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MECHANICAL PROPERTIES OF FRACTURED AND INTACT RAT FEMORA EVALUATED BY BENDING, TORSIONAL AND TENSILE TESTS

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Mechanical properties of healing fractures and growing, intact bones were studied in male rats aged 8 weeks at the beginning of the study period. A standardized, closed fracture was produced in the middle of the left femur. The fracture was not immobilized. At various intervals after the fracture, the healing fractured femora and the contralateral, intact femora were subjected to bending, torsional and tensile tests.

The fractured femora regained the strength and the ultimate deformation of the contralateral, intact femora after about 8 weeks when tested in bending, and after about 13 weeks when tested in torsion. In the first phases of fracture repair, the healing fractures could resist more torsional than bending load, whereas the opposite was found for solidly consolidated fractures and intact bones.

For intact bones, the ultimate bending and torsional moments increased with increase in age and weight of the animals, whereas the ultimate angular deformation remained constant. The ultimate bending and torsional stresses (bone material strength) increased to reach a plateau when the rats were about 14 weeks old.

No significant differences were observed between the bending, torsional and tensile test methods. For the evaluation of fracture repair, each test has its particular application.

Key words: biomechanics; bone and bones; bone deformation; bone development; callus; fracturing forceps; growth; stress, mechanical

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The healing of experimental fractures has mainly been studied with radiographic and histological techniques (Hulth & Olerud 1964, Slätis & Rokkanen 1965, Green et al. 1971). Neither of these, however, measure the functional properties of the healing bone. The functional recovery of a fractured limb is determined by the mechanical properties of the fracture. During mechanical tests, these properties may be compared with those of the contralateral, intact bone. In this way fracture repair may be evaluated quantitatively (Braden et al. 1973, Sarmiento et al. 1977, Paavolainen et al. 1979), and a more functional healing time assessed. This is an advantage when the influence of different factors (hormones, drugs, immobilization etc.) on fracture repair is investigated. The mechanical properties of bone can be determined by application of bending, torsional, tensile, compression and shear tests (Evans 1973). The first three tests are most commonly used, and bending (Weir et al. 1949), torsional (Burstein & Frankel 1971) and tensile (Evans 1973) tests are each claimed to have special advantages.

The purpose of the present study was to establish an experimental fracture model in rats, and

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evaluate the fracture repair by mechanical methods. Bending, torsional and tensile tests were employed for bone loading, and, in addition, the test methods themselves were compared and evaluated.

MATERIALS AND METHODS

Experimental animals

A total of 124 male Wistar/Af/Han/Mol SPF rats were used. They were about 8 weeks old when the study started, and the median weight was 214 grams. Four to 5 rats were kept in each cage, and they were given standard animal pellets containing 0.9 per cent calcium and 0.7 per cent phosphorus (Bl. nr. 3155, Möllesentralen i/s, Oslo, Norway) and water *ad libitum*.

Experimental fracture

Following intraperitoneal anaesthesia (fluanisone 5 mg/kg and fentanyl citrate, corresponding to fentanyl 0.1 mg/kg, Hypnorm Veterinary®, Mekos, Helsingborg, Sweden), a standardized, closed, transverse fracture was made in the mid-shaft of the left femora with forceps (Figure 1). In order to adjust the plate on the fracturing forceps correctly, three rats were killed immediately after the fracture. Both femora were dissected free of soft tissue. The length of the right femur was measured (from the top of the head to the distal end of the medial condyle) with a sliding caliper (accuracy of ± 0.01 mm), and compared with the length of the fragments of the left femur. The plate was then adjusted accordingly, to produce fractures in the middle of the femur.

