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METHODS FOR TESTING THE MECHANICAL PROPERTIES OF THE RAT FEMUR

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A modular apparatus to measure the bending and torsional properties of the rat femur is presented. Both intact femur diaphyses and diaphyseal fractures in different phases of healing can be tested. It is also possible to measure the bending-strength of the distal femur metaphysis and the epiphyseal plate.

The apparatus can be used to investigate the effect of drugs and hormones on the remodelling of the rat femur.

Key words: methods; mechanical properties; fractures; bones; femur; rats; epiphysiolysis; metaphysis; diaphysis

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Both the growth of bone and fracture healing are influenced by many factors. In a clinical situation, however, it is usually impossible to evaluate the effect of each of these factors separately. In animal experiments, factors of importance for bone remodelling can be tested separately and may provide relevant information for the understanding of clinical problems.

The rat is a widely used and convenient animal for experiments on bones. This paper presents an apparatus in which the mechanical properties of the rat femur can be tested in several ways. These include:

(1) Diaphyseal bending tests
(2) Epiphyseal plate/metaphysis bending tests
(3) Diaphyseal torsional tests.

DESCRIPTION OF THE APPARATUS

(1) Diaphyseal bending tests

For bending tests the femur is supported as a cantilever (Figure 1), where the bending force is transferred to the distal end of the femur by a cam mounted on a rotating vertical disc. The disc is rotated by a flexible wire attached to its circumference. The proximal end of the femur is fixed by a clamp so that the medullary canal is coincident with the axis of the disc.

To localize the fracture site in the bone, a metal pin is used as a fulcrum for the test bone (Figure 1). The pin can be moved horizontally, at the level of the disc centre, and adjusted to touch the bone. Accordingly, the fracture will also be located at a site coincident with the axis of rotation of the disc.

Figure 1c shows that the measured moment (T × a) equals the moment applied to bend the distal femur fragment ventrally relative to the proximal fragment (P × b). Because the arm of the applied force (i.e., the radius of the disc, a in Figure 1c) is constant, the known applied force (T) is directly proportional to the bending moment (P × b). It is therefore unnecessary to measure the distal fragment of the femur to find the applied moment.

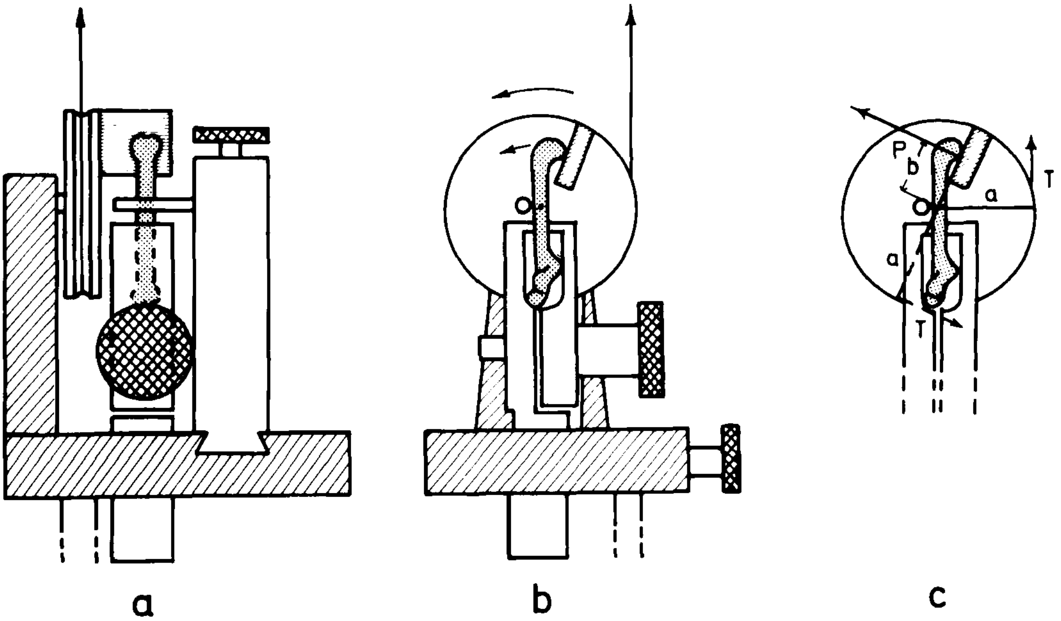

Figure 1. The bending apparatus — frontal (a) and lateral (b) view. Figure 1c shows that the moment measured (T × a) equals the moment needed to bend the distal part ventrally relative to the proximal part (P × b) (Rø et al. 1976).

