a simple model, it can be shown that the contracture must be stretched by 4.75 mm to straighten through 90°. Assuming that any initial lengthening of the contracture is from the transition tissue and that its length is of the order of 10 mm, this represents a strain of 47.5%. This, in turn, is less than half of the average strain needed to produce failure. This may explain why there was no fibre fracture or haemorrhaging in contractures treated by Messina's method since the flexed joint is extended at the rate of 2 mm per day (Brandes et al, 1994; Messina and Messina, 1991). The observations made by Luck (1959) that "contractures of interphalangeal joints occurred only when nodules developed in relation to these joints" and by Messina and Messina (1991) that "extension of the contracture is easier when nodules are present" seem to support this as transition tissue occurs between cord and nodule.

It is less clear, however, what effect occurs on transition tissues when they are placed under endogenous loading which must be part of the pathological progression of the Dupuytren's contraction.

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