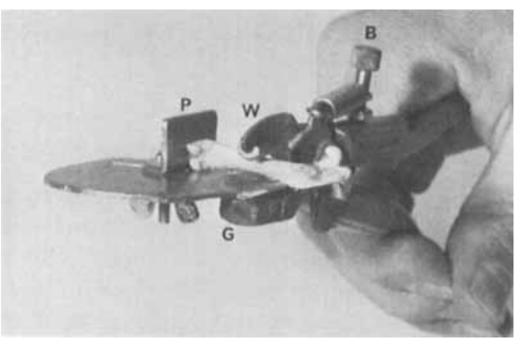

Figure 1. Forceps to make standardized femoral fractures in rats by three-point loading. In vivo, the flexed knee is resting against an adjustable plate (P) in order to localize the fracture to the middle of the femur. The thigh is loaded between the wedge (W) and groove (G) formed arms, resulting in a transverse femoral fracture as the forceps is clamped. To prevent substantial damage to the soft tissue, the deflection of the loading arms is kept constant by the adjustable check bolt (B). (×0.5).

Table 1. Number of rats in the test groups

Test day after fracture	Number of rats			
	Bending tests	Torsional tests	Tensile tests	Total
7	6	6	6	18
10	6	6	6	18
14	6	6	6	18
21	6	6	6	18
28	5	5	5	15
40	5	5	3	13
57	5	5	3	13
93	4	4		8

The fractures were not immobilized, and the rats walked on the broken limb within a few days.

Mechanical testing

At various intervals after the fracture, 8–18 animals were weighed and killed with ether (Table 1). Both femora were immediately dissected free and stored in cooled, isotonic, Ringer's solution. Within 2 hours, the healing fractures and the intact bones were subjected to either bending, torsional or tensile (only fractured femora) tests, and loaded to failure. The bending and the torsional test procedures have been described in detail previously (Engesæter et al. 1978). Briefly, the bending tests were performed by ventral deflection of the distal half of the bone. The distal end of the femora was rotated outwards in the torsional tests the tensile tests, the same clamp was used for fixation of the proximal femur as in the bending and the torsional tests. A wire sling was used for fixation of the femoral condyles. The test specimen was then mounted in the same hydraulic tensile testing machine as used for bending and torsional tests (Engesæter et al. 1978). The force was applied with a constant deformation rate, 0.9 mm/second in tensile tests, corresponding to 2.5 degrees/second (0.04 radians/second) in bending and torsional tests.

Biomechanical calculations

The strength of the bones was defined as the *ultimate moment* (maximal moment) during bending and torsional loading, and the *ultimate force* (maximal force) during tensile loading. These values were read as a y-coordinate from each load-deformation curve. The corresponding x-coordinate was defined as the *ultimate deformation* (Figure 2).

The dimensions of the femora were measured using a

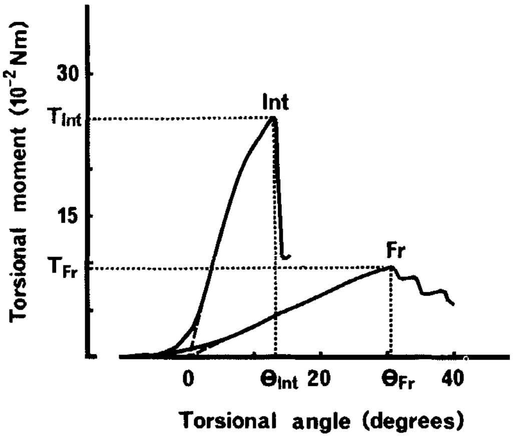

Figure 2. Load-deformation curves for a two-week-old fracture (Fr) and its contralateral, intact bone (Int) tested in torsion. The ultimate torsional moment (T) and the ultimate torsional angle (θ) are indicated. As the fracture healed, the configuration of Fr approached that of Int. Corresponding curves were obtained during bending tests, whereas only healing fractures were tested in tension.

sliding caliper. Before the mechanical tests, the lengths of both femora were recorded. The major (2a) and the minor (2b) diameters of the intact, right femur were measured in the middle of the bone. The thickness (t) of the ventral femoral cortex was also measured in the middle of the right femur immediately after the test.