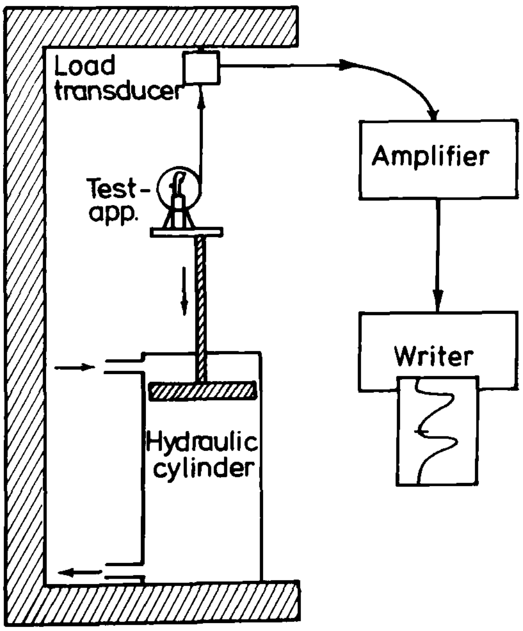

Figure 2. Equipment for measuring mechanical properties of the rat femur.

The force T is applied by a hydraulic tensile testing machine type 7-1/1 (AB Lorentzen & Wettres Maskinaffär, Stockholm, Sweden) (Figure 2). The tension in the wire is measured with a load transducer (HBM Kraftaufnehmer Type U1, load range 0–10 kp, Hottinger Baldwin Messtechnik, Darmstadt, W. Germany), which is connected via an amplifier (HBM Messverstarker KWS/T-5) to a chart-recorder (Riken Denshi Co. Ltd, Model SP-J5B, Tokyo, Japan). The force is applied with a constant deformation rate, 0.04 radians per second (2.5 degrees per second).

A typical bending stress-strain curve of the intact femur diaphysis is shown in Figure 3. The curve gives information about: the maximum stress, the stiffness of the bone in the linear elastic part of the curve (tg α) and the angle of deformation necessary to produce fracture (fracture angle). The area below the curve expresses the energy which the bone can absorb before fracture.

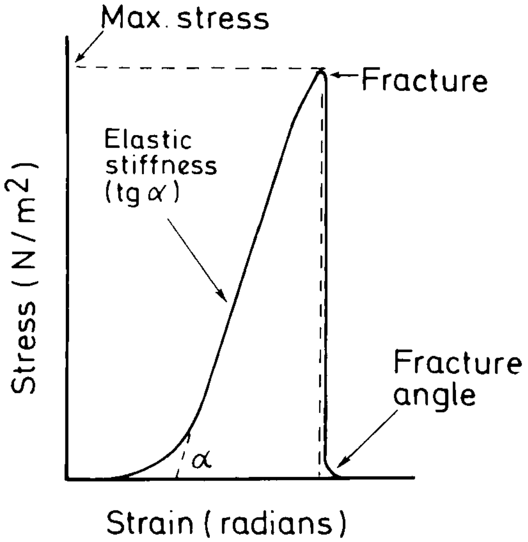

Figure 3. Stress-strain curve for bending of the rat femur diaphysis.

(2) Epiphyseal plate/metaphysis bending tests

The bending moment necessary to produce epiphysiolysis and metaphyseal fracture can also be tested. The bone is placed as shown in Figure 4a with the disc centre and the fulcrum pin just proximal to the epiphyseal plate.

With this arrangement the cam on the disc will first give epiphysiolysis followed by dislocation of the epiphysis. By continual rotation of the disc the distal part of the metaphysis will fracture. The resultant moment-deformation curve (Figure 4b) has two peak values, the first one represents epiphysiolysis and the second one represents metaphyseal fracture of the bone. As the epiphyseal discs of the long bones in rats do not close (Farris & Griffith 1949) this test method is not restricted to young rats.

