Effect of preservation medium on the mechanical properties of cat bones

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Mechanical properties of long cat bones after freezing at –20 °C and after storage in 10 percent buffered formalin were investigated. The right humeri and femora from 48 adult cats were divided into four groups: Groups 1 and 2 were stored in a freezer at –20 °C for 3 and 21 days, respectively, while Groups 3 and 4 were stored in 10 percent buffered formalin for 3 and 21 days, respectively. The control left bones were tested fresh. The humeri were subjected to a torsion test and the femora to a four-point bending test. Freezing had no effect on the mechanical properties evaluated, while formalin storage caused a 50 percent reduction in energy absorption and increased the brittleness of the bones.

Mechanical properties of bone are used to evaluate bone healing or remodeling in animal experiments. For practical reasons, some form of preservation is required, notably freezing below –20° C and formalin storage. However, controversy prevails about the effects of fixatives on mechanical properties of bone (Carothers et al. 1949, Calabrisi and Smith 1951, McElhaney et al. 1964, Evans 1964, Sedlin 1965).

We have studied effects of freezing and formalin storage on mechanical properties of diaphyseal cat bone.

Materials and methods

Forty-eight healthy adult cats weighing between 2.8 and 4.5 kg were used. The cats were killed with an overdose of sodium pentobarbitone, and the humeri and femora from both sides were harvested. The soft tissues were removed, and the left specimens were tested immediately to serve as controls; specimens were divided into four experimental groups:

Groups 1 and 2: Specimens were stored in a freezer (–20° C) for 3 and 21 days, respectively.

Groups 3 and 4: Specimens were preserved in formalin (10 percent) for 3 and 21 days, respectively.

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Specimens stored in the freezer were thawed to room temperature before testing. All the humeri were subjected to a torsion test and all the femora to a four-point bending test.

Torsion test

Both ends of the humerus were firmly fixed by quick self-curing dental cement (Vertex, Dentimex, Holland) with the length of the exposed central portion standardized to 40 mm. The specimen was mounted onto a torsional testing device (Shimadzu Corp., Kyoto, Japan) with the longitudinal axis of the humerus kept in alignment with the axis of rotation. The specimens were kept moist and tested to failure at a rate of 0.18 radian/min external rotation. The maximum torque, torsional stiffness, and energy absorption were recorded (Dataletty 401, Shimadzu Corp., Japan).

Four-point bending test

Using dental cement, both ends of the femur were embedded into 75 mm x 25 mm glass moulds leaving 40 mm of the central portion of the bone exposed. Alignment of the specimen was maintained by a jig, and the glass moulds were removed by breakage. The prepared specimen was then mounted onto a four-point bending apparatus (Augograph DCS 5000, Shimadzu Corp., Japan). The distance between the two supports was standardized at 140 mm, and the distance between the two loading points to 60 mm. Bending was performed in the anteroposterior plane. The central deflection of the specimen was measured using a linear variable dif-

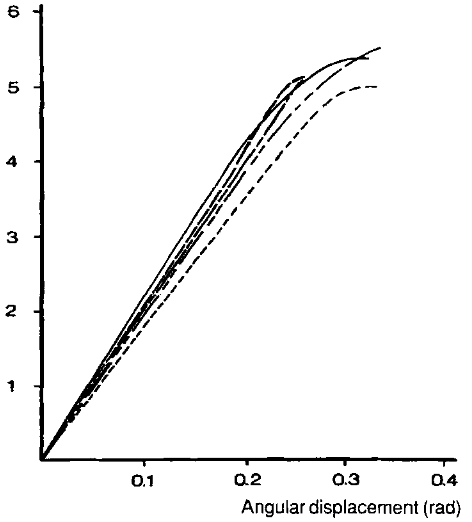

Figure 1. Typical torque versus angular displacement curves. Each curve represents a specimen from each group tested. ——— fresh, — — — 3 days formalin, — - - — 3 weeks formalin, — - — 3 days fresh, - - - - 3 weeks fresh.

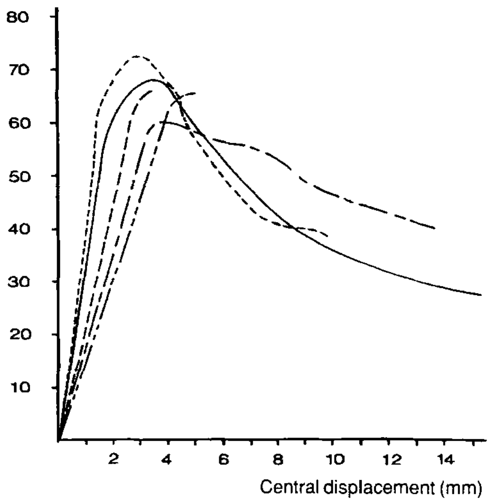

Figure 2. Typical bending load versus central displacement curves. Each curve represents a specimen from each group tested. Symbols as in Figure 1.

Table 1. Torsion test values as a percentage of fresh contralateral controls

		Group			
		1	2	3	4
Maximum torque	Mean	99.6	93.2	88.7	89.0
	SD	13.8	12.3	15.6	19.7
Torsional stiffness	Mean	93.7	96.1	102	99.6
	SD	16.4	18.9	10.7	12.6
Torsional energy	Mean	101	90.9	63.9	54.8
	SD	19.6	12.4	8.9	10.6

t-test. All the groups were compared; only significant differences are indicated. * $P < 0.05$, ** $P < 0.01$.

Table 2. Bending test values as a percentage of fresh contralateral controls

		Group			
		1	2	3	4
Maximum torque	Mean	97.2	101	88.3	90.4
	SD	17.9	17.1	18.0	15.2
Torsional stiffness	Mean	96.2	98.5	102	105
	SD	11.4	11.5	9.4	13.0
Torsional energy	Mean	102	93.2	46.3	49.9
	SD	7.8	14.6	8.9	8.5

t-test. All the groups were compared; only significant differences are indicated. ** $P < 0.01$.

ferential transformer (Schaevitz HPA series, Pennsauken, NJ, USA). The specimens were tested to failure at a crosshead speed of 5 mm/min. The maximum bending load, bending stiffness, and energy absorption were recorded.

Data from the experimental groups were expressed as ratios in relation to the fresh contralateral control bones. The Student's *t*-test was used to assess differences.

Results

Storage in formalin reduced the energy absorption value during torsion by 40 percent, and during bending by 50 percent (Tables 1 and 2 and Figures 1 and 2). Freezing did not change the mechanical properties.

Discussion

Our findings that freezing does not alter the mechanical properties of bone are in general agreement with the results reported in previous studies (Sedlin 1965, Pelker et al. 1984, Panjabi et al. 1985).

There have also been many reports on the effects of embalming on the physical properties of bone (Calabris and Smith 1951, Carothers et al. 1949, Evans 1964, McElhaney et al. 1964). However, information on the influence of 10 percent formalin alone has not been well documented. Sedlin (1965) found little change in the modulus of elasticity of human cortical bone after 3 weeks of storage in 10 percent formalin, but suggested that formalin and embalming solutions may affect biomechanical properties, and therefore should not be used in mechanical studies. Our study showed that preservation in formalin did not alter the maximum loading capacity and stiffness, but caused a marked change in energy-absorption capacity. The results indicate that preservation in formalin causes bone specimens to loose their nonelastic (plastic) behavior, so as to become more "brittle."

The results suggest that formalin changes the structural bond in the osteons. The consequence of this is loss of the energy-absorbing behavior of bone. Use of formaldehyde to preserve bone for transplantation as suggested by Lavrishcheva et al. (1981) has to be carefully reconsidered.

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