Both the mid-cross-sectional area and the *area* and the *polar moment of inertia* of bones are related to their load carrying capacity (Frankel & Burstein 1970, Asang 1976, 1978). The *ultimate* (maximal) *bending* and *torsional stresses* express the strength of bone as a material. These mechanical parameters were calculated for intact femora considering bone as an elastic, isotropic, homogenous beam with a hollow, elliptical cross-section. The mid-cross-sectional area (A) of the femora was obtained from the formula:

$$A = \pi(ab - (a-t)(b-t)) = \pi t(a+b-t)$$

The other equations used are given in a previous paper (Engesæter et al. 1978).

Statistical analyses

Median with 0.25- and 0.75-fractiles were used to express the average and the dispersion of the measured values. The statistical significance probability was calculated by Wilcoxon's two-tailed test for two samples (Diem & Lentner 1975), whereas the Kruskal Wallis test was used for three samples (Zar 1974). Differences were considered significant when $2P \leqq 0.05$. The coefficient of variance (V) was calculated from the formula:

$$V = \frac{(0.75\text{-fractile} - 0.25\text{-fractile}) \times 0.74}{\text{median}}$$

(Diem & Lentner 1975, Høyland & Walløe 1977).

RESULTS

Growth of the animals

The rats doubled their body weight during the experimental period (Figure 3). There were no significant differences between the weights of rats used for bending, torsional and tensile tests. The coefficient of variance of the weights was about 0.04 in all the groups.

The increase in bone dimensions from the 7th to the 93rd day after the fracture is given in Table 2. As the femoral fractures were not immobilized, a shortening was observed compared with the contralateral femur. This shortening was about 2.5 mm, and remained almost unchanged during the study.

When the length of the distal fragment of the left femur was related to the total length of the right femur in the 12 first rats tested for tensile strength, a ratio of 0.54 (coefficient of variance 0.04) was found. This shows that the fractures were located near the middle of the femur.

Bending tests

The strength of the healing femoral fractures increased with time, as did the strength of the intact femora as the animals grew in size (Figure 4A).

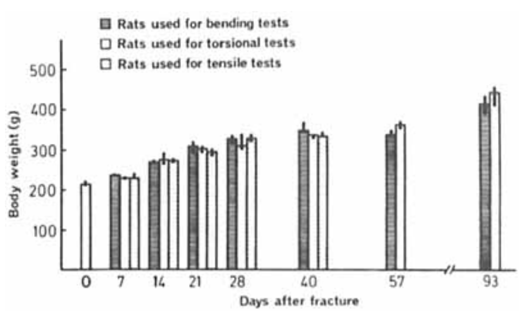

Figure 3. Body weight of the rats during the experimental period. Three to 6 animals in each group. (Median with 0.25- and 0.75-fractiles.)

Table 2. Growth of fractured and intact femora (tested for bending and torsional strength). (Median with 0.25- and 0.75-fractiles) n = Number of animals

Days after fracture	*n*	Body weight (grams)	Fractured femur	Intact femur				
			Length (mm)	Length (mm)	Major diameter (mm)	Minor diameter (mm)	Cortical thickness (mm)	Mid cross-sectional area (mm^2)
7	12	232 (230–238)	28.7 (28.1–29.6)	31.0 (30.8–31.6)	3.7 (3.7–3.9)	2.9 (2.9–3.0)	0.52 (0.49–0.56)	4.6 (4.4–4.9)
93	8	432 (390–446)	35.1 (34.2–37.4)	37.6 (37.3–38.6)	4.6 (4.4–4.6)	3.5 (3.4–3.6)	0.69 (0.67–0.70)	7.1 (6.6–7.5)
Increase (per cent)		86	22	21	24	21	33	54

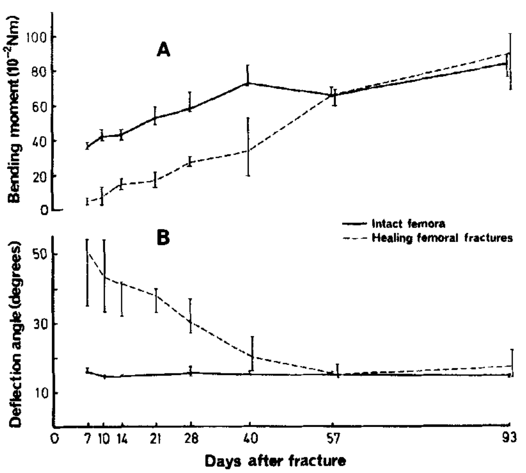

Figure 4. Ultimate bending moment (A) and ultimate deflection angle (B) for healing femoral fractures and the contralateral, intact femora as a function of time following the fracture. Four to 6 animals in each group. (Median with 0.25- and 0.75-fractiles.)