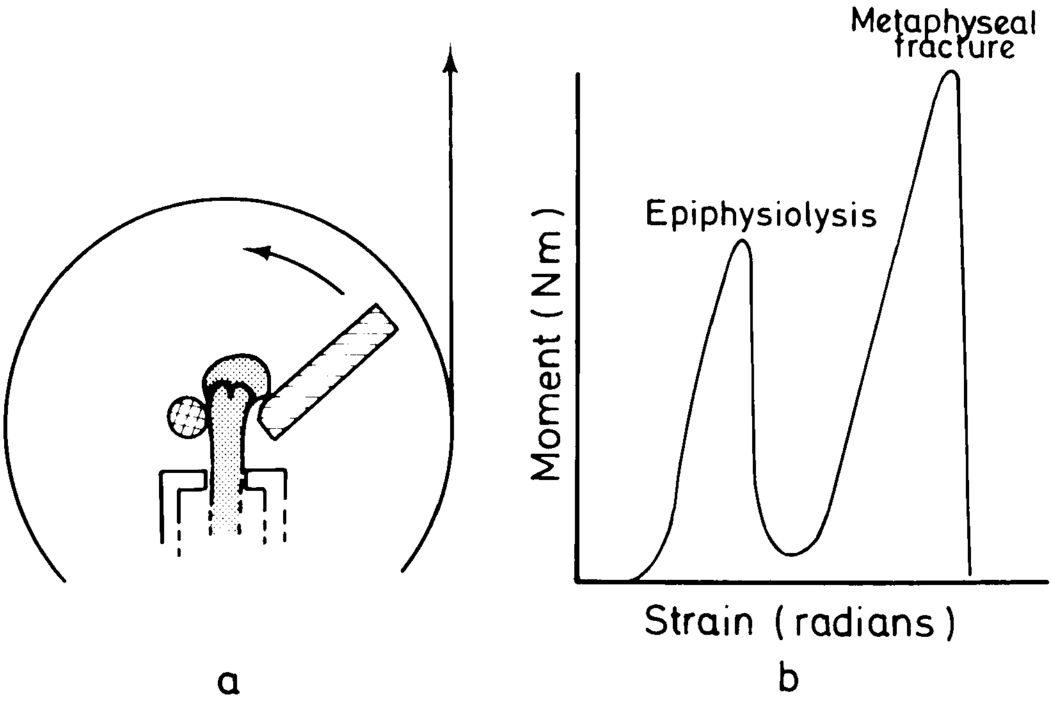

Figure 4. Testing of the epiphyseal plate and the metaphysis in bending (a). Rotation of the disc will first give epiphysiolysis and then metaphyseal fracture. This is recorded as two peak values in the moment-deformation curve (b).

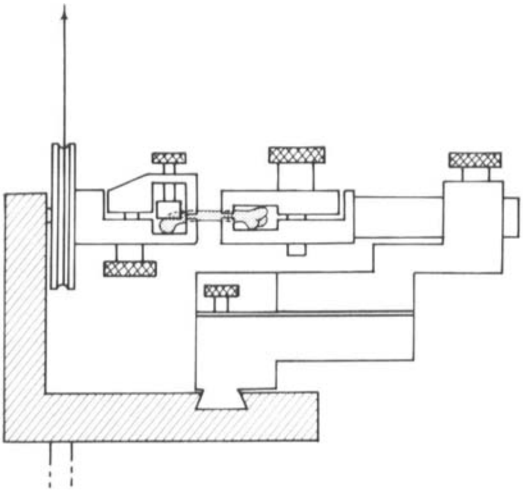

Figure 5. The arrangement for torsional testing. The force is applied via a rotating disc as in the bending apparatus.

(3) Diaphyseal torsional tests

The torsional testing system is illustrated in Figure 5. The torsional moment is, as in the bending apparatus, applied via a rotating disc. The distal end of the femur is attached to the disc via a special clamp. The clamp which fixes the proximal end of the femur can be moved horizontally and adjusted to different angles when testing fractures united in angulated positions. The clamps are constructed to envelop the ends of the bone (Figure 5). Therefore only part of the rotating force is transferred to the bone via the claws of the clamps, the rest being transferred by the body of the clamps. This arrangement reduces the stress concentration and the chance for fracture at the claws.

The tension of the wire, and thereby the applied moment, is continually measured as described in the bending tests. The stress-strain curve is also principally the same (Figure 3). The bones can be tested by both inward and outward rotation of the distal part relative to the proximal part.

STRESS CALCULATIONS

The stress in the test bone (force/cross-sectional area) depends not only on the applied moment, but also on the shape and the area of the cross section of the bone. Because of variation in the size and shape of the femurs, not only from one femur to another, but also along the diaphysis in the same bone, the stress calculations can only be approximate.