The ultimate deflection angle (ultimate deformation) was about 15 degrees (0.26 radians), and almost constant for intact femora (Figure 4B). For healing fractures, the ultimate deflection angle decreased as the fractures became more stable. At 57 days following fracture, the fractured femora had regained the same strength, and also had the same ultimate deflection angle as the contralateral, intact, femora. All the healing fractures failed through the callus.

Torsional tests

The fractured bones achieved the strength of the contralateral, intact femora at 93 days (Figure 5A). The ultimate torsional angle (ultimate deformation) of intact bones was almost unchanged during the study (Figure 5B). The angles were about 15 degrees (0.26 radians), as observed in the bending tests. The healing fractures reached

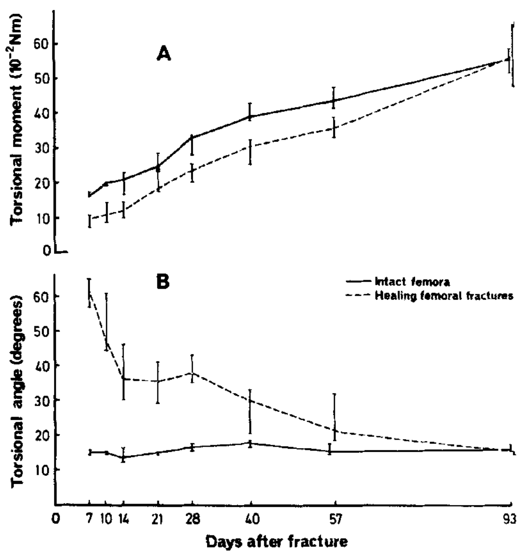

Figure 5. Ultimate torsional moment (A) and ultimate torsional angle (B) for healing femoral fractures and the contralateral, intact femora, as a function of time following the fracture. Four to 6 animals in each group. (Median with 0.25- and 0.75-fractiles.)

this value at 93 days. During torsional tests, the healing fractures first failed through the callus. When the fractures were solidly consolidated, the fractured femora failed at a site distal to the callus.

Tensile tests

The strength of the healing fractures increased monotonically (Figure 6A). At 57 days, the fractured femora had gained a strength beyond the upper load range (98.1 N) of the transducer used. The ultimate elongation (ultimate deformation) did not change significantly during the 40-day study period (Figure 6B). All the healing fractures failed through the callus.

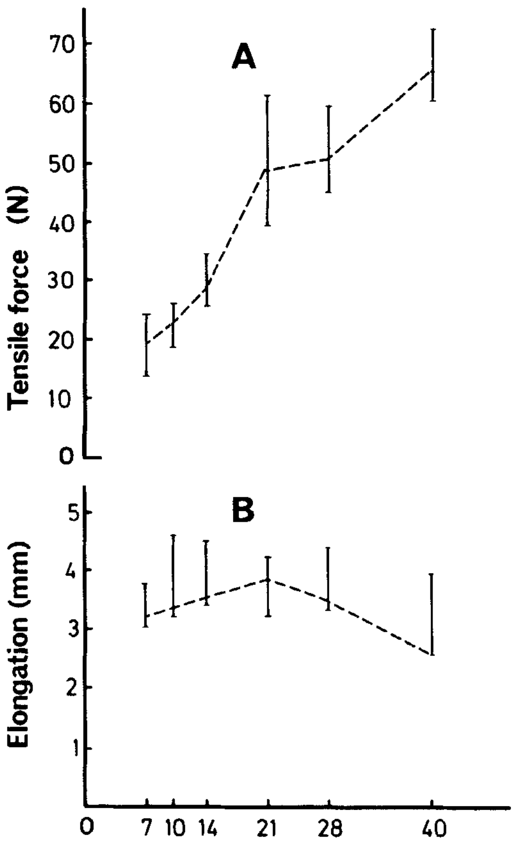

Figure 6. Ultimate tensile force (A) and ultimate elongation (B) for healing femoral fractures as a function of time following the fracture. Three to 6 animals in each group. (Median with 0.25- and 0.75-fractiles.)

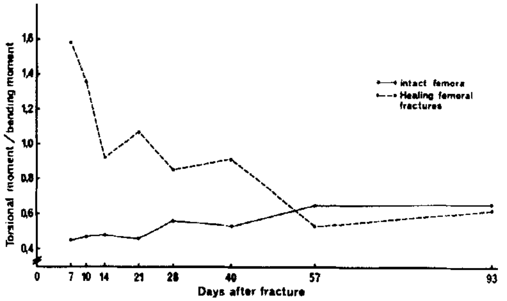

Figure 7. Ratio of ultimate torsional to ultimate bending moment for healing femoral fractures and the contralateral, intact femora as a function of time following the fracture. The ratios are calculated from the median values of the test data, and 4–6 rats were tested each time both in torsion and in bending.

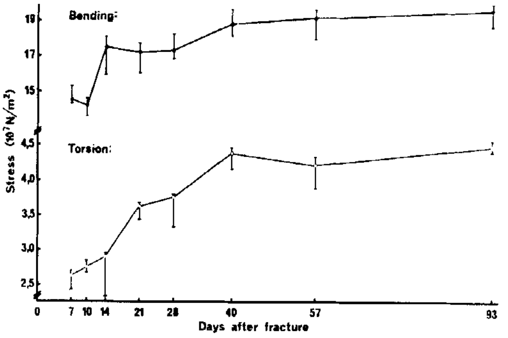

Figure 8. Ultimate bending and ultimate torsional stresses (bone material strength) for intact femora as a function of time following fracture of the contralateral bone. Four to 6 animals in each group. (Median with 0.25- and 0.75-fractiles.)

Comparison of data from bending and torsional tests

The ratio of ultimate torsional to ultimate bending moment was about 0.5–0.6 for intact bones, and increased slowly during the study (Figure 7). The corresponding ratio for healing fractures decreased from 1.6 to 0.6 as the fractures healed.

The strength of the bone material of intact femora, e.g. the ultimate stress, is shown in Figure 8. Both the ultimate bending and the ultimate torsional stresses increased to reach a plateau 40 days after the fracture. At that time the rats were 14 weeks old, and had gained a body weight of about 350 grams. The ratio of ultimate torsional to ultimate bending stress was about 0.22. The

Table 3. Mechanical properties of intact femora. (Median with 0.25- and 0.75-fractiles) n = Number of animals

Days after fracture	n	Bending tests			Torsional tests		
		Ultimate moment (10^{-2} Nm)	Area moment of inertia (mm^4)	Ultimate stress (10^7 N/m^2)	Ultimate moment (10^{-2} Nm)	Polar moment of inertia (mm^4)	Ultimate stress (10^7 N/m^2)
7	6	36.8 (35.2–38.8)	3.5 (3.4–4.0)	14.5 (14.3–15.3)	16.5 (16.5–17.0)	9.6 (8.8–10.4)	2.6 (2.4–2.7)
93	4	84.9 (76.6–91.1)	7.9 (6.3–8.8)	19.5 (18.6–19.9)	55.2 (51.1–58.3)	20.1 (17.2–23.8)	4.5 (4.4–4.6)
Increase (per cent)		131	126	35	235	109	73

Table 4. Coefficients of variance of the test data obtained in bending, torsional and tensile tests. The coefficient of variance was calculated for eight observations (test days) in bending and torsional tests, and for six in tensile tests. No significant differences between the coefficients of variance of the three test types. (Median with 0.25- and 0.75-fractiles)