In earlier investigations these problems have been simplified by calculating the cross-sectional area of the femur as a hollow ellipse (Bell et al. 1941). The formula for the maximal bending stress of an elastic, isotropic, homogeneous beam with a hollow elliptical cross section, derived from Roark & Young (1975), is:

$$\text{Max. bending stress: } \sigma_m = \frac{M \times b}{I} = \frac{4 \times M \times b}{\pi(ab^3 - (a-t)(b-t)^3)} \quad (1)$$

where M is the maximal bending moment, a and b are the major and minor external radii of the hollow ellipse, t is the thickness of the hollow ellipse and I is the area moment of inertia for an ellipse.

The maximal torsional stress for a hollow ellipse is:

$$\tau_m = \frac{T \times b}{I_p} = \frac{4 \times T \times b}{\pi(ab^3 + a^3b - (a-t)(b-t)^3 - (a-t)^3(b-t))} \quad (2)$$

where T is the maximal torsional moment and I_p is the polar moment of inertia for a hollow ellipse.

Obviously the cross section of the femur of the rat deviates from a hollow ellipse. To estimate the magnitude of this simplification, six rats (Wistar SPF, with mean weight 278 ± 12 g) were investigated. A slice from the middle of the right femur was photographed through a microscope and magnified (25 ×) (Figure 6). The cross-sectional area was measured by a planimeter. The area and polar moment of inertia were calculated by graphical integration. The area moment of inertia is $\sum x^2 dA$, where dA is the

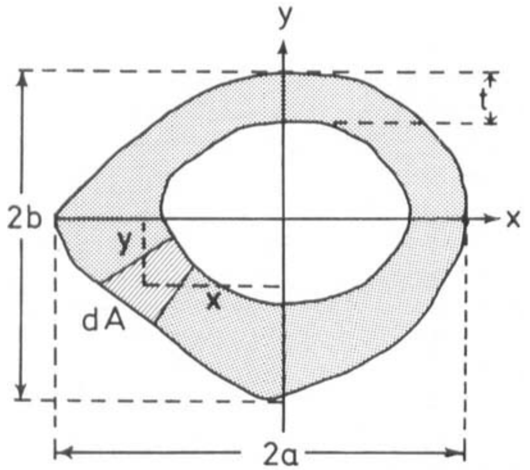

Figure 6. Cross section of the middle part of the rat femur diaphysis. 2a and 2b represent the major and minor diameters, t the ventral thickness, x and y the distance of the elemental area, dA, from the diameters.

elemental area (Figure 6). The polar moment of inertia is $\sum(x^2+y^2)dA$.

The femur dimensions were measured using a sliding callipers (accuracy of ± 0.01 mm). The thickness of the femoral cortex (t in the equations (1) and (2)) was measured ventrally in the middle of the femur (Figure 6). The major diameter (2a in the equations) and the minor diameter (2b in the equations) were also measured in the middle of the femur.

The calculated values for the area and polar moment of inertia firstly regarding the femur as a hollow ellipse, and secondly by direct measurements of the cross-sectional area using planimetry, are given in Table 1. The mean calculated area moment of inertia is 11 per cent higher than the measured value. The calculated polar moment of inertia is only 2 per cent higher than the measured value. These differences will of course give the corresponding differences in the maximal stress values (see equations (1) and (2)).

To get an impression of the absolute differences in the maximal stress with the measured and the "elliptical" moments of inertia, the maximal moment must be known. Therefore, fresh femurs of two groups of six rats were tested, one group in bending and the other in torsion. These rats were equivalent to the rats used for moment of inertia measurements. The mean maximal bending and torsional moments were found to be 0.44 Nm and 0.20 Nm, respectively. The measured and "elliptical" maximal bending and torsional stresses calculated from the equations (1) and (2) are shown in Table 1.

TESTING OF THE APPARATUS

To estimate the accuracy of the apparatus, polished steel rods (diameter 1.70 mm) were tested, ten for bending and eight for torsional strength. At the same rate of testing as the rat femurs the steel rods did not fracture, but exhibited plastic deformation. Therefore, the force value at 0.79 radians (= 45 degrees) deflection was chosen, since this was representative of the force level seen in the bone experiments (Figure 7).