Test data at ultimate failure	Healing fractures			Intact bones	
	Bending	Torsion	Tension	Bending	Torsion
Moment	0.32 (0.22–0.56)	0.24 (0.17–0.27)	0.24 (0.21–0.35)	0.11 (0.08–0.13)	0.10 (0.06–0.13)
Stress				0.06 (0.05–0.07)	0.08 (0.05–0.09)
Deformation	0.26 (0.19–0.36)	0.27 (0.18–0.43)	0.23 (0.19–0.30)	0.05 (0.04–0.06)	0.09 (0.04–0.12)

increase in ultimate stress during the study (from the 7th to the 93rd day after the fracture) and the corresponding values for moment of inertia and ultimate moment are given in Table 3.

By comparing the data given in Tables 2 and 3, some information about the influence of growth on the mechanical properties of intact bone is obtained. In a period when the body weight increased by about 85 per cent, the femur increased in length and width by about 20 per cent. The ultimate moment increased simultaneously by about 130 per cent in bending and by about 230 per cent in torsional tests. The ultimate angular deformation was, however, rather constant. The median in bending and torsional tests was 15 degrees (0.26 radians) (coefficient of variation 0.07), and 97 per cent of the deformations were below 20 degrees (0.35 radians).

Dispersion of the data

During mechanical evaluation of healing fractures, coefficients of variance from 0.23–0.32 were found both for ultimate moments and ultimate deformations (Table 4). There was less dispersion of the data obtained during testing of intact bones, the coefficient of variance here ranged from 0.05–0.11.

Judging by the coefficients of variance obtained for the test data, no significant differences were observed between the bending, torsional and tensile tests.

DISCUSSION

A fracture may be considered healed when it has reached the strength and the stability of the contralateral, intact bone. In the present study, the ultimate moment for healing fractures (giving information about fracture strength) (Figures 4A and 5A) and the ultimate deflection/torsional angle (giving information about fracture stability) (Figures 4B and 5B), reached values corresponding to those of the contralateral, intact bones after the same healing period. This occurred, however, first in the bending tests. Accordingly, the fractures seemed mechanically healed after about 8 weeks when tested in bending, and after about 13 weeks when tested in torsion. Similar fractures in male rats of about the same weight have been reported to be healed within 10 weeks as evaluated radiologically (Sudmann et al. 1979), whereas increased bone remodelling continues for at least half a year (Bohr 1955).

To determine the healing time of the fractures in the present study, some mechanical properties of fractured and intact femora were compared, assuming equivalence in pairs of intact bones. This assumption has been studied previously, and it was valid ($P < 0.025$) both for the ultimate bending and the ultimate torsional stresses of the bones (Engesæter et al. 1979).

Seven to 10 days after the fracture, the ratio of distal fragment length to intact femur length was 0.54. Since most of the longitudinal growth of the rat femur occurs at the distal epiphysis (Tapp 1966), the fracture location was probably still nearer the middle of the femur when the experiment started. With a coefficient of variance for the fracture location of only 0.04, our fracturing forceps proved to be a reliable device to produce standardized, closed femoral fractures.

Using the contralateral bone as a reference, the shorter healing time for fractures tested in bending compared to those tested in torsion is probably due to differences in the loading configuration of the tested bones, since the experimental fractures were very similar. Thus, during the three-point bending test, the maximum applied load was at the fulcrum in the middle of the bone. Consequently, all healing fractures tested in bending failed through the callus. In torsional tests, the load is equal along the bone, and failure occurs at the weakest section (Burstein & Frankel 1971). Therefore, the healing fractures tested in torsion first failed through the callus. Later, with increasing callus strength, they failed distal to the callus, where the polar moment of inertia of the bone was smaller. In the latter situation, callus strength exceeded the measured values, and this may explain the relatively slower increase in fracture strength (and longer healing time) in the torsional compared to the bending tests. Angulations in the united fractures will produce additional bending moment components during torsional tests. This may reduce the measured torsional moment, offering another explanation for the longer healing time observed in torsional tests. However, the angulations in the fractures were small, and these should not influence the ultimate torsional angle, which behaved in a similar way to the ultimate torsional moment (Figure 5). Moreover, addition of up to one-half of the ultimate bending load to the weakest part of human tibiae during torsional tests has produced the same ultimate torsional moments as those observed during pure torsion (Asang 1978).