Table 1. Calculated and measured values for the area moment of inertia/bending stress and the polar moment of inertia/torsional stress in the rat femur

	The area moment of inertia (± standard deviation)	Max. bending stress	The polar moment of inertia (± standard deviation)	Max. torsional stress
Calculated values	4.07 ± 0.60 mm^4	$1.62 \cdot 10^8$ N/m^2	10.63 ± 0.87 mm^4	$2.91 \cdot 10^7$ N/m^2
Measured values	3.63 ± 0.52 mm^4	$1.82 \cdot 10^8$ N/m^2	10.45 ± 1.14 mm^4	$2.96 \cdot 10^7$ N/m^2

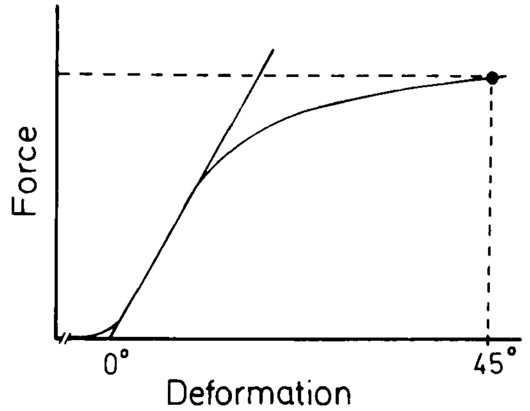

Figure 7. The force deformation curve for the steel rods in bending (principally the same for torsion). The force value at 45 degrees deflection is marked. The deflection is calculated to start where the tangent to the elastic part of the curve crosses the abscissa.

The formulas for maximal stress for a solid circular rod (Roark & Young 1975) are:

$$\text{Maximal bending stress: } \sigma_m = \frac{M \times r}{I} = \frac{4M}{\pi r^3} \quad (3)$$

where r is the radius of the circular rod. The mean bending stress at 45 degrees was $1.73 \times 10^9 N/m^2$ (Standard deviation (s.d.) 0.8 per cent).

$$\text{Maximal torsional stress: } \tau_m = \frac{T \times r}{I_p} = \frac{2T}{\pi r^3} \quad (4)$$

The mean torsional stress at 45 degrees was $6.04 \times 10^8 N/m^2$ (s.d. 1.3 per cent).

DISCUSSION

The test system which is described is a simple and accurate method for testing the mechanical properties of both intact rat femurs and fractured femurs in different phases of healing.

The objections to this test system can be divided into mechanical and biological factors.

Beside the limitations of the load transducer, amplifier, chart recorder and tensile testing machine, some mechanical inaccuracies are known: The weight of the cam on the disc gives tension in the wire, but this is below the sensitivity of the transducer/chart recorder (i.e. less than 0.05 N). Friction both in the ball-bearings and between the bone and the cam on the disc is probably greater, but difficult to estimate. The overall accuracy of the apparatus tested by the steel rods gave, however, a standard deviation of about 1 per cent. This indicates rather accurate measurements, especially since this deviation may partly be caused by variation in the steel rods.

The biological part, i.e. the test bones, is probably the main source of errors in the test system. Angulations in the united fractures will give an unwanted bending moment when tested in torsion, which may result in a reduced measured moment. The magnitude of this error depends on the extent of the angulation. Approximations have to be made in the calculation of the stress from the measured moments and dimensions. Firstly, in the calculations the stress-strain curve is assumed to be linear up to the fracture. Weir et al. (1949) found that air-dried rat femur remained elastic only up to three-quarters of the maximal bending stress. Also in the present study the bones displayed plasticity near fracture (Figure 3). This plasticity may make whole bones considerably stronger (Currey 1969 and Burstein et al. 1972). Secondly, bone is not a homogeneous material, but it is penetrated by stress concentrators such as blood vessels, canaliculi and osteocytic lacunae. In addition, the muscle insertions make the surface uneven. Thirdly, the calculations ignore the effect of shear stress, but this error was estimated to be very small (about 0.25 per cent) in another cantilever system (Smith & Walmsley 1959). The fourth assumption is that the cross section of the femur diaphysis is simplified to be a hollow ellipse. In our investigation this simplification is estimated to give a difference of 11 per cent for the area moment of inertia and 2 per cent for the polar moment of inertia. This is in accordance with

Mather (1967) who found that the mean value for the elliptical calculated area moment of inertia of human femurs was 14.5 per cent higher than the measured value.

These inaccuracies in stress calculations are very difficult to avoid, but some of them are systematic and will therefore be less important when different groups of bones are compared.

The method of testing the maximal bending moment of the epiphyseal plate and the metaphysis makes it possible to test the growing cartilage and newly produced bone. Both should be important, for example when investigating the influence of drugs and hormones on the bone growth.

The test apparatus described is constructed as a modular system. The main parts of it are used both in the testing of bending and torsional properties. It can also be used for tensile strength measurements, but bones are normally very seldom subjected to pure tensile stress.

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