The ratio of ultimate torsional to ultimate bending moment changed considerably for healing fractures during the study (Figure 7). This, which seems to be a new observation, is difficult to explain. It may be due to changes in the orientation and the quality of collagen fibres and minerals as the fractures healed, making young callus, unlike normal bone, more susceptible to bending than torsional loads.

The capacity of an object to carry load is proportional to the moment of inertia of its cross-section. In the present study, similar increases were observed in both the area and the polar moment of inertia for intact bones (Table 3). The increase in the ultimate bending moment approximately equalled the increase of the area moment of inertia. The increase in the ultimate torsional moment, however, was more than twice that of the polar moment of inertia (Table 3). This explains why the ultimate torsional stress increased more than the ultimate bending stress as the animals grew in size (Table 3, Figure 8). Also the increasing ratio of ultimate torsional to ultimate bending moment for intact bones by growth (Figure 7) is in accordance with these observations.

The ultimate deformation for intact femora was not influenced by growth, and remained remarkably constant both in bending and torsional tests (ultimate deflection/torsional angle about 15 degrees (0.26 radians), coefficient of variance 0.07). The ultimate bending deformation, unlike the ultimate bending moment, has also been reported to be rather constant in rabbit metatarsals (Currey 1969). These observations may suggest that deformation is a more important criterion than strength in limiting the load-bearing capacity of bones, as already proposed by Currey (1970).

Both the ultimate bending and the ultimate torsional stresses for intact femora increased to reach a plateau when the rats were about 14 weeks old. Similar findings have been reported by Vogel (1979) for ultimate bending stress. Our values for ultimate bending stress are in accordance with those given by Weir et al. (1949) and Vogel (1979), whereas the values of Sarmiento et al. (1977) are lower. Bell et al. (1941) have reported higher values, but a ratio similar to ours of ultimate bending to ultimate torsional stress.

The groups of rats tested in the present study had the same body weight at each test day, and the same fracture location (coefficient of variance about 0.04 for both). Comparison of the dispersions of the mechanical test data may therefore provide some information about the relative accuracy of the bending, torsional and tensile test methods. As expected, the scatter of the test data was much higher for healing fractures than for intact bones, the coefficients of variance being about 0.25 and 0.08, respectively (Table 4). The magnitude of these coefficients is probably due to biological variations in the material, since bending and torsional tests on steel rods, using the same test apparatus, gave coefficients of variance of only 0.01 (Engesæter et al. 1978). Judged by the scatter of the test data, none of the three test methods proved to be significantly better than the others. However, tensile tests are difficult to perform on intact bones without making standardized, machined test specimens. Tensile strength measurements of fractures are most useful during the first phases of fracture repair, constituting a sensitive method to indicate the increasing strength of the connective tissue of the callus (Figure 6). Bending and torsional tests were easily performed both on fractured and intact bones in the present study. In both tests the coefficient of variance for ultimate stress was less than that for ultimate moment (Table 4), probably because the former expression for bone strength is corrected for shape and cross-sectional area of the bone. For the study of fracture repair, the bending test presented is most suitable for determining the mechanical properties of the callus, since this is where the bone fails during the test. The torsional test is more functional, as it evaluates the mechanical properties of the entire healing bone, indicating the weakest section.

In conclusion, femoral fractures in male rats reached the strength and the stability of the contralateral, intact femora faster when tested in bending than when tested in torsion. Material strength of intact bone (ultimate stress) increased until the rats were about 14 weeks old. To evaluate the mechanical properties of healing fractures, bending, torsional and tensile tests each have their particular applications.

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